HISTOLOGIC ANALYSIS OF TRANSVERSE GROWTH IN THE RAT MAXILLARY ALVEOLAR COMPLEX

by

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Abstract

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The purpose of this study was to perform a histologic analysis of rebound growth in the transverse dimension of the rat maxillary alveolar complex. A previous study by Tsolaikis, Cain, and Hans (1990) examined the growth of the maxillary complex during and after passive transverse restraint on the maxillary molar teeth of female Sprauge-Dawley rats. The experimental group was subjected to twenty-one days of passive restraint applied cross arch to the maxillary molar dental units. After release from restraint, maxillary growth was monitored for twenty-seven more days. Results from serial dental impressions and serial cephalometric radiographs indicated complete restriction of maxillary molars during restraint and nearly complete rebound to control values during the post-restraint phase.

The current study has made a histomorphometric analysis of fluorochrome dyes injected during the post-restraint rebound phase. The relative frequency of new

bone deposition in controls (n=12) and experimentals (n=16) was specifically analyzed to evaluate differences between sutural bone at the mid palate and alveolar bone surrounding the molars. Representative sections of first, second, and third molars were selected for each rat. Each section was analyzed in right and left molar areas to assess alveolar bone and the mid palatal area to assess sutural bone. Microradiographs of each section were used to differentiate sutural from alveolar bone.

Results are that experimentals had a 16.7% higher frequency of new deposition in alveolar bone than did controls, while sutural deposition between the two groups was nearly the same with only a 1.8% higher frequency in experimentals. The results of fluorochrome dye analysis indicate that rebound growth occurred primarily by alveolar remodeling. These findings support a functional based paradigm of facial growth as opposed to a genetically based theory. Within the context of the cybernetic system, the alveolar bone fills the critical role of dynamically responding to feedback, thereby maintaining normal morphology. Clinically, it is important to account for the dynamic and adaptive nature of alveolar bone when treatment planning.

DEDICATION

This work is dedicated to the memory of my great-grandfather, Henry Oliver Cain, D.D.S. (Cincinnati College of Dentistry, 1900) and my grandfather, Joseph Marian Cain, D.D S (Western Reserve University School of Dentistry, 1915). Their hard work and sacrifice has allowed me to pursue my education to a high level, and their wide ranging love of life and people has always been an inspiration.

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INTRODUCTION

Orthodontics is both a clinical and theoretical discipline. Clinically, treatment decisions in orthodontics have been empirical If a given mode of treatment is shown to consistently succeed for a given problem, it is emulated and becomes doctrine. Morphologic changes are the criteria of clinical success, as should be the case. Unfortunately, the histologic changes that accompany changes in form are poorly documented and even less well understood. Consequently, a scientific basis of treatment is elusive.

Like clinicians, theoreticians of facial growth propose intellectually satisfying arguments that are consistent with gross observations. These gross observations take the form of serial analysis of radiographs, dental casts, and photographs. Despite extensive experimental data there is little support for theory at the tissue level.

It was the purpose of this study to histologically analyze sutural and alveolar bone during experimental post restraint rebound growth. This served to test two dominant and dichotomous theories of facial growth, genetic and environmental. This will also hopefully lead to a more scientific basis for treatment decisions.

In a previous study by Tsolaikis, Cain, and Hans (1990), a growing rat model was subjected to passive transverse restraint of the maxillary molar teeth, followed by

a post restraint phase Serial, radiographic, and dental cast analysis show that, during the post restraint phase, experimental animals rebounded and established essentially the same molar width as controls. Thus, significant transverse rebound growth occurred during the post restraint phase But did this growth occur primarily at the sutures, as would be predicted by facial growth theorists who support a genetic paradigm? Or did rebound growth occur primarily in the dental alveolus, as would be predicted by theorists who favor an environmental or functional effect to facial growth?

It was the purpose of this study to document not just what happened on a gross morphologic level, but where, specifically, at the tissue level did this change occur. It is hoped that by understanding morphologic changes on a histologic level that clinical treatment decisions can be made on a more scientific basis.

LITERATURE REVIEW

It is my intention to study facial growth. To understand the intellectual arguments and rationale for experimental design, it is important to have a historical perspective on theories and tests of them. This review of the literature will be in four sections. First will be a historical overview of theories of facial growth. Second will be a synopsis of clinical and experimental attempts to restrain growth. Third will be background on the histology of epifluorescence and use of histomorphometrics with a literature review of microradiography Lastly, a brief background of pertinent rat anatomy is provided.

Facial Growth Theories

As the face of any infant develops to that of an adult there are many morphologic changes. This is not just an increase in size, but a significant change in shape, structure and proportion (Enlow and Dale ,1994). That a baby does not look like an adult is obvious to even the most casual viewer. These changes are the purview of the study of facial growth. Facial growth theorists propose how and why these changes occur. These theorists can be seen as supporting basically one of two paradigms: either nature or nurture. The nature proponents explain the control mechanisms (the how and why) as genetic in nature. The nurture proponents explain environment as the important control factor. Both camps have extensive observational and experimental data to support their

beliefs. First, the genetic or sutural theory and supporting evidence will be reviewed. Second, the environmental or functional matrix theory and supporting evidence will be reviewed. Next, the nasal septum theory, an epigenetic one with relevant experiments will be reviewed. Finally, a synthesis of these theories as developed in a cybernetic model will be reviewed.

Sutural Theory

Genetic theorists believed that cranial form and facial development were overwhelmingly genetically controlled at the sutural joints between facial bones. Early observational evidence came from Milo Helmann (1933). Using accepted anthropometric techniques, he analyzed over 1100 orthodontically treated patients for changes. His conclusion was that orthodontics effects the dentition and supporting alveolus, but neither the direction nor the amount of growth of the facial bones.

Helmann's influential article ushered in the "nature", or genetic paradigm. He held that other than obvious gross traumatic effects, facial growth pattern and amount is genetically predetermined and cannot be altered, as had been previously thought. Jaw disharmonies, therefore, were genetic in cause. Interestingly, Helmann's theories were coincidental with the introduction of the lateral cephalometric radiograph as a diagnostic and research tool. Broadbent (1931) was responsible for the introduction of this technique as a tool. He did not propose any theory, but observed through longitudinal growth studies, a downward and forward growth of the face. Others were to try and

explain "why" and "how" Brodie, in 1938, made use of this technique and concluded that skeletal patterns were unalterable and orthodontic treatment is limited to the alveolus and teeth.

Among the genetic advocates, a more specific causal site, the "how" of facial growth was sought. Weiman and Sicher (1947, 1955) believed that growth at bony sutures was the motive force causing the downward and forward growth documented by Broadbent, as well as cranial vault growth. Weinman and Sicher specifically sited the frontomaxillary, zygomaticomaxillary, and the suture between the pyramidal processes of the palatine and pterygoid processes of the sphenoid to be responsible for downward and forward maxillary growth. The lateral cranial vault growth is specifically caused by the sagittal suture system composed of the medial palatine, intermaxillary and metopic sutures. According to Weinman and Sicher, connective tissue proliferation at the sutures was the driving force.

Elaboration of the sutural theory came from the work of Massler and Schour (1951) and Baer (1954) with work on rats. Their conclusion was that sutures are analogous to epiphyseal plates; that they are autonomous growth centers with intrinsic potential. Mednick's (1956) work on infant pigs concurred. All of this work focused on cranial vault sutures.

Further support for sutural immutability came from Lewis (1969) and Lewis and Irving (1970). Rat calvarium suture was grown in organ culture. The bony remodeling

pattern in culture was similar to that in the intact animal This implies an intrinsic control factor in the suture that is independent of influence from neighboring tissue (local environmental). Transplanted cranial base synchondrosis of the rat was studied by Koski and Ronning (1969, 1970). They concluded that the suture is more inherently controlled than condylar cartilage, but less so than epiphyseal plate cartilage of long bones.

Another organ culture study was done by Servos (1975) He examined synchondrosal cartilage in rats. He reported that synchondroses grown in organ culture grew normally, without contribution from neighboring tissue. Servos concluded that spheno-occipital synchondroses have an inherent growth potential beyond reactive or adaptive responses.

In a study using beagle dogs, Wijdevel et al. (1968) performed palatal surgery on different aged animals. Metal implants were placed in the palate to allow serial lateral and dorsoventral radiographs to study the effects of palatal surgery. The surgery had no influence on the anteroposterior or vertical growth, and very little on transverse growth despite alteration of the adjacent tissues. His conclusions support a genetic based cause for maxillary growth.

A clinical human study of patients with maxilonasal dysplasia was done by Nordenrain and Thilander (1989). An orthodontically treated group was compared to untreated groups as well as a group with orthognathic profiles and normal occlusion. Results showed no difference in growth between the dysplasia groups despite successful

results for the dentition of the treated group. In this study, orthodontic treatment did not influence sutural growth.

Functional Matrix Theory

In contrast to the sutural theory, which began in the 1930's, the functional theory has older roots. As early as the 1700's, functional based theories could be found in literature. Fauchard (1728) and Hunter (1778) stated that, "based on clinical observation, that the presence of healthy deciduous and permanent dentition is required for proper maxillary and mandibular development". Wolf (1870) combined engineering principles of his day to the cellular deposition of bone, stating that functional stresses on bone determine how new reparative bone is deposited; this pattern being best suited to distribute and withstand the induced stresses.

Specific to dentofacial orthopedics, Kingsley (1880) introduced extraoral headgear to effect both the maxillary dentition and supporting alveolus. John Koch (1917) later applied Wolf's theories of architectonics to facial bones. Wolf, Kingsley, Hunter, and others embraced the optimistic concept that carefully applied external forces could alter function and form of any bone, including those of the face.

During the 1930's, the functional approach to growth and the belief in the mutability of skeletal form fell out of favor. The sutural belief prevailed theoretically and clinically. The late forties and fifties, though, saw a resurgence of interest in a functional approach.

Helmann's (1933) and Brodie's (1938) influence prevailed until the 1960's, but was not unchallenged. A series of experimenters used various models to refute or support the sutural paradigm, contributing to a more refined understanding of the complexities of facial growth.

In 1947, Washburn unilaterally dissected out the temporalous muscle on growing rats. His results showed alteration in morphology of both the cranial bones and the mandible, thus challenging the genetic paradigm.

Another challenge to sutural based paradigm came from Selman and Sarnat (1957). They found that obliteration of the fronto nasal suture of the rabbit failed to arrest growth. Ford (1958) from his study of dried skulls attributed cranial vault growth to brain growth pressure. He had also attributed anterior vault growth to pneumatization of frontal and ethmoid sinus spaces. Another dissenter was Young (1959) who attributed the causal factor of growth to intracranial pressure. This transmitted forces to sutural fibers resulting in tension and osteoblast activity. Dubruland and Laskin (1961) in a study discussed the adaptability of the mammalian skull. When the spheno-occipital synchondrosis was removed in young animals, there were changes in shape and size of the entire skull of the adult animals. Interestingly, the adult skull form simulated the classical changes associated with the change to upright posture. These researchers had laid the ground work for Moss, (1960, 1962, 1968, 1969) who is credited with developing the functional matrix theory. Young and Ford suggested neural matter pressure and

pneumatic pressure, i.e. "nurture" causes for the "why" of facial growth. Moss extended this concept to include nerves, muscles, glands, organs, special sense organs, respiratory airways, as well as sinuses. In his work from the mid 1950's to late 1960's, he devised a variety of experimental conditions and analyzed results. In a comprehensive review of his own work, Moss (1969) draws on nearly 30 of his own articles and summarizes the role of functional matrices in facial growth. According to Moss, functions are carried out by a "functional cranial compartment" which is comprised of a "functional matrix" and a "skeletal unit". The functional matrix performs a task, the skeletal unit protects and/or supports the functional unit. More specifically the skeletal units consist of bones, cartilage and tendinous tissues. An individual bone is anatomically described as a "microskeletal unit" in Moss's terms. For example, the maxilla is a "skeletal unit" composed of several microskeletal units. The term "functional matrix" as mentioned earlier, comprises not only all the attached and associated soft tissues of muscles, nerves, vessels, glands, fat, etc., but also the functioning spaces, such as the oronasopharyngeal cavity. Moss divides functional matrices into two categories: periosteal and capsular. A periosteal matrix is composed of a muscle attached to a bone with periosteum, tendon, vascularity, innervations and lymphatics. Bone deposition or resorption occurs as the result of muscular demands or lack of demand. This opposes the concept of genetically predetermined bone morphology or framework onto which muscle grows attached. A capsular matrix must be conceived of as a volume; a space filled by neural tissue or a

space filled by air. The capsule surrounds and protects this volume. Moss defines two capsular matrices, neurocranium and orofacial. The neurocranial capsule matrix consists of the neural mass of brain leptomeninges and cerebral spinal fluid The orofacial capsular matrix consists of the nasopharyngeal functioning spaces that facilitate breathing and deglutition.

Behrents (1975) tested Moss's theory of a neural capsular matrix with an ingenious technique. He produced intracranial unilateral electrolytic lesions of the trigeminal nerve on 113 rats aged 10 to 20 days. There were four groups of experimentals; in one the trigeminal was severed, in another a lesion was placed in the gasserian ganglion, in a third the lesion was interior to the ganglion in the axon, and in a fourth the lesion was posterior to the ganglion. Animals were examined for motor impairment and a sensory loss just prior to sacrifice at 50 days. Trigeminal nerves were dissected out to histologically verify location of lesions. Dorsoventral and lateral cephalometric x-rays were made to analyze for asymmetries. Results showed a genuine neurotrophic effect, with the greatest asymmetries present in animals with the most severe ganglionic lesions. The effects, however, were slight and did not reflect the magnitude of input from the neuro capsular matrix attributed by Moss.

Frankle (1980,1983) designed his appliance to fill the orofacial capsule and encourage normal development where it did not occur. Frankle stresses the importance of stretching the periosteum and for lip seal exercises to stimulate bone growth and

retrain muscles.

An experiment of nature, cyclopia in man and pigs, was studied by Bacon and Mathis (1983). They specifically examined the basicranium and found it significantly shorter They postulated that the shortened basicranium was due, at least in part, to the absence of the septo-ethmoidal process This led to their contention that the ethmoidal strut is of major influence in development of a normal cranial base, contributing support to a neural capsular matrix theory.

Histologically, Oyen and Russel (1989) provided support for the functional matrix theory. They studied dried skulls of cercopithecoid monkeys, ceboid monkeys and anthropoid apes. They postulate the role of woven bone in facial growth and remodeling. Woven bone is characterized by rapid growth and relative independence from endocrine control. It responds to changes in functional biomechanical demands by rapid construction of a framework or scaffold that, despite a light weight per unit volume, can withstand high stress and strain. If new functional demands remain constant, the woven bone framework can be filled in by lamellar bone or remodeled to yield Haversian bone. Rapidly deposited woven bone provides a histologic model of how bone responds consistent with the functional matrix theory.

Human autopsy specimens were used to study the palatal maxillary region by Melsen and Melsen (1989). They postulate that the palatal bone plays a key role in facial growth as a buffer between the pharynx and the maxillary complex, these two areas

having significantly different inherent growth patterns and functions. The palatal bone is a boundary between areas of differing functional demands and is remodeled accordingly on an individual basis to meet differing airway demands. In a surgical experiment by Beck (1988) a soft tissue palatal cleft was created in utero in lambs. Observation and measurements showed the experimental lambs to have significantly transversely restricted palates. The author attributes this difference to lack of a normally functioning soft tissue matrix.

Thimaporn, Goldberg and Enlow (1990) used a rat model and, with cyanoacrylate, unilaterally prematurely fused the zygomatico-maxillary suture. Effects on growth were analyzed both cephalometrically and histologically. Cephalometric results revealed no induced asymmetry. Histologic analysis showed compensatory periosteal bone deposition at the junction of the inferior aspect of the maxillary process of the zygoma and the maxilla proper on the fused side. There was also resorption in the corresponding areas of the non-fused side. These compensations prevented gross asymmetry. The authors concluded that environmental factors and biologic demands stimulated the compensation to maintain symmetry.

Another experiment of nature, craniosynostosis, or premature fusion of cranial sutures, in human infants, was examined by Richtsmeier et al. (1991). Pre-operative computed tomographic (CT) scans were compared to one year status postoperative images in eleven patients. Every basicranial landmark measured showed a development

to normalcy, supporting the concept of a neural cranial capsular matrix having a direct effect on basicranial form.

Nasal Septum Theory

While Moss was developing his functional matrix concepts and the sutural camp continued to compile evidence, a third and different concept was being developed by Scott (1953,1954,1956). He proposed that the cartilaginous nasal septum, projecting from the middle of and dividing the craniofacial complex, was responsible for the observed downward and forward growth. He claimed that the projecting septum exerted a traction force on the sutural system that pulled the complex forward. Lathum (1969) provided a critical anatomical aspect of the system, the septo-premaxillary ligament. This ligament, in theory, ties the cartilaginous septum to the bony facial skeleton, thus transmitting the traction force. Lathum's further study (1970) stressed the contribution of the retromaxillary fat pad in providing a cushion and pushing force. This developed into Scott and Lathum's theory of multiple assurances in which there are genetic controls with mechanical failsafes to assure normal facial development. One can see that this concept is neither purely genetic nor functional, but its own category of theory. Experimental support for this theory came from Kvinnsland (1973a, 1973b). Rat nasal septum was autotransplanted (surgically removed but placed in another sight of the same animal with blood supply) and exhibited significant growth, indicating genetic predisposition to growth.

Hans (1981) in a unique non-traumatic experiment used an anti-rat nasal septum antibody in an attempt to arrest growth of the nasal septum. The result was rats with slightly shorter snouts, again supporting the role of the nasal septum in facial growth.

As can be seen even from this brief overview, evidence for a genetic, a functional, and a nasal septum explanation to craniofacial growth gathered. Support for a middle ground, which takes into account aspects of these three theories can be seen in the work of Bjork, Enlow and Petrovik.

Synthesis of Concepts

Bjork (1964, 1970) made extensive studies of maxillary and mandibular growth by means of metallic implants placed in human subjects. In the craniofacial complex pairs of implants were placed on either side of sutures. By means of superimposition the distance between implants could be measured on serial cephalometric radiographs. Using the analysis of these results growth of the maxilla has been plotted. Increase in anteroposterior dimension occurs at the palatine maxillary suture and by deposition periosteally at the maxillary tuberosity. Growth in the vertical dimension occurs at the maxillary frontal and zygomatic processes at the suture and by periosteal deposition at the inferior boarder of the alveolar process. Growth in the transverse direction occurs by mid palatal sutural growth and by buccal alveolar deposition. According to Bjork then, growth in all dimensions has both sutural and alveolar process contributions. Remodeling of periosteal surfaces accounts for the nasal floor and the nasal spine migrating inferiorly.

Bjork also concluded that both sutural and condylar growth can be modified by external forces.

Rather than studying serial radiographs with implants, Enlow (1963, 1965, 1966, 1968, 1975, 1982, 1990) used histology to analyze facial growth. He used individual dried skulls of specific ages. By making cuts perpendicular to the surface of bones and examining these cross sections, he mapped the distribution of endosteal and periosteal bone deposition. In this way he was able to map the surface of craniofacial and mandibular bony surfaces as either resorptive or depository through time. For any given bone this mapping quantifies the two basic factors in bone deposition, i.e. amount and direction. This mapping accounts for the tremendous subtleties of morphologic variation in the human face, even within racial types. The idea that a surface could be resorptive yet move forward, as shown by cephalometric analysis, was novel. Enlow explained it by the very important concept of secondary displacement. Secondary displacement of an individual bone occurs when other bones that are superior and posterior to it (i.e., towards the cranial base) grow individually. For example, the lacrimal bone will be carried downward and forward, independent of its own growth, by virtue of growth of the zygoma, sphenoid and nasal bones. Primary displacement, on the other hand, results from the individual bones own growth. This occurs by a combination of sutural and periosteal growth. Concerning the genetic versus functional paradigms, Enlow's work requires both. Genetics predisposes to facial form, but individual subtle variation within

the form requires input from the periosteal and capsular matrices

It was Petrovik (1972) who applied cybernetics to facial growth. Cybernetics is "the study of control processes especially in mathematical analysis of the flow of information". (American Heritage Dictionary, 1976) This method of analysis was broadly applied at the time to electronic, mechanical, biologic and social systems Cybernetics originates from the greek word for control or govern These models then, present self regulation mechanisms so that the system functions within certain parameters. Petrovik presented a flow diagram to explain the control mechanisms for mandibular growth, but the approach can be applied to the entire cranial facial complex. It provides for input from genetic and epigenetic factors, as well as from the functional matrix. This synthesis of ideas is held by most facial growth investigators today, although the degree of influence from any one factor is still arguable.

In conclusion, some generalizations can be made. The cranial base and the growth of cartilage, especially of the spheno-occipital synchondrosis seems to be controlled by intrinsic genetic factors. The growth of intramembranous bone of periosteal and sutural connective tissues are genetically influenced, but depend on epigenetic and functional factors for full expression of form. Experiments of nature and by man have shown that growth processes are influenced by adjacent structures, so are under local epigenetic control. Clinical experience (Frankel 1980, 1983) and experimental evidence (Stutzman and Petrovic 1976, 1977) indicate that growth processes are highly influenced

by external functional stimuli. Genome determines general characteristics such as basic form, growth period and possibly growth potential. Epigenetic and environmental factors, such as muscle forces and airway demands, produce significant modifications. Consequently, we can allow for consistency of racial types, but allow for significant variation within the types.

Experimental Results of Growth Restraint

Now that the sutural, functional matrix, nasal septum and cybernetic models of facial growth have been explored, I will examine clinically and experimentally induced growth restraint.

That external force can affect bone formation has been apparent for centuries. Flathead Indians of North America, the papoose board effect on Indian infant skulls, and the binding of young Chinese women's feet to keep them small are ancient examples of growth modification.

In the early 1800's Cellier (1809) and Fox (1803) attempted to modify mandibular growth with a chin cup attached to a hairnet for occipital anchorage. Kingsley (1855) was the first to describe extraoral force directed against the maxillary arch. Care, in 1895, used the term "orthopedic". He felt that to correct facial deformity "movement of other parts" was equally important as dental correction.

In the early twentieth century though, orthopedic treatment fell out of vogue. The genetic immutability of the growth pattern, as expressed by Hellman (1933) and Brodie

(1938) deemed this treatment to be hopeless or even harmful.

Oppenheim (1934, 1936) reintroduced extraoral headgear and Kloehn (1947) popularized it. The contemporary concept was that the maxillary dention was moved posteriounly or at least held in place while the maxilla and mandible grew forward. Kloehn optimistically discussed changing and guiding the growth pattern. Watson (1955), Klein (1957) and Newcomb (1958) reported actual skeletal treatment changes, specifically to the palatal plane. Likewise, a rotation of the sphenoid bone was reported by Wieslander (1963) and Sanduskey (1965) from extraoral headgear.

Greater use and more sophisticated analysis of extraoral headgear lead to greater understanding. Direction of force (Poulton, 1959; Nelson and Evemark, 1969) magnitude and duration of force (Armstrong, 1971; Watson, 1972; Badell, 1976) and consideration of the vertical dimension of growth and orthopedic force all received attention.

Several animal studies have investigated the effect of extraoral force. Henry (1976) used very heavy posteriorly directed extraoral forces on the craniofacial complex of growing Macaca mulatta. The maxilla, facial bones and cranial vault were all significantly effected. Kantomoa and Ronning (1985) subjected 78 ten day old rats to premature synostosis of sutures with cyanoacrylate. The sutures glued were fronto-nasal, fronto-premaxillary and fronto-maxillary. This created a restriction is in the anteroposterior direction. Results showed diminished mandibular length and increased mandibular

height. Histologically there was a difference in condylar cartilage with the experimental groups being thinner. No anterior crossbites developed. These interesting results illustrate the significance of the maxillary/mandibular growth relationship.

Experiments Exploring Post Restraint Compensatory Changes

Effects of restraint on growing patients and animals is well studied. Few researchers however, have followed with post restraint studies.

On human subjects Watson (1972) used high pull headgear. One to three months after cessation of treatment he found rebound from periodontal space condensation, tipping of teeth, bone bending and sutural fiber deformation. Watson, Wieslander and Buck (1974) used lighter forces (less than half of Watson in 1972) and found little tendency for post treatment change.

Tuenge and Elder (1974) studied post treatment changes in the maxilla of monkeys following extraoral high pull traction. They used implant markers for cephalometric analysis as well as double fluorochrome dye analysis. Four experimental animals were used. Headgear wear averaged seventy-one days. One animal was sacrificed at the end of headgear use, the others at two weeks, six months and ten months post treatment. Cephalometric and histologic evidence showed rapid reversal of alveolar changes in the two week post treatment sample. The six and ten month sample animals showed continued relapse with most change occurring at the sutures. Although there was significant relapse in all samples, the cephalometric growth pattern of experimentals

showed a significant difference from controls. All analysis of changes in this study was in the antero-posterior direction.

Henry (1976) used a 1,600 gram force with high pull headgear on growing monkeys. The result was alteration of the maxilla, facial bones and cranial vault. During a twenty-one day post restraint phase he observed partial relapse of both skeletal and dental changes. Unfortunately no long term cephalometric nor histological analysis was made. He theorized that rebound was due to recovery of bone deformation and sutural compression. He further theorized that, after this initial relapse, little if any change would occur because all remaining force would be dissipated.

In a rare study of transverse palatal restraint and rebound, Fotis (1985) examined dental and skeletal post treatment changes in humans who had been treated with maxillary splints designed to prevent maxillary development. He found that, after appliance removal, there was significant rebound and change in both the transverse and vertical dimensions. Results were from radiographic analysis and were not well detailed in the published report.

Histologic Studies

Most studies report results from analysis of changes in gross morphology, most commonly using the serial radiographs. These studies provide much information on how a face changes in space over time, but provides no way to know where in the cranial facial complex this change is occurring. In Bjork's famous implant studies (1970) he

placed tantalum implant markers on either side of facial sutures to measure rate of bone deposition across that suture and better explain, by means of radiographs, where specifically the growth is occurring. But even this technique does not account for all possible types of bone deposition and facial growth. Considering Enlow's (1976) description of primary and secondary displacement, clearly only primary can be accounted for by implant studies. The only way to confidently analyze where facial growth is occurring is at the tissue and cellular level. This is a histological approach to facial growth. Histologic analysis of bone and it's investing tissues has been done since the early use of a microscope. Most bone histology, prior to the 1950's, was done as decalcified paraffin sections. The invention of plastic, however, allowed for development of a non-decalcified technique (Woodruff and Norris, 1955; Yaeger, 1958; Burkhardt, 1966; Velde, 1977). After proper preparation of the bone, polymethylmethacrylate perfuses osteoid and spaces while air is removed under vacuum. The result, after complete polymerization of all the clear plastic is a totally infused specimen which can be sectioned very thin to 100 microns and still maintain dimensional and anatomic integrity. These plastic embedded nondecalcified bone sections can be stained, microradiographed, or used in conjunction with autoradiography or fluorochrome dye analysis. The technique best suited to analyze deposition of bone over time and therefore, bone growth, is fluorochrome dye analysis. It is equally valid for intramembranous or cartilaginous bone deposition, and remodeling deposition. This marks the bone deposition on the day

of injection only, and therefore is a time marker (Frost, 1960). These dyes exhibit epifluorescence. When struck with the appropriate wavelength or band of light, the dye kicks off an electron and fluorescenses. A special light source with custom filters is used on the microscope to visualize these dyes in the thin prepared sections of whole specimens.

The most common use of fluorochrome dye analysis is in biopsy of human patients with Type I osteoporosis and related calcium metabolic disorders. (Eriksen, et. al, 1994) Specific techniques vary, but all are based on the principle of multiple dyes; i.e. one type of tetracycline being given followed by a waiting period of seven to twelve days, followed by a different type of tetracycline that will fluoresce a different color. Some techniques call for a second lag phase and third tetracycline dose. A biopsy specimen is taken from the iliac crest of the patient and is prepared as previously outlined for calcified tissue. Although there are other dyes available, only Food and Drug Administration approved tetracycline is used in humans because of it's bio-compatibility. Iliac crest bone is a common biopsy site because it is easily obtained and provides cortical bone. In cortical bone, deposition is in distinct layers. These layers are marked when the tetracycline dose is given. These layers are measured by a technique to be described shortly. Although bone biopsy for osteoporosis is a common use of vital or fluorochrome dyes (Recker, 1985), they have been used for centuries to study bone growth.

Lemnius (1567) was the first to mention red colored root of madder. Madder had

been used for centuries to stain cloth. Interestingly, the cloth stained better if soaked in a lime solution rich in calcium. Belchier (1736) was the first to associate feeding madder to animals to vitally stain the bone While eating dinner at a friend's home, he noted the red stain in the pork bone. His friend was a printer who used madder for color printing. Rather than throw out the mash it was fed to the pigs. Duhamel (1742) was the first to demonstrate that the madder die was taken up only by newly forming bone. Hunter (1771, 1778) reported on the growth of the mandible in the pig by periods of feeding madder and withholding madder. He also demonstrated wide red and white bands in sectioned long bones that corresponded to periods of madder feeding. Brash (1924, 1934) repeated Hunter's early work with similar results. Specifically in the mandible he measured amount and rate of growth in the vertical and anteroposterior direction. Alizarin red S is the principle tinctorial agent found in madder. It is available in synthetic form and can be injected intraperitoneally or intravenously in a 2% solution It has been used in various growth studies such as Schour et. al, (1941) to study bones and teeth. Cleall et. al, (1968) studied normal craniofacial skeletal growth of the rat with alizarin. His study was of sagittally cut sections. It later became apparent that other calcium chelators such as tetracycline were useful for bone growth study as well. Soni and Malloy (1973) used alizerin and tetracycline to study the effect of temporalis muscle removal on the coronoid process in guinea pigs. Interestingly, Soni and Malloy's findings oppose those of the nearly identical study by Boyd, Castelli and Huelke (1967) which was

not a histologic analysis, but based on a macroscopic analysis

Multiple fluorochrome injections, when deposited on cortical bone, can be read as time markers like the rings of a tree. By measuring the distance between dye layers and providing the interval time in days, a mineral apposition rate (MAR) is obtained. (Anderson, 1982; Eriksen et. al, 1994) This when used with osteoporotic biopsy analysis tells the physician the severity of the patient's disease. This technique, of obtaining numerical data from histologic sections, is termed histomorphometrics. Histomorphometrics gets around a common problem in histology; that it is descriptive. Descriptive history is very important and interesting, but is not amenable to statistical analysis. Histomorphometrics digitizes the analog image producing numbers that can be statistically analyzed.

Histomorphometrics can be applied to non-cortical bone also, such as trabecular bone. Trabecular bone is not deposited into distinct measurable layers. To analyze vital dyes in trabecula bone, a grid, i.e., an eye piece reticle, is used. It is a well accepted technique of data collection (Weible et. al, 1966; Merz, 1968; Frost, 1969, 1977; Jee and Parfitt, 1980; Recker and Frost, 1982). The technique uses a ten line by ten line grid. Intersections of lines are referred to as "points" The grid is super imposed on the microscope section. If a "point" lands on an area of interest (example, a specified dye) it is referred to as a "hit" and records them. Although there are automated techniques using sensors and computers, the manual technique is well documented (Bromley et. al, 1966;

Frost, 1969, 1977; Merz and Schenk, 1970; Schenk, 1976) for skeletal tissue The principle disadvantage of the manual technique is considered to be that it "demands excessive personnel time" (Jee and Smith, 1984). Despite the usefulness and reliability of histomorphometric analysis of fluorochrome dyes, the technique has been used only sparingly in the study of facial growth.

Tuenge and Elder (1974) used a double fluorochrome analysis in their headgear study on monkeys. Their histologic analysis found initial rebound from restraint to be dento-alveolar and further rebound to be primarily sutural. Stutzman and Petrovic (1976) review an extensive series of experiments on rat maxillae in attempts to study accelerated and modified growth. Use of bovine somatotropic hormone (STH) and nasal septum obliteration with and without STH, were analyzed. STH increased growth in both nonresected and nasal septum resected animals. Three experiments on changing tongue volume were reviewed; volume was decreased by partial and nearly complete glossectomies, increased by inserting plastic wedges and increased by STH injections. Glossectomies resulted in decreased sutural activity. Macroglossia resulted in increased sutural growth. Another series of experiments used hooks embedded on either side of the suture to restrict growth. This was done across the pre-maxilla/maxillary suture and across the mid palatal suture. The result in both cases was decreased growth. There was no post restriction phase of the experiment. The fluorochrome technique used in these studies is unusual in that only one dye was used. It was injected prior to the experiment

so that growth analysis was made by examining bone laid down after the dye From the 1970's through the 1980's to the present, Roberts has contributed greatly to the study of bone growth by applying the most sophisticated histologic analytic techniques available to orthodontic and orthopedic research. In reviewing his own work, (1992) Roberts outlines the various techniques to analyze nondecalcified, plastic embedded, bone sections. These are microradiography, fluorescent labels, birefringence of polarized light, autoradiography, nuclear volume morphometry, and finite element modeling. A large part of his work relates to endosseous implants, their loading and the bone implant interface. Fluorochrome analysis of titanium dental implant healing sites has revealed the rate of new bone deposition and maturation. It has confirmed what Frost (1950) has called RAP, or regional accelerated phenomenon. RAP occurs when bone has been surgerized or otherwise traumatized. There is a higher concentration of osteoblasts to produce new bone. This bone is first laid down in a scaffold-like framework. This allows for maximum support with minimum calcified material in the least time. This is the same woven bone matrix described by Oyen and Russel (1989). This framework is later more highly calcified in the maturation phase. Rynearson (1987) used unspecified "bone labels" to study orthodontic movement of corticotomised teeth. Scott and Kudlick

In addition to alizarin red and tetracycline compounds, there are other calcium chelators that are used for fluorochrome analysis in experimental animals. Two of these are calcein and xylenol orange. These compounds fluoresce at different wavelengths

producing different colors thereby allowing for multiple dye analysis by histomorphometrics. An important question then, is, do different fluorochrome labels give equivalent histormorphometic information? This question was taken up by Sun et al. (1992). Their method used six groups of rats. Group one had calcein (10 mg./kg.), group two tetracycline hydrachloride (30 mg./kg.), group three had alizarin complexone (25mg./kg.), group four had xylenol orange (90 mg./kg.), group five had two dyes; calcine followed by tetracycline and group six had tetracycline followed by calcine. Rat tibias were analyzed histomorphometrically for mineral apposition rate and bone formation rate. The results indicate that all these compounds do give equivalent values.

Microradiography

Despite the usefulness of fluorochrome dyes the technique best suited to study bone to implant interface is microradiography. Microradiography allows for quantitative analysis of mineral content of bone. The term is reserved for the technique in which microscopic aspects of bone are imaged onto a photographic emulsion using soft or ultra soft x-rays. Soft x-rays are those with very low miliamps (eg. 3 mA) and very long exposure times (15 minutes to 1 hour). The technique also requires a very fine grain emulsion for the photographic plate. The energy of the x-ray produced must be very low because of the amount of material absorbing the x-rays per unit area is very small. Soft x-rays have a wavelength of greater than one Angstrom. By comparison, x-rays used for clinical diagnosis or therapeutic purposes have wavelengths of less than one Angstrom.

Wavelength of an x-ray depends on the type of metal the anode is made of. Soft x-rays are produced by a copper or nickel filtered copper anode. (Jee and Smith, 1984) The process was pioneered by Goby (1913), Dauvilliers (1930) and Lamarque (1936). It has been refined more recently by further work of Hall et al. (1972) and Sharpe (1979)

Anatomy and Postnatal Development of the Rat Maxillary Aveolar Complex

The cranium of the rat is illustrated in Figures 2 and 3 to demonstrate the vertical and lateral aspect of the skull. Anatomical description will be limited to the maxilla and palatal bones because these are the areas of histologic investigation in this study.

The maxilla is of membranous origin. It is an integral part of the nose, the lacrimal apparatus and the mouth. The maxilla encloses the rostral portion of the maxillary sinus. The orbital process of the maxilla, along with the lacrimal, forms the shell of the snout lateral to the sinus. Zygomatic arch growth corresponds to the growth of the masseter and temporalis muscles. The masseter originates at the zygomatic arch and the temporalis muscle lies beneath the arch.

The maxilla contains the maxillary molar dentition and serves as the origin of the majority of muscles which elevate the mandible. The formative end of the incisor and the roots of the molars are contained in the alveolar portion of the maxilla which is a linear mass lateral to the palatal process. The buccal wall of the buccinator muscle originates at the alveolar process of the maxilla. The superficial and deep masseter muscles originate

from the zygomatic arch and the adjacent maxillary bone

The greatest growth in mass of the maxilla is in its alveolar portion. This corresponds with growth, eruption and utilization of the dentition. Growth in width of the maxillary portion of the bony palate occurs primarily at the intermaxillary suture.

The intermaxillary suture of the rat is characterized by pleomorphic development. As early as nineteen days in utero, cartilage forms in the suture (Mohammed, 1957). This early cartilage is transient though and disappears until fifteen to twenty days postnatally. The reappearance of cartilage at the suture is coincidental with eruption of the maxillary first molar. The suture remains as a synchondrosis, presenting a bilateral epiphyseal plate until seven weeks age. At that time the suture returns to a syndesmosis. Remember that a syndesmosis has interosseous ligaments. Eventually the syndesmosis becomes a fibrous tissue suture. Several features of the cartilage formation at the intermaxillary suture are significant. First, the type of cartilage that forms is designated as secondary It develops after the appearance of bone. It is noteworthy that this suture is present only between the first molars. Distal to that the midline is characterized by an interpalatine suture.

The palatine bone is formed in membrane and is located at the junction of the nose, mouth and pharynx. Its horizontal or transverse portion is the caudal part of the bony palate and of the floor of the nasopharyngeal canal. The bony palate is displaced ventrally by deposition on its oral aspect and resorption of its dorsal surface. The median suture becomes denticulated. Note especially that the palatine bone of the rat, unlike the

human, projects anteriorly to the mesial aspect of the second molar. In the sagittal section this is revealed as three sutures: palatine, palatine at the midline and maxilla/palatine bilaterally at the junction of the alveolar. Figures 1 and 2 diagram the rat cranial anatomy.

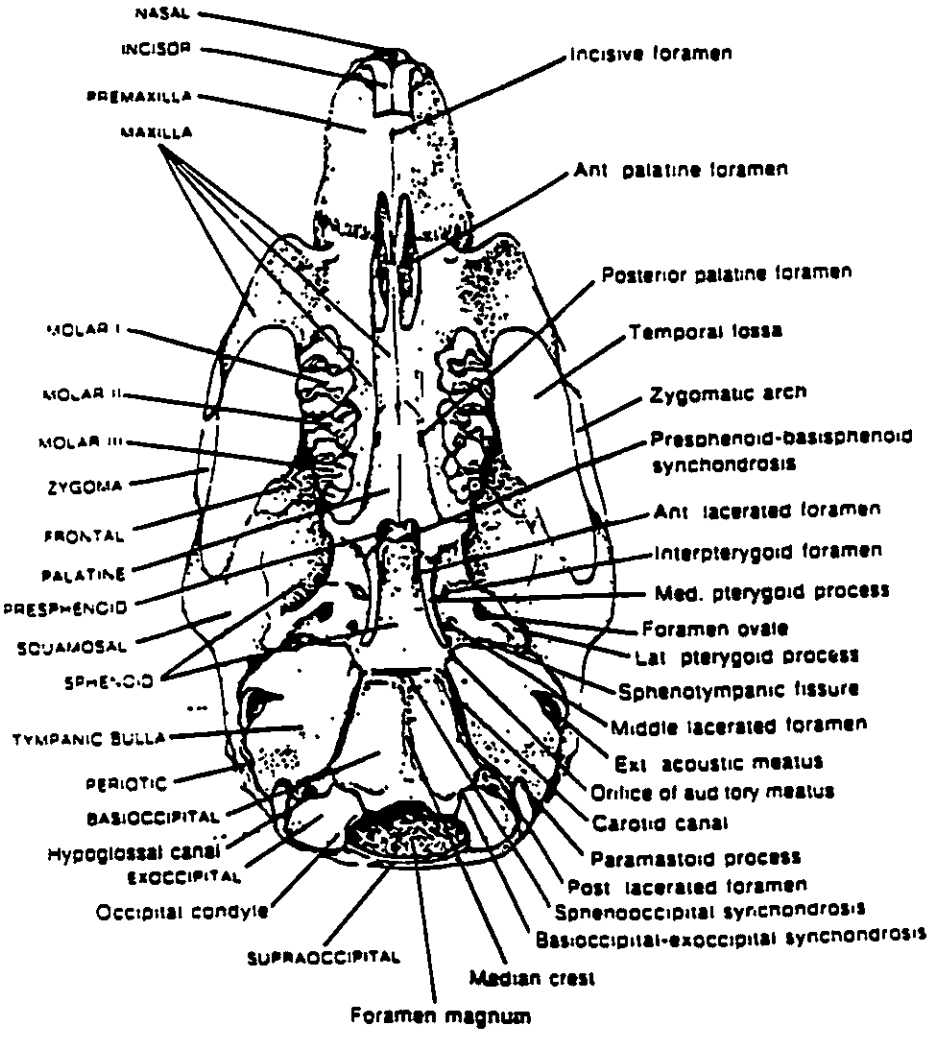


Figure 1. Thirty-three day old rat cranium-vertical aspect
(From "The postnatal development of the rat skull"
Baer et. al, 1984)

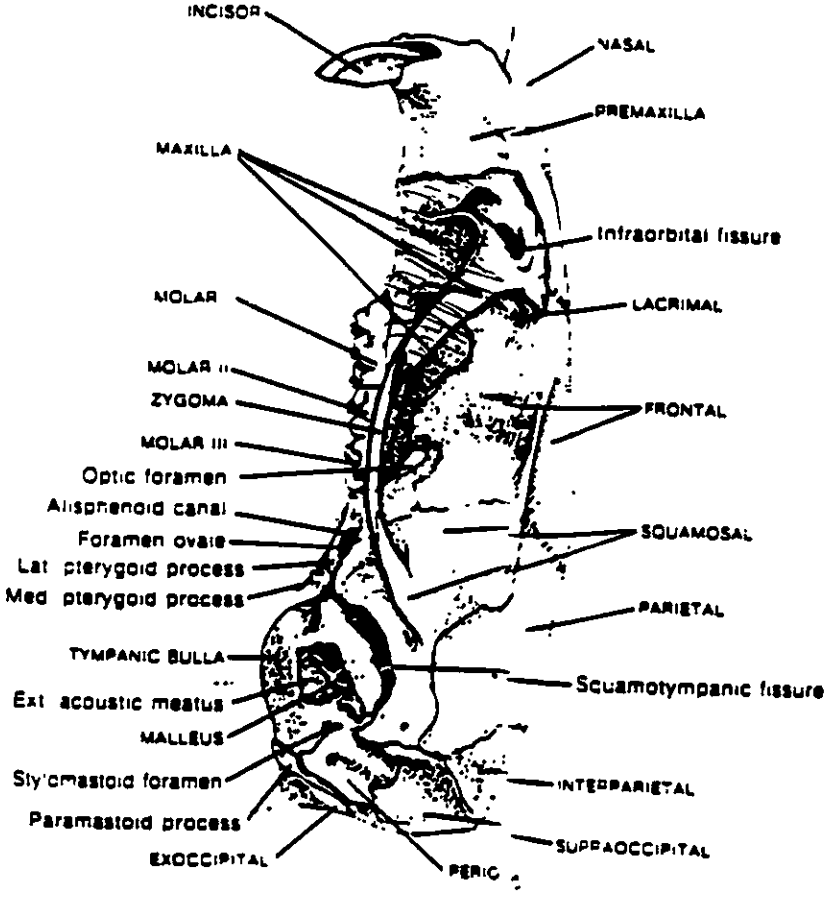


Figure 2 Thirty-three day old rat cranium-lateral aspect
(From "The Postnatal development of the rat skull"
Baer, et. al, 1984)

STATEMENT OF THESIS

A previous study (Tsolaikis, Cain, and Hans, 1992) applied passive transverse restraint to the maxillary molars of young growing rats, followed by a post-restraint observation phase. Results from dental impressions and radiographs indicate that experimentals had no transverse maxillary alveolar growth during restraint but had significant post-restraint rebound growth, nearly catching up to unrestrained controls by experiment's end.

Accelerated (rebound) growth in the transverse dimension could only have occurred via two possible mechanisms: at sutures in the mid palatal region, secondarily displacing the molars, or via remodeling in the dental alveolus effecting translation of molars. A combination of these two methods constitutes a hybrid possibility.

It was the intention of this study to histomorphometrically analyze fluorochrome dyes, injected during the rebound phase, to determine the mechanism of rebound growth in the rat maxillary alveolar complex

It is hypothesized that rebound growth occurs primarily in alveolar bone. Implications of results as they relate to dominant theories of facial growth will be discussed.

MATERIALS AND METHODS

Previous Study

Sample

In a previous study by Tsolaikis, Cain, and Hans (1992), thirty-nine female Sprauge-Dauley rats were divided into controls and experimentals. Each of four litters was split into the two groups to avoid litter bias. All animals were aged twenty days at the start of the experiment.

The rat was chosen as an experimental model because of its rapid growth and ease of handling. The twentieth day after birth was chosen as the start of the experiment because the prepubertal growth spurt takes place at this time. Prior to twenty days age, growth is less predictable, and due to a weaning age of twenty days, is influenced by maternal nutrition and litter size. Growth from twenty-one to fifty-five days is rapid and relatively constant. (Park and Nowsielski-Slepowron, 1976), animals were individually identified by the ear notching technique (Stark, 1989).

Experimental Design and Procedures

This experiment consisted of a maxillary growth restraint phase of from twenty days age to forty-two days age and a post-restraint, or rebound, phase of from

forty-two days to sixty-nine days. These times were chosen because of the growth characteristics of the rat maxillary alveolar complex. The greatest maxillary growth corresponds to molar eruption (Bear, Bosma, and Ackerman, 1984) and maxillary molar eruption occurs at day seventeen (first molar), day nineteen (second molar) and day thirty or later (third molar) (Mohammed, 1957)

To create growth restraint, twenty-one experimental animals had custom fabricated composite BIS-GMA appliances bonded to acid etched maxillary molars From day twenty to day forty-two, rats were checked daily for loose appliances, which were immediately replaced

At day forty-two, appliances were removed and the control and experimental groups further subdivided. Half of each group had maxillary molars reduced to the gingival margin with a dental diamond bur and formocreosol pulpotomies performed to fix any vital tissue. The rational for this tooth reduction was to reduced the surface area for lateral tongue forces, thereby upsetting the equilibrium between cheek and tongue forces and testing the functional matrix theory. If there was less transverse growth in the reduced molar groups, Moss's theory would be supported. All unrestrained animals were left to grow until sacrifice at day sixty-nine.

Anesthesia and Sacrifice

There are three critical times in the experiment; T-1 at twenty days, T-2 at forty-two days, and T-3 at sixty-nine days. At T-1 and T-2, all procedures and

records taken were performed under general anesthesia. The animals were anesthetized by intramuscular injection of a mixture of 0.9 cc of ketamine hydrochloride (Ketalar 100 mg/ml [Parke-Davis, Division of Warner Lambert Company, Morris Plains, New Jersey, 07950 U.S.A.]) and 0.1 cc of Xylarime (Rompun 20 mg/ml [Haver, Moby Corporation. Animal Health Division, Shawnee, Kansas, 66261, U.S.A.]). The dose was 0.12-0.15 cc/100 grams of body weight.

At T-3, animals were sacrificed, conforming with contemporary experimental criteria. The specific euthanasia agent was a saturated solution of sodium pentobarbital used at 3-6 ml/kg at minimum concentration of 65 mg/ml injected intraperitoneally. Pentobarbital is a common anesthetic agent whose mode of action is central nervous system depression.

Data Collection and Dye Injection

At T-1, prior to appliance placement, specific data was collected. Each animals weight was documented. Impressions of the maxillary were made using a thermoplastic dental temporary crown material. (Temporary Crown Matrix Button, Advance Dental Products, Inc., P. O. Box 214767, Aubury Hills, Michigan 48391). This material was chosen because of its accuracy and stability. Additionally, frontal (dorso-ventral) and lateral x-rays were taken, using a standardized holder and technique. Finally, xylenol orange (60 mg/kg) was injected intraperitoneally.

At T-2, the animals were weighed, x-rayed and had impressions made in the same manner The dye injected was calcein green (5 mg/kg).

At T-3, minus one day, the third dye, tetracycline (10mg/kg) was injected. One day prior to sacrifice was chosen to allow the dye to be taken up by depositing bone.

At T-3, animals were sacrificed as described and weighed, x-rayed, and had impressions made exactly as before. Table 1 is a diagramatic review of the experimental design.

Table 1.--A Diagramatic Review of Experimental Design

TIME	GROUPS	VARIABLE	DATE COLLECTED	DYE
T - 1	C E	appliance placed (E)	weight, x-rays impressions	xylenol orange
T - 2	C_1 C_2 E_1 E_2	appliance removed (E) molars reduced (C_1, E_1)	weight, x-rays impressions	calcein green
T - 3	C_1 C_2 E_1 E_2	sacrifice	weight, x-rays impressions	tetracycline (at T-3 minus 1)

Previous Results

Weight gain over the experimental period varied. Weight gain from T-1 to T-2 was greater for control animals, but from T-2 to T-3 was greater for experimentals so that by T-3, all groups were physically similar. (Table 2)

Table 2 --Summary Data of Weight Gain

TIME 1

GROUP	N	MEAN	S.D.
C_1	9	54.7	4.2
C_2	9	51.1	4.8
E_1	10	55.3	3.4
E_2	11	53.7	3.9

TIME 2

GROUP	N	MEAN	S.D.	WEIGHT GAIN
C_1	9	184.2	12.1	M = 129.5
C_2	9	165.1	11.5	M = 113.9
E_1	10	173.6	13.2	M = 118.3
E_2	11	153.8	13.1	M = 100.1

TIME 3

GROUP	N	MEAN	S.D.	WEIGHT GAIN
C_1	9	263.7	15.7	M = 79.4
C_2	9	245.6	11.4	M = 80.6
E_1	10	258.0	16.9	M = 85.3
E_2	11	246.1	20.1	M = 92.3

Successive x-rays for each animal were magnified times three and traced. Specific landmarks were measured. Maxillary impressions were measured transversely. Specific results can be found in the 1990 Thesis by Tsolaikis at CWRU Most significant was the change in intermolar width (Table 1). In the control group transverse was increased with time, on average, from 11.8 mm to 16.2 mm from T-1 to T-2. In the experimental group there was complete restriction with no detectable increase in transverse molar width. For the same experimental period. From T-2 to T-3, the control groups continued nearly the same rate of transverse increase as from T-1 to T-2. The control group with reduced molars (C_2) had slightly less transverse increase. T-2 to T-3 in both experimental groups increased at a significantly accelerated rate, nearly catching up, or rebounding, to the control group. Again E_2 (the group with molars reduced) had slightly less growth. This rebound growth is demonstrated in data from both x-rays and impressions. It has been determined that rebound growth, in the transverse direction of the rat maxillary alveolar complex, occurred in the experimental animals to a significant degree. (Figure 3).

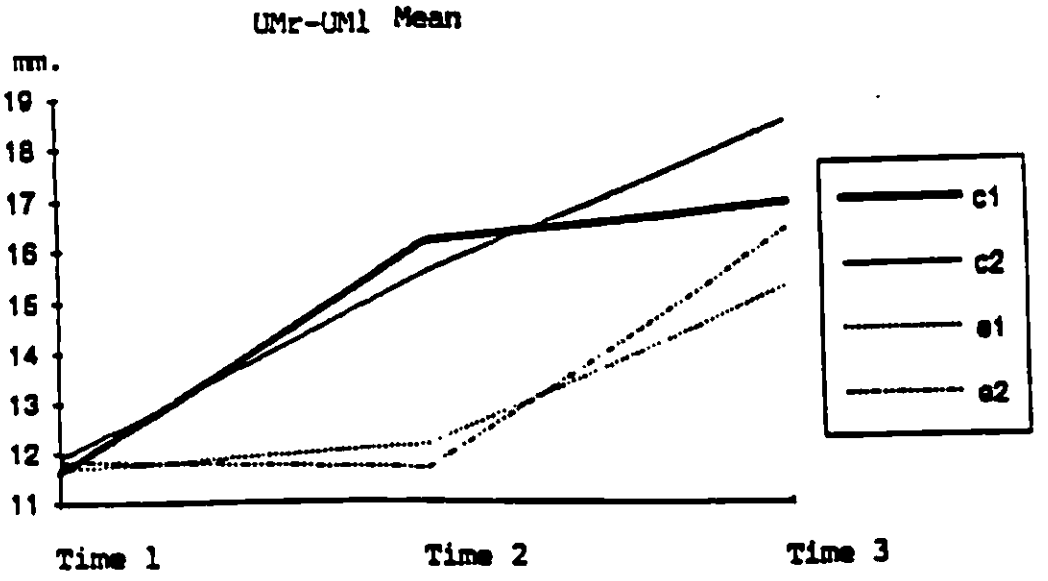


Figure 3.--Plot of transverse dimension of upper molar right (UMR) and upper molar left (UML) as a function of time for each rat group. (From Tsolaikis master thesis, CWRU, 1991)

Materials and Methods of the Current Study

Nondecalcified Tissue Preparation

To accomplish a histologic analysis of bone deposition, it was necessary to prepare and section the rat heads (this technique was generously provided in personal conversation by Lawrence Garretto, Ph.D. of Indiana University School of Dentistry, Department of Orthodontics). The remaining twenty-eight rat heads were manually defleshed, using a #15 Bard-Parker scalpel blade in a standard blade holder. Care was taken to not strip away periosteum and risk damaging the last dye deposited. For the same reason, palatal mucosa and periodontal soft tissues were not removed. Defleshed heads were returned to 70% alcohol, as formalin leaches out vital dyes.

To maintain individual identification after defleshing, individual mental tabs, fabricated from orthodontic band material, had rat numbers engraved on them, then wired to the skulls.

Prior to embedding in plastic, it is necessary to dehydrate and defat the skeletal remains. Dehydration is accomplished by a series of acetone washes as follows: seven, one-hour washes with the seventh wash standing overnight. In the morning, an eighth and final wash for one hour is done. Defatting follows dehydration. The routine is as follows: the last acetone wash is followed by seven, one-hour ethyl ether washes, the last one standing overnight. The next morning, an eighth and final ethyl wash is accomplished. Following the last ethyl ether wash, a batch of up to fifteen rat

heads was placed in a 1 0 liter plastic beaker with .5 liters of polymethylmethacrylate resin.

This beaker was placed under a bell jar with a vacuum provided by an electric pump The beaker required constant monitoring to prevent overactive bubbling and to keep skulls immersed in liquid plastic. After 4-8 hours under vacuum, heads were removed and placed in custom made, coffin-shaped aluminum foil molds. These molds, each with a rat head and filled to the top with vacuumed polymethylmethacrylate, were placed in a vented curing oven at 100° F for two days. They were then left for final polymerization for one month at room temperature.

The polymerized blocks were then carefully trimmed to the smallest possible size without cutting into the skulls. A dental cast trimmer was used to accomplish this. Because metal identification tags were ground away in this process, indelible marker was used to mark the rat numbers on each plastic block. These trimmed plastic blocks were scribed with orientation lines corresponding to midsagittal and maxillary occlusal planes. These orientation lines were critical in orienting the heads in the sample holding device for sectioning. The desire was to have the saw blade cut at 90 degrees to the sagittal plane and 90° F to the maxillary occlusal plane. This consistent orientation ensured the best possible sections for comparative study.

Heads were sectioned on a Buehler slow speed saw (11-1180 isomet low speed saw, Buehler Ltd. Lake Bluff, Illinois 60044, U.S.A.) using a 0.3 mm thick fine

grain diamond wafering blade at 250-300 rpms with a counter weighted pivot arm sample holder weighted to 50 gms Each section took approximately twenty minutes cutting time. Heads were sectioned from distal of the third molar to mesial of the first molar. Sixteen to twenty-two sections were cut for each head, numbered sequentially and identified for sample number and right and left All sections and heads were stored in the dark to preserve maximum epiflourescence.

After all twenty-eight heads were sectioned, each section was measured for thickness with a micrometer (Federal, Providence, Rhode Island) and best molar sections with consistent thickness chosen for study. Average section thickness was 150 microns. Sections taken from the same sample varied by less than 10 microns.

Experimental Design

After proper preparation of nondecalcified plastic embedded rat head sections had been accomplished, histologic analysis could begin. The central question of this study is, did rebound growth occur primarily at the suture or in the dental alveolar bone? To answer this question, it is imperative to clearly differentiate the two types of bone. The anatomy of the rat maxillo alveolar complex is such that alveolar bone, especially in the second molar region, is directly in contact with sutural bone. Because of this close proximity of two types of bone, for which growth is being tested, it was necessary to clearly differentiate between the two types of bone. The technique to best differentiate alveolar from sutural bone was microradiography. For this reason,

microradiographs of each section were taken. Each molar section of each rat was divided into three sections, molar left, molar right, and mid palatal (See Figure 4)

Sutural bone is present primarily in the mid palatal region Alveolar bone is present primarily in the left molar and right molar regions. Over each of these regions, an eye piece reticle, i.e., a 10 x 10 line grid, was superimposed.

In the case of the molar areas, the grid was superimposed so as to include only alveolar bone and absolutely exclude sutural bone. An orientation landmark of the buccal crest of bone was chosen to orient the grid. The x,y coordinate system was used so that the 10 x 10 line grid could be superimposed over a molar region and include only alveolar bone, excluding sutural bone. When the palatine-maxillary suture is present, as is often the case in the second molar region, it is often contiguous with alveolar bone It is then necessary to shift the 10 x 10 line grid buccally and occlusally and record the position of the x,y grid relative to the buccal crest of bone.

In the case of the mid palatal region, the central abscissa (x=5, y=5) of the 10 x 10 line grid was superimposed on the superior aspect of the mid palatal suture Dimensions of the grid were defined by surrounding bone and recorded.

By using the same grid, and same magnification of the same sample section as the microradiograph, the grid orientation was transferred from microradiograph to the fluorochrome section for analysis. This technique assured differentiation between sutural and alveolar bone. This allowed for confident collection of histomorphometric data from fluorochrome sections.

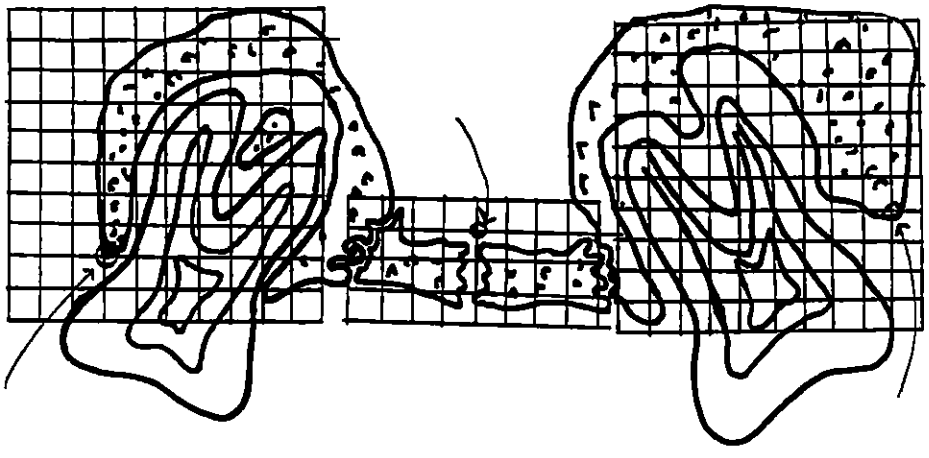


Figure 4. Diagramatic representation of grid superimposition over molar alveolar areas and mid-palatal sutural areas. Circles here correspond to orientation landmarks. For alveolar bone the orientation point was the buccal alveolar crest The grid was superimposed to eliminate any sutures from the alveolar sample. The mid-palatal sutural grid was always oriented with x=5, y=5 coordinate just superior to the mid-palatal suture.

Contact Microradiography

Selected representative section of the first, second, and third molars of each rat were microradiographed. The film used was 2 inch x 2 inch x .06 inch ultra flat, high resolution, glass plate film. (Imtec, Menlo Park, California) The x-ray unit used was a Hewlett-Packard cabinet x-ray system (Faxitron Series 43856A). Exposure with soft x-rays was at 3 mA at 40 KV for one hour The three molar sections were oriented on the film with a ten layered step-wedge. A step-wedge is a radiographic tool that is composed of an increasing number of layers of aluminum. Each thicker step projects a lighter shade of grey due to less radiation penetrating to the film. In this way a grey scale for each microradiograph is established. By using the same step-wedge for all microradiographs, there is a standard established. This is critical for detailed analysis of microradiographs because it eliminates variables such as fluctuations in mA or KV by the radiograph tube, and variations in developing solutions such as concentration, age, and temperature.

Identification of rat sample number was scratched onto the corner of the film where the step-wedge was placed. Section numbers were scratched in at the corner for each section. Each film, with three sections plus step wedge, was placed in the port hole like device emulsion side up. A circular piece of mylar was carefully placed over the plate, and a threaded brass ring or cap of the port hole screwed to place. An "O" ring creates a seal. A vacuum line connected under the glass film was activated.

This vacuum sucked the mylar flat, flattening the plastic sections. This assures no magnification of any section It is because of this complete and uniform contact of the sample with the film emulsion that the technique is called contact microradiography (Boivin and Baud, 1984)

Exposed films were developed at 72 degrees in D-40 solution for five minutes, rinsed, fixed for five minutes, neutralized for five minutes, rinsed for ten minutes in water, dipped in Photoflo (all Kodak, Rochester, New York) and allowed to stand to dry. They were then ready for use. Microradiographs were analyzed with a Reichert Microstar IV microscope with a Javelin chrome chip camera attached to a Reichert-Jung RGB monitor 80 to view images on the screen. There is a Yashica 35 mm camera attached to the microscope which is controlled and operated by a Reichert-Jung Photostar system from which photo microradiographs were made. This microscope, with the eye piece reticle, was used to establish grid coordinate to differentiate bone types.

Additionally, a descriptive analysis of bone closest to the mid-palatal suture and bone adjacent to the periodontal ligament space was made. These two locations were categorized as sutural and alveolar respectively. By using the step-wedge on each microradiograph, a grey scale of one to five was visually perceived. A number of one to five was assigned to the sutural and alveolar bone in the described location. This was done in an attempt to compare new bone density at sutural and alveolar sites.

Histomorphometry

The method of data collection is a point-hit technique as reviewed by Parfitt (1982). The technique uses a grid, already described in this work. All fluorochrome and microradiograph data was collected by the same blinded researcher Of interest here were the post restraint phases of growth, T-2 to T-3. Calcein (green) and Tetracycline (yellow) were injected at the start and near the end of this period, so the frequency of these dyes to total bone was of most interest.

To obtain this frequency, it is necessary first to establish the total amount of bone present Orienting the grid over the fluorochrome bone section, as described earlier, allows one to see that the x,y coordinate or point can be over three possible items; space, tooth, or bone. When a point lies over bone, it is a "hit" and is counted on a hand held numerical counter (ENM, England). Total number of bone hits is recorded. Without moving the grid or specimen, the number of green dye hits is recorded, then in the same manner, the number of yellow hits is recorded. In this way a frequency can be established; green + yellow hits/total bone hits. Xylenol (orange) was injected during the restraint phase of the previous experiment. Although the current study focused on the post-restraint phase with green and yellow dyes, orange hits were recorded for sake of completeness.

This technique of recording bone hits, orange hits, green hits, and yellow hits was repeated for the left molar, right molar, and mid palatal areas of each of three

molar sections for all twenty-eight samples. This was a total of eighty-four slides and 252 areas of study. There were twelve control animals and sixteen experimental animals analyzed

This data was gathered with a Zeiss Standard Model Microscope with a xenon light source and filters specific for the dyes used. Two filters were needed. One for xylenol orange which emits at a spectrum of 610 to 615 nm and a second filter specific for calcein green which emits at 520 to 540 nm. Tetracycline emits at 525 nm so that a third filter was not needed. (Rahn, 1978) A manually operated Leica R4 35 mm camera recorded the photomicrophraphs. Figures 5a, 6a, 7a, 8a, 9a are photomicrographs of fluorochrome sections. Figures 5b, 6b, 7b, 8b, 9b are photomicroradiographs of these same sections.

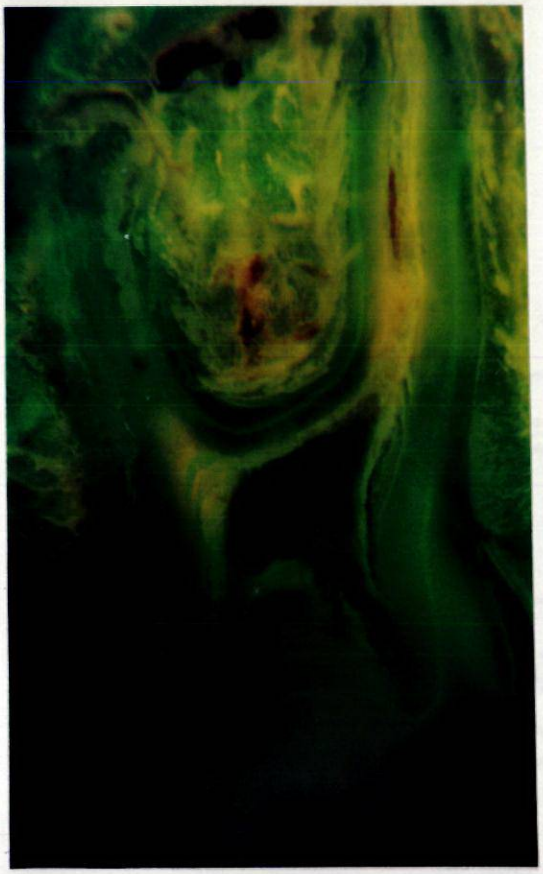


Figure 5a

Photo micrograph of fluorochrome section of upper left molar, 3rd molar section of rat #5. (experimental) Successful uptake and deposition of all three dyes is demonstrated in the dentin. A faint orange line is outer most, followed by green, and finally by a yellow line adjacent to the pulp. (43x)

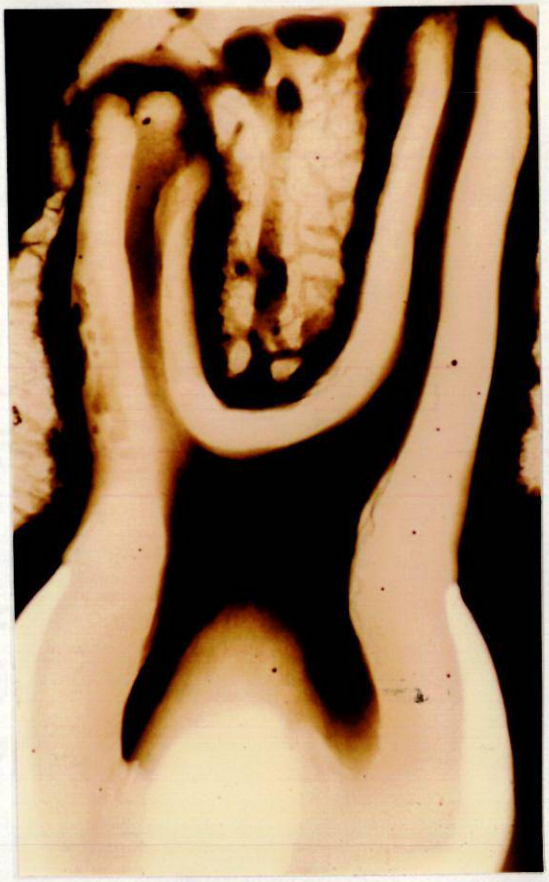


Figure 5b

Photo microradiograph at same magnification and of the same section as figure 7a. The irregular topography of alveolar bone is much more evident here. Microradiographs of each of the 258 areas analyzed were used to differentiate between bone and spaces (vessels and PDL space) (43x)



Figure 7 a

Photomicroradiograph of mid palate of control rat #13 at second molar. This is the junction of bimaxillary, bipalatine and maxillary-palatine sutures. (43x)

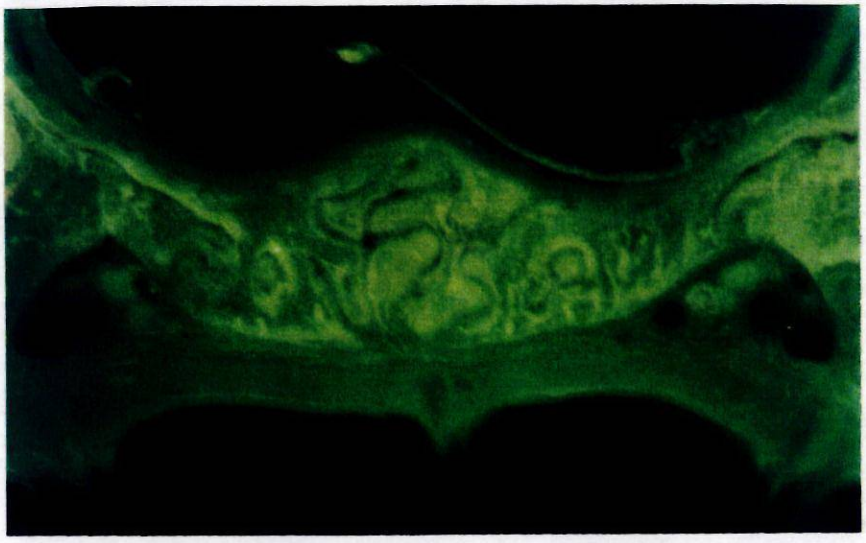


Figure 7 b

Flourochrome photomicrograph of mid palate of control rat #13 at second molar.(43x)

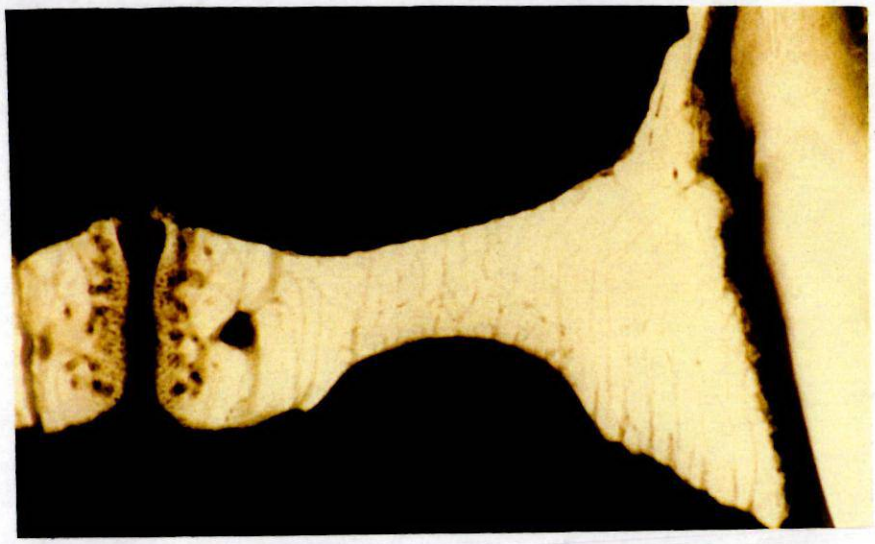


Figure 18a

Photomicroradiograph of right palate, experimental rat #19 at the first molar. Mid palatal suture is at left and molar root at right. (43x)

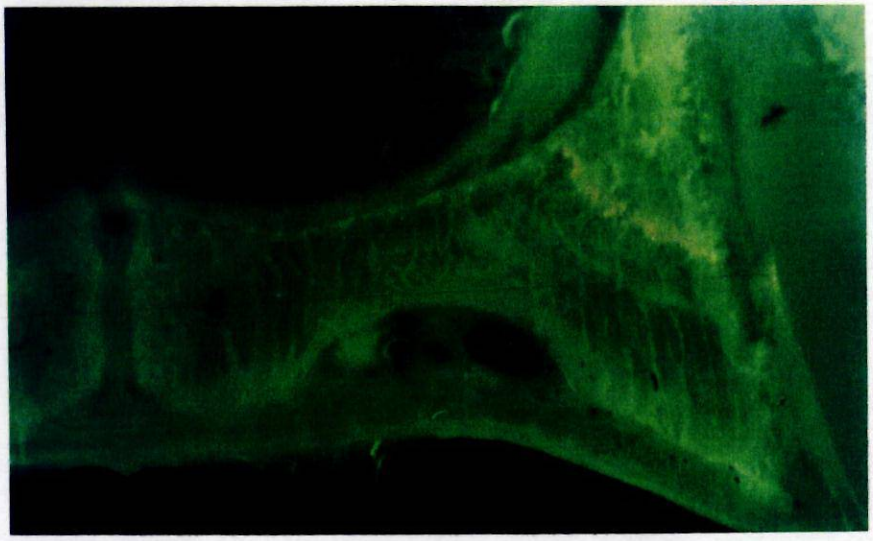


Figure 18b

Flourochrome photomicrograph of right palate, experimental rat #19 at the first molar. Note orange and green lines upper right of Plate 7. This demonstrates Enlow's (1969) concept of an expanding "V" contributing to increase transverse width. (43x)

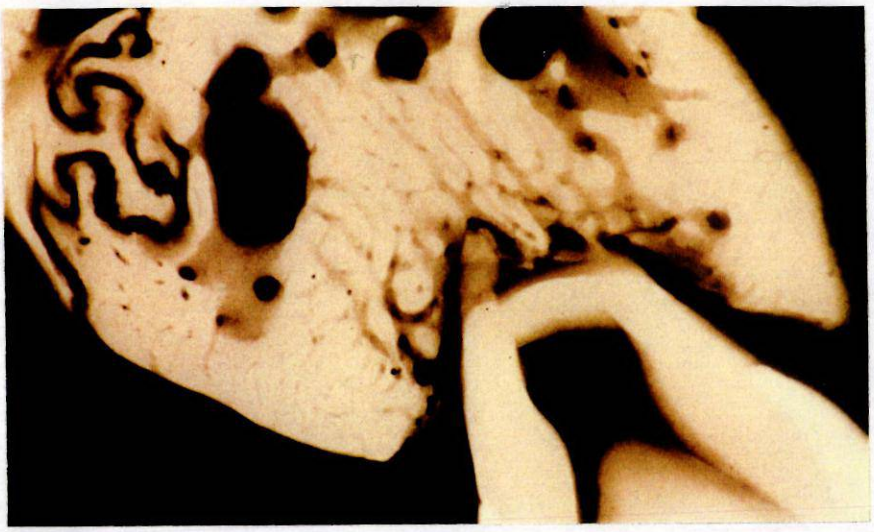


Figure 9 a

Photomicroradiograph of right buccal alveolus of experimental rat #29, at the third molar. (43x)

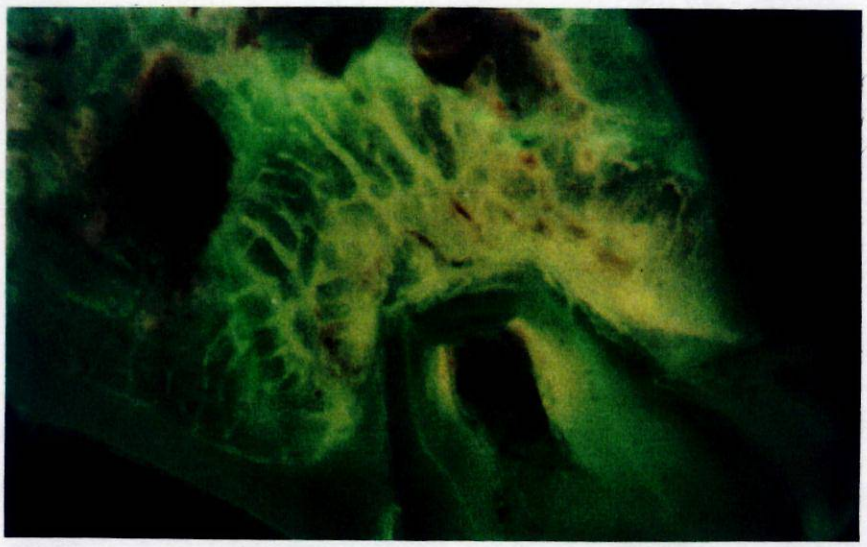


Figure 9 b

Flourochrome photomicrograph of right buccal alveolus of experimental rat #29 at the third molar. Note presence of orange, green, and yellow dye lines. (43x)

RESULTS

Results of histomorphometric analysis of the current study will be presented in five parts. The first results are the test of the hypothesis. Was rebound growth primarily in sutural bone or alveolar bone? Second will be a test of consistency Are dye analysis results consistent with radiographic and dental case analysis results from the same group of animals? Third will be a test for internal reliability. Is there a significantly different dye frequency in right and left molars? Significantly asymmetrical dye frequency results would not be consistent with gross morphology. (Tsolaikis, 1991) Third, comparisons will be made between animals that had maxillary molars reduced (C_1, and E_1) and those who did not (C_2, and E_2). A non-histomorphometric, descriptive analysis of microradiographs was made to evaluate density of new bone. Those results will be reported.

Before examining or interpreting results, it is important to understand how the numbers are generated. All histomorphometric data analyzed used only green and yellow dye frequencies. These are the dyes injected during the rebound phase. For this reason, any time the term "dye frequency" is used it refers to the frequency of green plus yellow dye hits divided by bone hits. Orange, injected at the start of passive restraint, was not calculated, except in the original raw data for each section and a mean for each sample. (Table 4 in appendix)

It is important to remember that all data here is reported as a frequency, not as an actual amount The reason for this lies in the nature of the way data was collected Recall that a grid was superimposed on a histologic section. The number of "points" of the grid that "hit" bone for each sample was recorded as a bone hit. Bone hits is the denominator of a fraction. Without moving the grid, dye hits are recorded. Dye hits becomes the numerator. Because green and yellow dyes were injected during post-restraint phase, it is the ratio of green + yellow hits divided by bone hits that provides the dye frequency for rebound growth.

Remember that each rat was sampled in three representative sections: first, second, and third molars. Also, each section was divided into three areas: left molar, right molar, and mid-palate. Only when comparing like sections, e.g., midpalate of a control to midpalate of an experimental, can these frequencies give use useful information. Confidence in accuracy can be gained by taking the mean of many samples. For example, the mean value for dye frequency of mid-palate of experimentals is calculated by averaging forty-eight frequencies; sixteen experimental samples times three sections for each rat. This, then, is the dye frequency for sutural bone of experimentals. It can be compared to mean dye frequency of sutural bone of controls. Alveolar bone was calculated by averaging the means of right and left molars.

Test of the Hypothesis

Was rebound growth alveolar or sutural? Results can be summarized as follows.

A_C = alveolar control, A_E = alveolar experimental

S_C = sutural control, S_E = sutural experimental

Mean frequencies are used. % differences = E - C / C

A_C = .4453 S_C = 2833

A_E = .5195 S_E = .2884

% difference = 16 7% % difference = 1.8%

There was a 16.7% greater frequency of dye hits in A_e than A_c, and only a 1 8% greater frequency of dye hits in S_e than S_c. The hypothesis then is supported; post-restraint rebound growth occurred primarily in alveolar bone.

Confidence and Reliability

Because the raw data here is generated as frequencies a t-test is not applicable. As explained earlier, whole numbers here have no real meaning, only the frequencies generated from them do. Consequently, other methods must be employed to test confidence and reliability.

Confidence here is tested by analyzing consistency of histologic data with radiographic and dental cast data. Both of these previous tests indicated post-restraint rebound, therefore, it would follow that there would be a higher frequency of dye in

experimental versus controls By combining alveolar and sutural bone frequencies, these new frequencies are generated·

C = .3912

E = .4424 (.4424 - .3912) / .3912 = 13.1%

Confidence is established because there is significantly greater total dye frequency in experimentals than controls at the same time they were growing faster than controls.

Test of Reliability

If dyes were taken up successfully, distributed evenly, and reported accurately, one would expect a nearly symmetrical distribution of dye. This can be tested for controls and experimentals by dividing the left molar over the right molar. If results are perfectly symetrical, results would equal 1.000. Doing this we get these results.

$ML_C / MR_C = 1.065$ $ML_E / MR_E = 1.063$

This represents about a 6% difference in the two sides in both groups, and demonstrates a highly reliable data collection.

Difference Between Subgroups

In the previous study (Tsolaikis, Cain, and Hans, 1990) the controls and experimentals were subdivided. Group one of controls and experimentals had maxillary molars entirely reduced. Recall that this was an effort to test the functional matrix theory. Radiographic results (Figure 3) show less transverse growth in both

controls and experimentals that had molars reduced (group 1). Dye frequency means of subgroups are summarized as follows.

$AC_1 = .4532$ $SC_1 = .2984$

$AC_2 = .4357$ $SC_2 = .2622$

$AE_1 = 5872$ $SE_1 = 2409$

$AE_2 = 4668$ $SE_2 = .3254$

In all cases except SE_1 compared to SE_2, dye frequency means are greater in the reduced molar subgroups than in non-reduced molar subgroups.

Descriptive Evaluation of New Bone at Sutural and Alveolar Site from Microradiographs

Bone density sites of new bone deposition were graded one to five with the aid of a step wedge. Results are in Table G. Results are summarized as follows:

$S_E = 2.71$ $S_C = 2.58$

$A_E = 3.08$ $A_C = 2.92$

New alveolar bone appears to have a slightly denser quality than new sutural bone, and experimentals slightly denser than controls.

DISCUSSION

Review of Experimental Design

The current study has histologically analyzed accelerated growth. This accelerated growth was the result of previous passive restraint. Post-restraint accelerated growth is generally referred to as rebound growth. Implicit in this term, "rebound growth" is the assumption that this is a return to normalcy, or for statisticians, a regression to the mean. Rebound growth in the Sprauge-Dawley rat model did in fact nearly catch up to unrestricted controls (Tsolaikis, et. al, 1992). This rebound occurred in all dimensions so that overall morphology returned to normal. That a restrained, growing animal should catch up when released, seems intuitively satisfying Perhaps it appeals to a sense of fairness. But what is the reason, the teleologic cause of this accelerated growth? The cause may be that overall growth potential is predetermined genetically by the nature of the animal. White lab rats do all look the same after all. Certainly epigenetic factors such as neighboring facial bone growth contributes. Perhaps the very large tongue of the rat has an effect by how the muscle mass functions. How can we test for these causes? That is the question answered by specific experimental design.

If we want to examine rebound growth, first we must restrict growth and see if there is rebound. So how do we restrict? The very least intrusive technique will introduce minimal variables and inspire confidence in results. On the other hand, it seems difficult to draw confident conclusions from traumatic surgical experiments such as nasal septum obliteration (Selman and Sarnat, 1957), which is akin to studying the workings of a fine watch with a toolbox of hammers. Buccal segment teeth were acid etched, rinsed, and bounded with composit down to the gingival margin, the main difference being that it is a single piece of acrylic with no expansion screw. Trauma to the rats is probably less than to our patients. Although the appliances were time consuming and difficult to place, they were stable and well tolerated by the rats. Only six came loose and they were immediately replaced. Although weight gain was less while the experimental group had the appliance on, no long term ill effect is apparent, as final weight of all animals was within normal variation. Another factor in design is the animal model chosen. In this experiment, we chose the Sprauge-Dawley rat because of its ease of handling, rapid growth, and well documented anatomy. All animals received identical food, water, anesthesia, dyes and treatment, with the exception of the appliance. As a result of this single variable, the design of the experiment is a simple block for the restriction phase. Rebound growth has been verified with dental impressions and radiographs (Tsolaikis, et. al, 1992). The remaining question is, where did the growth occur? This was to be answered by

fluorochrome dye analysis. During all data collection, the single researcher was blinded. Because of half of all experimentals and controls had molars reduced, it was impossible to distinguish between groups. The researcher remained blinded for the data gathered from microradiographs as well. Inevitable bias has been avoided by having a single, blinded data collector.

Theoretical Implications of Results

Returning to our research question, "where did rebound growth occur?" Why is this important? On a theoretical level it is important because sutural growth and alveolar growth have specific implications. The genetic based theorists (Helmann, 1933; Brodie, 1938; Weinman and Sicher, 1947) proposed sutures to be the modus through which genetic control of growth operates. The functionally based theorists (Moss, 1969) attribute control of growth to soft tissues and airway demands. Where as a consequence of these demands, bone growth would occur, is of secondary importance to the theory, but significant contribution from the alveolar bone would be expected. The alveolus is highly plastic in nature and would respond most readily to a changing environment. If we then, examine for dye frequency during rebound in a sutural and alveolar bone, and compare frequencies in those areas between experimentals and controls, we can evaluate relative rates of bone deposition.

This would be set up as:

S_C vs. S_E and A_C vs. A_E

where S_C = sutural control, S_E = sutural experimental,

A_C = alveolar control and A_E = alveolar experimental

Doing this we get the following values:

$S_C = .2833$, $S_E = .2884$

$A_C = .4453$, $A_E = .5195$

Sutural values are nearly the same (1.8% difference) but alveolar values are quite different, A_E showing 16.7% higher frequency of dye than A_C. Rebound growth occurred primarily in the alveolar bone. This supports a functional based teleology of facial growth and casts significant doubt on a suturally based genetically driven force to account for rebound growth. These results are not inconsistent with previous research using excised sutures grown in culture exhibited (Lewis, 1969; Lewis and Irving, 1970; Servos, 1975). These sutures exhibited independent growth without environmental influence. Our results show sutural bone deposition frequency to be nearly the same in controls and experimental groups. Sutures may exhibit an inherent growth capacity but seem to be neither a controlling factor, nor an adaptive mechanism.

The concept of an adaptive mechanism is critical in the cybernetic model of facial growth. (Petrovik and Stutzmann, 1969) Cybernetic models diagram how very

complex systems operate within specific functional parameters. These diagrams include aspects such as feed back loops, servo mechanisms and circular systems. Cybernetics by Norbert Wiener was published in 1948. It was an offshoot of early work with computers. Wiener introduced the concept in the technical field, but he developed this further and applied it to biology, sociology, and psychology. It is the standard method of conceptualizing biologic systems in Western thought. An important distinction should be made though, between a "system theory" and a "growth theory". Growth theories are inherently teleological. That is, they assign cause and effect relationships and try to explain "why?". "These changes occurred in the alveolus because this caused it" is a teleologic statement. System theories, though make no attempt to explain why a cause and effect are linked. It is recognized that any effect is the result of multiple causes. Each of these causes is controlled by one or several inputs. To account for this complex level of input, it is necessary to devise systems. In his highly regarded General Systems Theory, Ludwig von Bertalanffy (1968) describes how the need for systems theory developed. "Its necessity resulted from the fact that the mechanistic scheme of insoluble causal trains and meristic treatment had proved insufficient to deal with theoretical problems, especially in the biosocial sciences, and with the practical problems posed by modern technology." Conceptually, the cybernetic model cannot be equated with a growth theory. Although teleologic questions are interesting, it is more important to understand the

system. A system will have some input that is constant and some input that responds to feedback

In the case of the facial growth system, and specifically in terms of this experiment, sutural growth and alveolar growth have different types of input Sutural growth was virtually the same in controls and experimentals during the post-restraint phase, despite significantly different environments. From these results one may infer that sutural growth was dependable, not easily modified, and (for the group) predictable. In the cybernetic model then, input from the suture would be of a constant value, and have little response to feedback. A cybernetic model though, relies on forms of input that are dynamic and respond. It is this response, the feedback, that is the essence of cybernetics. If an entire system is going to adapt to different environmental input and be moderated to the norm, then some aspect of physiologic input must respond appropriately. If sutural growth is a mechanism which is unvaried despite system changes, then alveolar bone must be the adaptable and dynamic factor that accommodates to change and maintains normal morphology.

Returning briefly to the teleologic concepts of control mechanisms, let us review the factors at work here. It would seem logical that sutural growth is under direct genetic control for amount and duration of bone deposition. Alveolar bone, however, seems to have multiple control factors. There may be some direct genetic control. Certainly there is epigenetic control in the form of growth hormones and

influence from approximating facial bones. Breathing and deglutition effect tongue pressure and posture, thereby having a primary influence on soft tissues effects Remembering the anatomy of the rat head, the masseter and temperalis muscles are very large and are intimately associated with the zygomatic arch. These muscles are in dynamic equilibrium with tongue muscles. Despite this, the zygomatic arch width was only slightly constricted in the experimental group and growth rate remained the same during the post-restraint phase. The zygomatic arch laterally contains the temporalis muscle and it suspends the masseter. The zygomatic arch dimension is a very significant factor in establishing gross morphology and it is a key factor in determining the forces on the teeth and alveolus from the cheeks. If lateral tongue forces are causal factors of alveolar remodeling, the medial forces of the cheek had be to be overcome, and therefore less than tongue forces. If constricted molars are contained in an envelope of muscle that is of nearly normal dimensions, then there is room for growth. Had bi-zygomatic width been constricted proportionally to molar width, the medial soft tissue forces would have been greater and possibly allowed for less molar rebound Returning again to the cybernetic model, accelerated alveolar remodeling, not accelerated sutural growth accounted for rebound of molar width, but sufficient bi-zygomatic width allowed for cheek forces to be overcome by tongue forces, which in turn allowed for the alveolar remodeling. Despite that the discussion here is limited to the transverse dimension only, the complexity of the multifactorial causal

relationships can begin to be seen. Although it is educationally and intellectually convenient to simplify cause and effect relationships, it is apparent that a single effect requires multiple causes. If though, instead of looking for a single cause, we accept the cybernetic model, it becomes most interesting to look for the most adaptable and plastic mechanism that allows for maintenance of normalcy. According to these results, that important adaptive mechanism in this experiment is clearly the dental alveolus. A cybernetic model incorporating experimental results is seen in Figure 10.

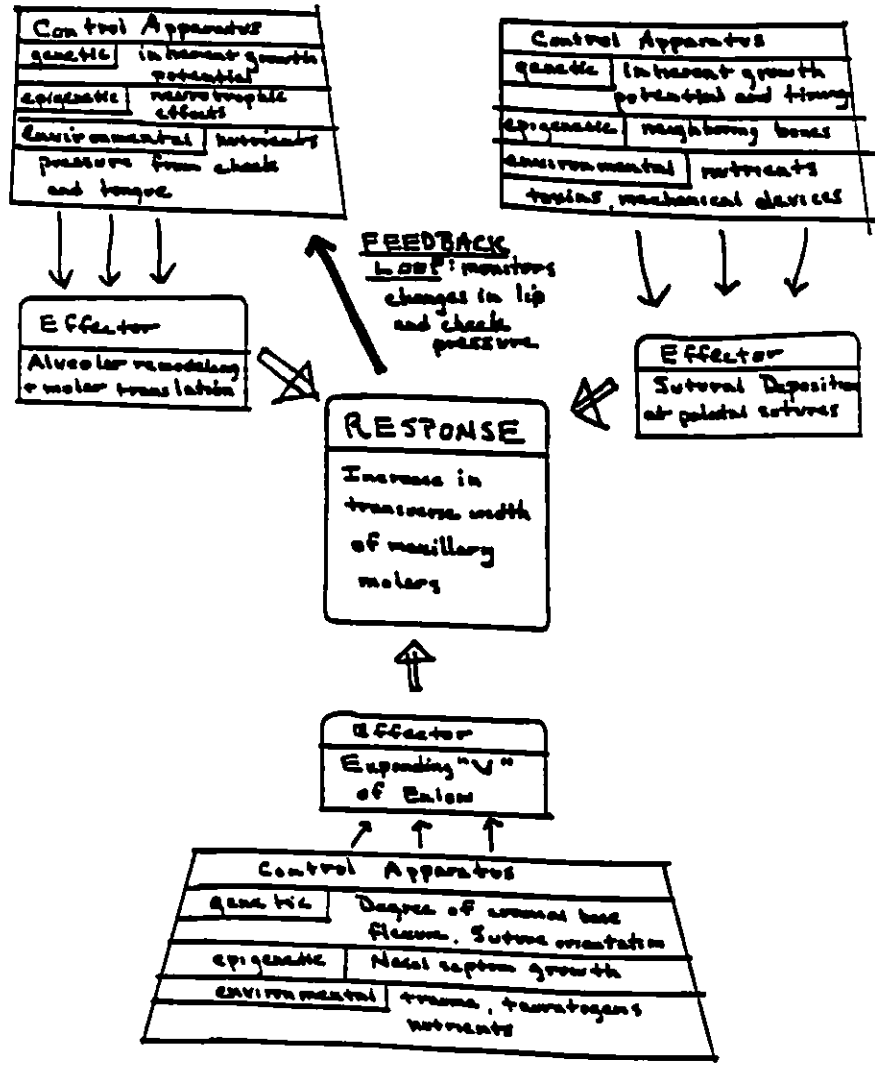


Figure 10. Model for influences on molar width in maxilla

Other Fluorochrome Results

Reliability

Discussion thus far has been focused on the thesis question and answer. But can we be confident enough with these results to draw these conclusions? This was tested by dividing left molar frequencies by right molar frequencies. If results were perfectly bilaterally symmetrical, the result of ML/MR would be 1.000. Results of ML_C/MR_C is 1.065 and of ML_E/MR_E is 1.063. Even in an experimental rat model some differences can be expected, so the small difference is quite acceptable. It indicates that dyes were deposited and reported reliably.

Differences Between Subgroups

Recall that molars were reduced to the gingival margin in half the controls and half the experimentals at T-2. Radiographic results indicate slightly less transverse growth in both reduced groups when compared to the unreduced groups. Dye frequency analysis is not consistent with this. SE_1 had less dye frequency than SE_2, but in all other subgroup comparisons, the reduced group actually had greater dye frequency. The magnitude of difference in frequencies varied considerably as well. These variable and inconsistent results may again be due to subdividing the groups too far and introducing another variable.

Microradiograph Analysis

The primary purpose of making microradiographs was to provide for more accurate differentiation between alveolar and sutural bone. This provides for greater confidence in results. But these microradiographs can be used to describe density of newly deposited bone in terms of a grey scale Dense bone is equated with more mature and higher quality bone. A grey scale of 1 to 5 was used with the aid of a 10-step wedge. At the suture, the bone closest to the midline was examined. In the alveolus, the bone adjacent to the periodontal ligament space to the lingual of roots was examined. Results show slightly less dense bone in the alveolus. This is consistent with greater frequency of dye, because more rapid deposition implies a higher percentage of woven bone (Oyen and Russel, 1989) which would be less radiopaque.

Clinical Application

Can any use be made of this theoretical information by the clinician?

It is important to remember our patients represent an extremely diverse population, so greater variation can be expected clinically than in any animal study This is not to say though, that no clinical application can be made from this study. It has been shown that alveolar bone is highly plastic and mutable and sutures are significantly less so. Clinically we depend on the plasticity of the alveolus to move teeth. We change not only tooth position but also arch form, arch width, and interarch

relationships by manipulating the alveolus. In light of these experimental results, we must treatment plan alveolar changes to not violate soft tissue constraints. Transverse arch width cannot be increased so much as to violate bizygomatic constraints. A square arch form cannot be changed to an oval arch form and maintained in the presence of tense circum-oral musculature. The alveolus will adapt to the oral environment over time. It seems wise then, that in the absence of surgical or significant orthopedic change of environment, that we be conservative in the amount of change we attempt to accomplish by alveolar means alone

SUMMARY AND CONCLUSION

This study made a histologic analysis of rebound growth in the transverse dimension of the rat maxillary alveolar complex. A previous study by Tsolaikis, Cain, and Hans (1990) examined the growth of the maxillary complex during and after passive transverse restraint on the maxillary molar dental units of female Sprauge-Dawley rats. The experimental group was subjected to twenty-one days of passive restraint applied cross arch to the maxillary molar dental units. After release from restraint, maxillary growth was monitored for twenty-seven more days. Data analysis was made from serial dental impressions and serial cephalometric radiographs. Results indicated complete restriction of maxillary molars during restraint and nearly complete rebound to control values during the post-restraint phase.

The current study has made a histomorphometric analysis of fluorochrome dyes injected during the post-restraint rebound phase. The relative frequency of new bone deposition in controls (n=12) and experimentals (n=16) was specifically analyzed to evaluate differences between sutural bone at the mid palate and alveolar bone surrounding the molars. Best representative sections of first, second, and third molars were selected for each rat. Each section had two areas of analysis: combined left and

right molars and the mid palate Microradiographs of each section were used to differentiate sutural from alveolar bone. An eye piece grid was used to orient and transfer that information to the fluorochrome sections. Standard histomorphometric technique was used to establish relative dye frequency between alveolar and sutural bone of experimentals and controls.

Results indicate that experimentals had a 16.7% higher frequency of new bone deposition than controls. We can conclude the following about post-restraint rebound growth: 1) Histomorphometric results are consistent with dental impression and radiographic results from the previous experiment (Tsolaikis, 1991), 2) Bone deposition was essentially bilaterally symmetrical, and 3) The pattern of new bone deposition is different between controls and experimentals. Both groups had nearly the same frequency of new bone suturally, but significantly greater frequency of new bone deposition was seen in alveolar bone. These histologic results imply that transverse post restraint rebound growth occurs nearly exclusively in alveolar bone. This supports a functional rather than genetic paradigm of growth control. Although greater variation can be expected in the diverse genetic pool of human patients, alveolar rebound growth should be accounted for in treatment planning. Alveolar remodeling can be conceived of as the adaptive mechanism that responds to feedback in the dynamic multi-factorial facial growth system

APPENDIX

TABLE		PAGE
1	Microradiograph grey scale values	75
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3	Calculated means for mid-palate, molar right and molar left	79
4	Raw Data	80

TABLE 1.--Microradiograph Grey Scale Evaluation

Rat #	Molar #	C or E	Suture Value	Alveolar Value
2	1		3	2
2	2	E	3	3
2	3		3	2
4	1		2	2
4	2	C	2	3
4	3		3	3
5	1		3	4
5	2	C	2	3
5	3		2	3
7	1		3	2
7	2	E	2	2
7	3		2	2
8	1		3	2
8	2	E	3	3
8	3		2	3
9	1		3	2
9	2	E	3	3
9	3		2	2
13	1		3	2
13	2	C	2	2
13	3		2	3
15	1		2	3
15	2	C	2	3
15	3		2	2
16	1		2	3
16	2	E	2	2
16	3		2	2
19	1		2	3
19	2	E	2	3
19	3		2	3
21	1		3	3
21	2	C	4	4
21	3		2	3
23	1		3	4
23	2	E	3	4
23	3		2	3

TABLE 1. (continued)

Rat #	Molar #	C or E	Suture Value	Alveolar Value
24	1		3	4
24	2	E	3	3
24	3		4	3
25	1		2	4
25	2	C	2	2
25	3		3	3
27	1		2	2
27	2	E	2	3
27	3		2	3
28	1		3	3
28	2	E	2	3
28	3		3	3
29	1		3	4
29	2	E	3	3
29	3		2	3
30	1		3	3
30	2	E	3	2
30	3		3	2
32	1		2	3
32	2	C	4	4
32	3		2	3
34	1		3	3
34	2	C	2	2
34	3		3	4
39	1		3	4
39	2	C	3	3
39	3		3	3
41	1		3	2
41	2	C	3	3
41	3		3	3
42	1		3	3
42	2	E	3	3
42	3		2	3
43	1		3	3
43	2	E	3	3
43	3		3	4

TABLE 1. (continued)

Rat #	Molar #	C or E	Suture Value	Alveolar Value
44	1	C	3	3
44	2		2	3
44	2		2	3
45	1	E	3	4
45	2		3	3
45	3		3	4
46	1	E	3	3
46	2		4	3
46	3		4	3
47	1	C	3	2
47	2		3	3
47	3		3	2

TABLE 2.–Data for green + yellow/bone hits

Area	C or E	Mean
MP	E	0 3003

Area	C or E	Mean
MP	C	0 2844

C or E	Mean
E	0.4612

C or E	Mean
C	0.4051

(G+Y)/B
(MR+ML)/2
0 45213589
0.43567708
0 44527805
(MR+ML)/2
0.5872737
0 4663286
0 51952333

			(G+Y)/B
Area	C or E	Group	Mean
MR	C	1	0.2984
Area	C or E	Group	Mean
MR	C	2	0.2622
Area	C or E	Group	Mean
MR	C	both	0.2833
Area	C or E	Group	Mean
MR	E	1	0.2409
Area	C or E	Group	Mean
MR	E	2	0.3254
Area	C or E	Group	Mean
MR	E	both	0 2884

Area	C or E	Mean
ML	E	0.5581

Area	C or E	Mean
ML	C	0.4602

Area	C or E	Mean
MR	E	0.5250

Area	C or E	Mean
MR	C	0.4507

			(G+Y)/B
Area	C or E	Group	Mean
ML	C	1	0 4804
Area	C or E	Group	Mean
ML	C	2	0.4329
Area	C or E	Group	Mean
ML	C	both	0 4606
Area	C or E	Group	Mean
ML	E	1	0.5950
Area	C or E	Group	Mean
ML	E	2	0 4916
Area	C or E	Group	Mean
ML	E	both	0 5368

			(G+Y)/B
Area	C or E	Group	Mean
MR	C	1	0 4238
Area	C or E	Group	Mean
MR	C	2	0.4384
Area	C or E	Group	Mean
MR	C	both	0 4299
Area	C or E	Group	Mean
MR	E	1	0.5795
Area	C or E	Group	Mean
MR	E	2	0 4421
Area	C or E	Group	Mean
MR	E	both	0 5022

TABLE 3 --Calculated means for mid-palate, molar right and molar left

			(G+Y)/B		
Area	C or E	Molar #	Mean	Std. Dev	Variance
MP	C	1	0.3315	0.1832	0.0338
MP	C	2	0.2819	0.1383	0.0186
MP	C	3	0.2388	0.1665	0.0284
MP	E	1	0.2838	0.1088	0.0118
MP	E	2	0.2490	0.1969	0.0387
MP	E	3	0.3335	0.2129	0.0453

1,2,3

3,1,2

			(G+Y)/B		
Area	C or E	Molar #	Mean	Std. Dev	Variance
MR	C	1	0.4298	0.1286	0.0165
ML	C	1			
MR	C	2	0.4874	0.1100	0.0121
ML	C	2			
MR	C	3	0.4086	0.1224	0.0150
ML	C	3			
MR	E	1	0.5428	0.2823	0.0797
ML	E	1			
MR	E	2	0.4639	0.2034	0.0414
ML	E	2			
MR	E	3	0.5521	0.2041	0.0417
ML	E	3			

TABLE 4.--Raw Data

Rat#	Sectiond	C or E	Group	Area	Base	O	G	Y	O/B	G/B	Y/B	(G+Y)/B	O/B Ave	G/B Ave	Y/B Ave	Total Dye Wt %
2	8	E	1	MP	24	2	7	0	0.0833	0.0833	0.0000	0.0833				0.1667
2	8	E	1	MP	31	3	7	1	0.0968	0.2258	0.0323	0.2581				0.3548
2	8	E	1	MU	41	8	8	1	0.1951	0.1951	0.0244	0.2195				0.4146
2	13	E	1	MP	36	6	4	0	0.1667	0.1111	0.0000	0.1111				0.2778
2	13	E	1	MP	49	1	7	6	0.0204	0.1429	0.1020	0.2449				0.2653
2	13	E	1	MU	58	2	7	5	0.0345	0.1207	0.0862	0.2069				0.2500
2	18	E	1	MP	34	0	1	2	0.0000	0.0294	0.0588	0.0882	0.1944	0.1863	0.0686	0.0882
2	18	E	1	MP	78	1	12	8	0.0128	0.1538	0.1154	0.2692	0.0064	0.3760	0.2312	0.2396
4	4	C	1	MP	20	0	1	1	0.0000	0.0500	0.0500	0.1000	0.1112	0.3286	0.3192	0.3646
4	4	C	1	MP	36	1	2	10	0.0278	0.0556	0.2778	0.3333				0.1000
4	4	C	1	MU	31	0	3	14	0.0000	0.0968	0.4516	0.5484				0.3611
4	8	C	1	MP	25	0	1	3	0.0000	0.0400	0.1200	0.1600				0.5484
4	8	C	1	MP	42	0	3	13	0.0000	0.0714	0.3095	0.3810				0.1600
4	8	C	1	MU	68	1	2	41	0.0147	0.0294	0.6029	0.6324				0.3810
4	13	C	1	MP	20	0	0	8	0.0000	0.0000	0.4000	0.4000	0.0009	0.0667	0.5387	0.6471
4	13	C	1	MP	31	0	1	10	0.0000	0.0323	0.3226	0.3548	0.0083	0.1722	0.7247	0.4000
6	13	C	1	MU	32	0	1	13	0.0000	0.0313	0.4063	0.4375	0.0147	0.0818	1.1987	0.3548
5	3	C	2	MP	18	0	0	2	0.0000	0.0000	0.1333	0.1333				0.4375
5	3	C	2	MP	36	0	7	12	0.0000	0.1944	0.3333	0.5278				0.1333
5	3	C	2	MU	31	0	8	4	0.0000	0.1613	0.1290	0.2903				0.5278
5	7	C	2	MP	19	0	2	4	0.0000	0.1053	0.2105	0.3158				0.2903
5	7	C	2	MP	61	1	13	18	0.0164	0.2131	0.2623	0.4754				0.3158
5	7	C	2	MU	58	1	17	8	0.0172	0.3621	0.1466	0.4845				0.4918
5	13	C	2	MP	22	0	3	8	0.0000	0.1364	0.2273	0.3636	0.0000	0.2419	0.4822	0.4727
5	13	C	2	MP	36	5	8	18	0.0847	0.1356	0.2542	0.3898	0.1011	0.4132	0.6278	0.3636
5	13	C	2	MU	59	8	18	18	0.0726	0.2174	0.2319	0.4493	0.0809	0.9982	0.4203	0.4746
7	8	E	1	MP	18	0	2	8	0.0000	0.1333	0.4333	0.5667				0.5217
7	8	E	1	MP	30	1	3	17	0.0333	0.1000	0.5667	0.6667				0.5667
7	8	E	1	MU	44	8	2	18	0.0000	0.0455	0.4091	0.4545				0.7000
7	12	E	1	MP	36	1	2	1	0.0278	0.0556	0.0278	0.0833				0.4545
7	12	E	1	MP	45	1	5	10	0.0222	0.1000	0.2041	0.3061				0.1111
7	17	E	1	MU	37	0	2	12	0.0000	0.0541	0.3243	0.3784				0.3265
7	18	E	1	MP	18	0	2	2	0.0000	0.1111	0.1111	0.2222	0.0278	0.2111	0.3187	0.3784
7	18	E	1	MP	36	4	3	25	0.0556	0.0556	0.4028	0.4444	0.0788	0.1783	0.7899	0.2222
7	18	E	1	MU	33	1	2	12	0.0288	0.0871	0.3478	0.4060	0.0286	0.1263	0.8035	0.4884
8	4	E	2	MP	9	0	0	1	0.0000	0.0000	0.1111	0.1111				0.4286
8	4	E	2	MP	33	0	3	9	0.0000	0.0909	0.1818	0.2727				0.1111
8	4	E	2	MU	28	0	4	11	0.0000	0.1379	0.3793	0.5172				0.2424
8	8	E	2	MP	26	0	1	2	0.0000	0.0385	0.0769	0.1154				0.5172
8	8	E	2	MP	36	0	8	12	0.0000	0.1667	0.3333	0.5000				0.1154
8	8	E	2	MU	34	2	8	10	0.0588	0.1471	0.2941	0.4412				0.5000
8	12	E	2	MP	24	0	1	2	0.0000	0.0417	0.0833	0.1250	0.0880	0.0891	0.1973	0.9000
8	12	E	2	MP	50	0	8	16	0.0000	0.1200	0.3800	0.4800	0.0000	0.3170	0.7436	0.1250
8	12	E	2	MU	45	0	8	16	0.0000	0.1111	0.3556	0.4667	0.0586	0.3041	0.7761	0.4800
9	10	E	2	MP	12	0	1	1	0.0000	0.0833	0.0833	0.1667				0.4667
9	10	E	2	MP	27	0	5	14	0.0000	0.1852	0.5185	0.7037				0.1667
																0.7037

TABLE 4 --Raw Data (continued)

Ref	Subject	C or E	Group	Area	Score	O	G	Y	O/B	G/B	Y/B	(G+Y)/B	O/B Ave	G/B Ave	Y/B Ave	Total Dye Mt %
6	10	E	2	MU	75	0	1	7	0.0000	0.0400	0.2800	0.3200				0.3200
6	9	E	2	MP	19	0	0	4	0.0000	0.0000	0.2105	0.2105				0.2105
6	9	E	2	MR	44	4	3	8	0.0909	0.0682	0.1364	0.2045				0.2956
6	9	E	2	MU	31	0	3	8	0.0000	0.0968	0.2581	0.3548				0.3548
6	2	E	2	MP	23	0	4	3	0.0000	0.1739	0.1304	0.3043	0.0000	0.2017	0.3867	0.3043
6	2	E	2	MR	42	2	5	7	0.0476	0.1190	0.1667	0.2857	0.1349	0.2490	0.4798	0.3333
6	2	E	2	MU	39	1	6	15	0.0256	0.1282	0.3846	0.5128	0.0256	0.2393	0.7380	0.6345
13	9	C	1	MP	19	0	0	1	0.0000	0.0000	0.1000	0.1000				0.1000
13	9	C	1	MR	39	0	2	17	0.0000	0.0513	0.4359	0.4872				0.4872
13	9	C	1	MU	28	1	4	7	0.0345	0.1379	0.2414	0.3793				0.4138
13	8	C	1	MP	21	0	0	6	0.0000	0.0000	0.2857	0.2857				0.2857
13	8	C	1	MR	76	0	3	36	0.0000	0.0400	0.0067	0.9467				0.9467
13	8	C	1	MU	58	1	4	36	0.0169	0.0678	0.6441	0.7119				0.7288
13	13	C	1	MP	20	0	0	4	0.0000	0.0000	0.2000	0.2000	0.0000	0.0000	0.9189	0.2000
13	13	C	1	MR	84	0	0	32	0.0000	0.0000	0.3810	0.3810	0.0000	0.0571	1.0329	0.3810
13	13	C	1	MU	72	1	4	39	0.0139	0.0556	0.5417	0.5972	0.0423	0.1693	1.2982	0.6111
15	3	C	2	MP	15	0	0	4	0.0000	0.0000	0.2667	0.2667				0.2667
15	3	C	2	MR	27	0	2	12	0.0000	0.0741	0.4444	0.5185				0.5185
15	3	C	2	MU	24	0	2	15	0.0000	0.0833	0.6250	0.7083				0.7083
15	8	C	2	MP	21	0	2	5	0.0000	0.0952	0.2381	0.3333				0.3333
15	8	C	2	MR	37	1	1	15	0.0270	0.0270	0.4054	0.4324				0.4586
15	8	C	2	MU	46	0	3	33	0.0000	0.0652	0.7174	0.7826				0.7826
15	8	C	2	MP	20	0	2	7	0.0000	0.0000	0.2800	0.3500	0.0000	0.1782	0.8070	0.3800
15	8	C	2	MR	81	1	8	29	0.0123	0.1173	0.4321	0.5679	0.0498	0.1594	1.0437	0.6275
15	8	C	2	MU	67	3	8	28	0.0448	0.0896	0.4179	0.5075	0.0448	0.1679	1.3436	0.5672
16	3	E	1	MP	21	0	2	2	0.0000	0.0952	0.0952	0.1905				0.1905
16	3	E	1	MR	22	1	6	9	0.0455	0.2727	0.4091	0.6818				0.7273
16	3	E	1	MU	28	1	1	7	0.0357	0.0357	0.2500	0.2857				0.3214
16	2	E	1	MP	18	0	0	1	0.0000	0.0000	0.0556	0.0556				0.0556
16	2	E	1	MR	58	2	1	20	0.0345	0.0172	0.3448	0.4138				0.4483
16	2	E	1	MU	63	1	1	27	0.0159	0.0159	0.4286	0.4762				0.5472
16	1	E	1	MP	19	0	1	2	0.0000	0.0526	0.1750	0.1875	0.0000	0.0942	0.2123	0.1875
16	1	E	1	MR	80	1	8	36	0.0125	0.0875	0.0875	0.7000	0.0621	0.1701	1.2704	0.7625
16	1	E	1	MU	91	0	3	49	0.0000	0.0330	0.5385	0.5714	0.0000	0.0857	1.1312	0.5714
19	12	E	2	MP	18	0	0	7	0.0000	0.0000	0.3889	0.3889				0.3639
19	12	E	2	MR	30	1	2	18	0.0333	0.0667	0.5333	0.6000				0.6333
19	12	E	2	MU	44	1	4	17	0.0227	0.0909	0.3864	0.4773				0.5000
19	9	E	2	MP	23	0	0	10	0.0000	0.0000	0.4348	0.4348				0.4348
19	9	E	2	MR	61	2	4	23	0.0362	0.0784	0.4510	0.5294				0.5686
19	9	E	2	MU	44	2	5	20	0.0455	0.1136	0.4545	0.5682				0.6136
19	7	E	2	MP	22	0	1	6	0.0000	0.0455	0.2727	0.3182	0.0000	0.0450	0.8371	0.3182
19	7	E	2	MR	45	2	3	21	0.0444	0.0667	0.4667	0.5333	0.0848	0.1873	1.0984	0.6778
19	7	E	2	MU	38	2	2	21	0.0526	0.0526	0.5526	0.6053	0.1057	0.1989	1.1389	0.6579
2	8	C	2	MP	12	0	0	2	0.0000	0.0000	0.1667	0.1667				0.1667
2	8	C	2	MR	20	0	0	8	0.0000	0.0000	0.4000	0.4000				0.4000
2	8	C	2	MU	32	0	0	16	0.0000	0.0000	0.5000	0.5000				0.5000
2	10	C	2	MP	23	0	0	1	0.0000	0.0000	0.0435	0.0435				0.0435

TABLE 4.--Raw Data (continued)

Rat#	Sectional	C or E	Group	Area	Sheet	O	E	Y	O/E	O/E	Y/E	(O+Y)/E	O/E Ave	O/E Ave	Y/E Ave	Total Dye Hit %
21	10	C	2	MR	38	1	2	7	0.0263	0.0526	0.1842	0.2368				0.2632
21	10	C	2	MU	29	1	1	7	0.0400	0.0400	0.2800	0.3200				0.3600
21	13	C	2	MP	20	0	0	5	0.0000	0.0000	0.2500	0.2500	0.0000	0.0000	0.3450	0.2500
21	13	C	2	MR	47	5	3	12	0.1064	0.0638	0.2553	0.3191	0.1327	0.1196	0.8729	0.4255
21	13	C	2	MU	47	4	0	3	0.0851	0.0000	0.0638	0.0638	0.1251	0.0400	0.5106	0.1489
23	15	E	2	MP	21	0	0	3	0.0000	0.0000	0.1429	0.1429				0.1429
23	15	E	2	MR	42	0	1	21	0.0000	0.0238	0.5000	0.5238				0.5238
23	15	E	2	MU	33	1	2	19	0.0303	0.0606	0.5758	0.6364				0.6667
23	18	E	2	MP	22	0	1	9	0.0000	0.0455	0.4091	0.4545				0.4545
23	18	E	2	MR	70	2	3	34	0.0286	0.0429	0.4857	0.5286				0.5571
23	18	E	2	MU	48	4	5	27	0.0833	0.1042	0.5625	0.6667				0.7500
23	20	E	2	MP	18	0	0	7	0.0000	0.0000	0.4667	0.4667	0.0000	0.0455	0.9234	0.4667
23	20	E	2	MR	53	1	6	20	0.0189	0.0943	0.3774	0.4717	0.0674	0.1451	1.0287	0.4906
23	20	E	2	MU	57	1	7	20	0.0175	0.1228	0.3509	0.4737	0.1110	0.2672	1.1983	0.4912
24	4	E	2	MP	11	0	0	4	0.0000	0.0000	0.3636	0.3636				0.3636
24	4	E	2	MR	38	0	3	8	0.0000	0.0789	0.1579	0.2368				0.2368
24	4	E	2	MU	23	0	3	10	0.0000	0.1304	0.4348	0.5652				0.5652
24	7	E	2	MP	23	0	1	1	0.0000	0.0435	0.0435	0.0870				0.0870
24	7	E	2	MR	36	2	4	6	0.0556	0.1111	0.1667	0.2778				0.3333
24	7	E	2	MU	42	1	6	12	0.0238	0.1429	0.2857	0.4286				0.4524
24	12	E	2	MP	21	0	1	3	0.0000	0.0476	0.1429	0.1905	0.0000	0.0911	0.3078	0.1905
24	12	E	2	MR	61	3	2	8	0.0492	0.0328	0.1311	0.1639	0.1047	0.1702	0.3504	0.2131
24	12	E	2	MU	64	1	3	12	0.0156	0.0469	0.1875	0.2344	0.0384	0.2332	0.6181	0.2500
25	18	C	1	MP	13	0	0	2	0.0000	0.0000	0.1538	0.1538				0.1538
25	18	C	1	MR	42	0	2	9	0.0000	0.0476	0.2143	0.2619				0.2619
25	18	C	1	MU	53	0	3	3	0.0000	0.0943	0.0566	0.1509				0.1509
25	14	C	1	MP	22	0	1	3	0.0000	0.0455	0.1364	0.1818				0.1818
25	14	C	1	MR	60	0	5	21	0.0000	0.0833	0.3500	0.4333				0.4333
25	14	C	1	MU	57	0	2	22	0.0000	0.0351	0.3860	0.4211				0.4211
25	12	C	1	MP	27	0	1	2	0.0000	0.0370	0.0741	0.1111	0.0000	0.0822	0.2617	0.1111
25	12	C	1	MR	78	1	2	30	0.0133	0.0267	0.4000	0.4267	0.0133	0.1298	0.8214	0.4400
25	12	C	1	MU	68	1	2	22	0.0147	0.0294	0.3235	0.3529	0.0147	0.0868	0.7284	0.3676
27	3	E	1	MP	18	0	1	3	0.0000	0.0556	0.1667	0.2222				0.2777
27	3	E	1	MR	28	1	3	18	0.0357	0.1071	0.5714	0.6786				0.7143
27	3	E	1	MU	39	0	0	27	0.0000	0.0000	0.6923	0.6923				0.6923
27	8	E	1	MP	21	1	1	11	0.0476	0.0476	0.5238	0.5714				0.6190
27	8	E	1	MR	36	1	2	26	0.0278	0.0556	0.7222	0.7778				0.8056
27	8	E	1	MU	48	0	0	46	0.0000	0.0000	0.9583	0.9583				0.9583
27	10	E	1	MP	19	0	0	6	0.0000	0.0000	0.3158	0.3158	0.0476	0.0861	0.8952	0.3158
27	10	E	1	MR	85	1	1	47	0.0118	0.0118	0.5545	0.5727	0.0578	0.1092	1.7872	0.5909
27	10	E	1	MU	88	0	3	89	0.0000	0.0455	0.8636	0.9091	0.0000	0.0456	2.0830	0.9204
28	5	E	2	MP	14	0	0	6	0.0000	0.0000	0.4286	0.4286				0.4286
28	5	E	2	MR	37	0	12	6	0.0000	0.3243	0.1351	0.4595				0.4595
28	5	E	2	MU	38	0	8	10	0.0000	0.2105	0.2632	0.4737				0.4737
28	10	E	2	MP	28	0	0	2	0.0000	0.0000	0.0769	0.0769				0.0769
28	10	E	2	MR	28	1	4	8	0.0385	0.1538	0.3077	0.4615				0.5000
28	10	E	2	MU	25	1	9	7	0.0400	0.3600	0.2800	0.6400				0.6800

TABLE 4.--Raw Data (continued)

Rat#	Sections	C or E	Group	Area	Beta	O	S	T	OB	IB	YB	(G+Y)B	OB Ave	GB Ave	YB Ave	Total Dye Int %
28	13	E	2	MP	24	1	1	6	(.0417	.010	0.2500	0.3750	0.0417	0.1250	0.4636	0.4167
28	13	E	2	MR	32	1	3	7	(.0238	.032	0.3077	0.4359	0.0641	0.3906	0.8804	0.4815
28	13	E	2	MU	35	0	6	1	(.0000	.031	0.1579	0.8789	0.0400	0.8512	0.9256	0.8788
28	8	E	2	MP	22	0	1	5	(.0000	.005	0.2273	0.2727				0.2727
28	5	E	2	MR	41	0	2	9	(.0000	.018	0.4390	0.4878				0.4878
28	5	E	2	MU	35	0	3	9	(.0000	.001	0.3486	0.4643				0.4545
28	8	E	2	MP	23	1	2	7	(.0435	.010	0.4783	0.5652				0.8087
28	8	E	2	MR	36	0	2	7	(.0000	.014	0.3889	0.4643				0.4843
28	8	E	2	MU	37	0	2	4	(.0000	.031	0.5135	0.5526				0.8378
28	12	E	2	MP	11	0	3	3	(.0000	.000	0.8081	0.8081	0.0435	0.1021	1.4631	0.8091
28	12	E	2	MR	36	0	2	7	(.0000	.003	0.8417	0.8250	0.0000	0.1710	1.0809	0.8250
28	12	E	2	MU	27	0	1	7	(.0000	.010	0.4074	0.4444	0.0000	0.1478	1.0411	0.4444
28	12	E	2	MU	27	0	1	7	(.0000	.010	0.4074	0.4444	0.0000	0.1478	1.0411	0.4444
30	11	E	1	MP	18	0	3	7	(.0000	.010	0.2500	0.2500				0.2500
30	11	E	1	MR	15	0	2	7	(.0000	.000	0.7867	0.8286				0.8286
30	11	E	1	MU	43	0	1	4	(.0000	.000	2.2222	2.2778				2.2778
30	7	E	1	MP	23	1	3	1	(.0217	.032	0.1087	0.1739				0.1957
30	7	E	1	MR	33	1	2	9	(.0000	.030	0.9000	1.0000				1.0000
30	7	E	1	MU	25	0	1	3	(.0000	.030	0.6340	0.4828				0.4528
30	3	E	1	MP	15	0	3	3	(.0000	.000	0.2000	0.2000	0.0217	0.0652	0.3561	0.2000
30	3	E	1	MR	37	1	2	9	(.0000	.000	1.1676	1.3126	0.1725	0.2728	2.3494	1.3750
30	3	E	1	MU	23	1	3	4	(.0213	.004	0.8511	0.8574	0.0213	0.1436	2.0258	0.8787
30	3	E	1	MU	23	1	3	4	(.0213	.004	0.8511	0.8574	0.0213	0.1436	2.0258	0.0887
32	11	C	2	MP	13	0	2	1	(.0000	.007	0.0400	0.0667				0.2308
32	11	C	2	MR	35	0	3	3	(.0000	.007	0.1429	0.2286				0.3333
32	11	C	2	MU	12	0	1	3	(.0000	.000	0.2333	0.3333				0.5000
32	13	C	2	MP	17	0	1	3	(.0000	.000	0.2400	0.2000				0.4615
32	13	C	2	MR	34	1	1	3	(.0000	.000	0.2857	0.4201				0.3704
32	13	C	2	MU	27	0	1	3	(.0000	.011	0.2963	0.3704				0.2773
32	18	C	2	MP	22	0	1	1	(.0000	.000	0.1818	0.2273	0.0000	0.3043	0.4452	0.3519
32	18	C	2	MR	36	0	3	7	(.0000	.001	0.2037	0.3519	0.0385	0.3309	0.8206	0.2545
32	18	C	2	MU	35	3	3	1	(.0000	.000	0.1486	0.2000	0.0845	0.1950	0.4825	0.3810
34	8	C	1	MP	21	0	3	1	(.0000	.000	0.3810	0.3810				0.3065
34	8	C	1	MR	32	1	1	7	(.0161	.031	0.2742	0.2903				0.0417
34	8	C	1	MU	7	1	1	35	(.0139	.017	0.4881	0.5278				0.1905
34	10	C	1	MP	21	0	3	1	(.0000	.000	0.1905	0.1905				0.4390
34	10	C	1	MR	41	0	2	9	(.0000	.008	0.3902	0.4390				0.6349
34	10	C	1	MU	42	1	3	9	(.0182	.005	0.5769	0.6184				0.7895
34	14	C	1	MP	19	0	3	2	(.0000	.000	0.6316	0.7895	0.0000	0.1879	0.9490	0.8780
34	14	C	1	MR	39	1	3	9	(.0189	.009	0.8771	0.9810	0.0222	0.0961	1.1098	0.8140
34	14	C	1	MU	37	0	3	2	(.0000	.008	0.8514	0.8140	0.0238	0.1050	1.3004	0.3000
39	4	C	2	MP	10	0	3	1	(.0000	.000	0.3000	0.2000				0.8788
39	4	C	2	MR	37	2	3	7	(.0741	.027	0.3704	0.5946				0.4483
39	4	C	2	MU	32	1	3	1	(.0345	.034	0.3103	0.4138				0.3333
39	8	C	2	MP	15	0	3	1	(.0000	.000	0.3333	0.3333				0.6552
39	8	C	2	MR	33	1	3	3	(.0345	.024	0.4483	0.8207				0.7333
39	8	C	2	MU	30	1	3	9	(.0333	.017	0.8333	0.7000				0.2727
39	11	C	2	MP	22	0	3	1	(.0000	.034	0.1364	0.2727	0.0000	0.1364	0.9697	0.5778
39	11	C	2	MR	45	4	3	7	(.0889	.011	0.3778	0.4889	0.1481	0.3452	0.9495	

TABLE 4.--Raw Data (continued)

Rat#	Section#	C or E	Group	Age	Bone	O	G	Y	O/B	G/B	Y/B	(G+Y)/B	O/B Ave	G/B Ave	Y/B Ave	Total Dye Wt %
39	11	C	2	16	35	2	4	10	0.0571	0.1143	0.2857	0.4000	0.1020	0.3154	0.9725	0.4571
41	7	C	1	16	19	0	2	8	0.0000	0.1250	0.3125	0.4375				0.4375
41	7	C	1	16	33	0	3	7	0.0000	0.0909	0.2121	0.3030				0.3030
41	7	C	1	16	31	0	2	8	0.0000	0.0645	0.2581	0.3226				0.3226
41	14	C	1	16	26	1	2	8	0.0385	0.0769	0.3077	0.3846				0.4731
41	14	C	1	16	55	0	2	26	0.0000	0.0345	0.4483	0.4828				0.4828
41	14	C	1	16	60	0	2	28	0.0000	0.0333	0.4667	0.5000				0.5000
41	20	C	1	16	28	0	3	6	0.0000	0.1500	0.3000	0.4500	0.0385	0.2888	0.7119	0.4500
41	20	C	1	16	84	3	8	35	0.0357	0.0952	0.4167	0.5119	0.0357	0.1243	0.9344	0.5119
42	8	E	1	16	21	0	0	7	0.0000	0.0000	0.3333	0.3333				0.3333
42	8	E	1	16	35	0	7	11	0.0000	0.2000	0.3143	0.5143				0.5143
42	8	E	1	16	34	0	2	18	0.0000	0.0588	0.4706	0.5294				0.5294
42	8	E	1	16	22	1	1	0	0.0455	0.0455	0.0000	0.0455				0.0909
42	8	E	1	16	38	1	6	7	0.0263	0.1579	0.1842	0.3421				0.3684
42	11	E	1	16	51	1	2	10	0.0196	0.0392	0.1961	0.2353				0.2549
42	11	E	1	16	20	0	0	8	0.0000	0.0000	0.4000	0.4000	0.0455	0.0455	0.8111	0.4000
42	11	E	1	16	53	0	1	17	0.0000	0.0189	0.3208	0.3396	0.0263	0.2434	0.9097	0.3396
43	7	E	1	16	45	1	1	14	0.0222	0.0222	0.3111	0.3333	0.0418	0.0810	0.8641	0.3556
43	7	E	1	16	9	0	0	3	0.0000	0.0000	0.3333	0.3333				0.3333
43	7	E	1	16	42	0	3	7	0.0000	0.0714	0.1667	0.2381				0.2381
43	11	E	1	16	32	2	5	7	0.0625	0.1563	0.2188	0.3750				0.4375
43	11	E	1	16	23	0	1	2	0.0000	0.0435	0.0870	0.1304				0.1304
43	11	E	1	16	63	1	4	18	0.0159	0.0635	0.2540	0.3175				0.3333
43	14	E	1	16	85	3	9	20	0.0353	0.0588	0.2588	0.3176				0.3059
43	14	E	1	16	13	0	0	8	0.0000	0.0000	0.3846	0.3846	0.0000	0.0632	0.8827	0.3846
43	14	E	1	16	47	0	0	38	0.0000	0.0000	0.8085	0.8085	0.0159	0.0873	1.1120	0.8085
44	3	C	1	16	43	0	0	38	0.0000	0.0000	0.8837	0.8837	0.0764	0.1430	1.1203	0.8837
44	3	C	1	16	17	0	0	2	0.0000	0.0000	0.1176	0.1176				0.1176
44	3	C	1	16	40	1	3	11	0.0250	0.0750	0.2750	0.3500				0.3750
44	11	C	1	16	48	1	3	11	0.0208	0.0625	0.2292	0.2917				0.3125
44	11	C	1	16	17	0	2	1	0.0000	0.1176	0.0588	0.1765				0.1765
44	11	C	1	16	38	2	1	12	0.0526	0.0263	0.3158	0.3421				0.3947
44	13	C	1	16	34	0	1	14	0.0000	0.0294	0.3824	0.3947				0.3947
44	13	C	1	16	18	0	2	0	0.0000	0.1250	0.0000	0.1250	0.0000	0.2429	0.0980	0.1250
44	13	C	1	16	62	0	2	9	0.0000	0.0323	0.1731	0.2115	0.0610	0.0886	0.8805	0.2115
45	3	E	2	16	28	0	2	8	0.0000	0.0714	0.3036	0.3214	0.0089	0.0769	0.7978	0.3824
45	3	E	2	16	22	0	4	10	0.0000	0.0769	0.2308	0.3077				0.3077
45	3	E	2	16	28	1	5	6	0.0357	0.1818	0.4545	0.6364				0.6364
45	8	E	2	16	77	1	3	11	0.0130	0.1169	0.2143	0.3636				0.4286
45	8	E	2	16	47	2	5	11	0.0426	0.1064	0.2340	0.3404				0.5556
45	8	E	2	16	50	0	6	14	0.0000	0.1200	0.2800	0.4000				0.3636
45	10	E	2	16	18	0	1	4	0.0000	0.0556	0.2222	0.2778	0.0370	0.1923	0.7066	0.4000
45	10	E	2	16	39	0	3	7	0.0000	0.0769	0.1795	0.2564	0.0478	0.3721	0.8880	0.2778
46	8	E	2	16	14	0	0	8	0.0000	0.0000	0.4286	0.4286	0.0315	0.4739	0.8987	0.3846
																0.4286

TABLE 4 --Raw Data (continued)

Rep	Section	C or E	Group	Area	Beam	O	G	Y	O/B	G/B	Y/B	(G+Y)/B	O/B Ave	G/B Ave	Y/B Ave	Total Dye Hit %
46	6	E	2	MR	34	0	3	11	0.0000	0.0882	0.3235	0.4118				0.4118
46	6	E	2	MU	45	0	7	20	0.0000	0.1556	0.4444	0.6000				0.6000
46	11	E	2	MP	18	1	1	5	0.0556	0.0556	0.2778	0.3333				0.3889
46	11	E	2	MR	36	0	4	12	0.0000	0.1026	0.3333	0.4359				0.4359
46	11	E	2	MU	44	1	6	11	0.0227	0.1364	0.2500	0.3864				0.4091
46	14	E	2	MP	17	0	0	7	0.0000	0.0000	0.4118	0.4118	0.0556	0.0556	0.8324	0.4118
46	14	E	2	MR	45	1	5	18	0.0222	0.1111	0.4000	0.5111	0.0222	0.2431	0.8412	0.5333
46	14	E	2	MU	43	1	6	16	0.0233	0.1395	0.3721	0.5116	0.0460	0.3278	0.7702	0.5349
47	8	C	1	MP	13	0	0	8	0.0000	0.0000	0.6154	0.6154				0.6154
47	8	C	1	MR	41	1	1	22	0.0244	0.0244	0.5366	0.5610				0.5854
47	8	C	1	MU	53	1	1	25	0.0189	0.0189	0.4528	0.4717				0.4906
47	13	C	1	MP	23	0	2	9	0.0000	0.0870	0.3913	0.4783				0.4783
47	13	C	1	MR	45	0	1	27	0.0000	0.0222	0.6000	0.6222				0.6222
47	13	C	1	MU	35	1	0	21	0.0286	0.0000	0.6000	0.6000				0.6286
47	17	C	1	MP	14	0	0	6	0.0000	0.0000	0.4286	0.4286	0.0000	0.0870	1.0250	0.4286
47	17	C	1	MR	54	1	2	28	0.0185	0.0370	0.5185	0.5556	0.0286	0.0674	1.2974	0.5741
47	17	C	1	MU	50	1	2	29	0.0200	0.0400	0.5800	0.6200	0.0549	0.0462	1.1581	0.6400

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