1979

Parameters of the Dento-Gingival Junction: A Post Operative Healing Study in Humans

Richard J. Rizzo
Loyola University Chicago

PARAMETERS OF THE DENTO-GINGIVAL JUNCTION: A POST OPERATIVE HEALING STUDY IN HUMANS

By

Richard J. Rizzo

A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

March 1979

ACKNOWLEDGEMENTS

To Dr. Anthony Gargiulo my sincere gratitude and appreciation for his advice, guidance and concern these past two years.

To Dr. Daniel Grant who was instrumental in the initiation and completion of this research project. My thanks for his constructive criticism and encouragement.

LIFE

Richard J. Rizzo was born on April 9, 1944 in Chicago, Illinois.

He was graduated from Lake Park High School in Medinah, Illinois in June 1962. He attended Cornell College in Mt. Vernon, Iowa where he received a Bachelor of Arts degree with a major in Biology in 1966.

In August 1966 he enlisted in the United States Army and served four years in the Infantry and Chemical Corps. He was honorably discharged with the rank of Captain in August 1970.

In September 1970, he entered Loyola University School of Dentistry where he received the degree of Doctor of Dental Surgery in June 1974. After spending one year as a Career General Practice Resident at the Veterans Administration Hospital, Hines, Illinois he began graduate study toward the degree of Master of Science in Oral Biology and Clinical Specialty training in Periodontics under Dr. Anthony Gargiulo at Loyola University, Maywood, Illinois.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

Extensive research has been devoted to developing an understanding of the exact nature of the dentogingival junction, morphologically and histologically. The classic work of Gargiulo, Wentz and Orban[1] established certain morphologic quantitative relationships of the tissue at the dentogingival junction and found these relationships to be in accord with the concept that the dentogingival junction is a functional unit composed of epithelial and hard and soft connective tissue attachments to the tooth. Various researchers have established utilizing electron microscopic techniques,[2,3,4] the attachment apparatus of the junctional epithelium to be similar in nature to all epithelial-connective tissue junctions; that is, consisting of hemidesmosomes and a basal lamina of probable glycoprotein nature and epithelial origin. Their research clarified the nature of the epithelial attachment apparatus and shed further light on Gottlieb's (1921) contention that the epithelium is organically connected to enamel and cementum.

If the findings of Gargiulo and co-workers are verified then the understanding of the morphometric relationships of the soft connective and epithelial tissues of the dentogingival junction can provide statistical comparative parameters for evaluating the status of repair following periodontal surgery and for recognizing any morphometric

changes which may have occurred as the result of the surgery or from disease processes.

Few earlier studies were concerned with the morphometric relationships of the tissues at the dentogingival junction following surgery. Morris[5] in a 1961 study of the position of the epithelial attachment following the creation of periodontal wounds determined that intimate contact of the surgerized tissue to the tooth is required for the induction of the connective tissue fiber attachment and to halt the apical proliferation of the sulcular and junctional epithelium. Morris described no morphometric relationships of tissues. In addition his use of a notch in the tooth as a landmark may have influenced the post surgical positioning of the junctional epithelium.

Marfino[6] evaluated the repair of the dentogingival junction following surgical intervention in dogs; however, no specific morphometric criteria were established. Wilderman[7] in an animal and human study of repair following osseous surgery delineated the healing sequence of the tissues comprising the dentogingival junction, yet no morphometric criteria were established.

The studies of repair following various periodontal treatment modalities were initially concerned with the feasibility of re-attachment of the epithelium and connective tissues to the enamel or cementum. Later studies were concerned with sequential order of healing as it related to those hard and soft tissues which comprise the dentogingival junction.

The purpose of this investigation is to evaluate the histological

parameters of the tissues of the dentogingival junction following periodontal flap procedures and to relate these findings to the 1961 Gargiulo study which established the morphometric norms for the disease free dentogingival junction of humans. This will give a comparative basis upon which we can better understand the overall effects of surgical procedures upon the ultimate relationship of the tissues of the dentogingival junction following repair.

CHAPTER II

REVIEW OF THE LITERATURE

A. Introduction

Early investigators paid much attention to the ability of the gingival tissue to reattach to the tooth following pathologic or surgical separation. Management of the hard tissue was of great concern. The studies generally revealed that the gingival tissues will reattach at some point providing the tooth surface is free of deposits and that any diseased cementum is removed.

Attention was later directed toward an understanding of the sequential events of healing following various surgical modalities. Clinical and histologic criteria were established for numerous animals as well as humans.

Currently, due to the more extensive utilization of electron microscopic and biochemical techniques, attention is being directed toward determining the exact nature of the reattachment and repair of the dentogingival junction on an ultrastructural and biochemical basis.

B. Wound Healing - Light Microscopic Studies

1. Animal Studies

Linghorne and O'Connell[20] studying reattachment following flap surgery on dogs found that new cementum deposition was preceeded by cementum and dentin resorption, and that the reattachment of the soft

tissues was linked to the process of new cemental deposition.

Linghorne and O'Connell[21] in a subsequent study on surgically created defects in dogs stated that morphodifferentiation of undifferentiated mesenchymal cells occurred only where resorption had occurred or was occurring. They felt that the presence of resorbing calcified tissues was a stimulus for osteoblastic and cementoblastic differentiation. Histologically no difference was detected between osteoid and cementoid. Linghorne and O'Connell's evidence favored the bone rather than the gingival corium as being the source of the undifferentiated mesenchymal cells.

In a later study utilizing dogs, Linghorne[22] found that coronal reattachment of gingival soft tissue to tooth followed the creation of pockets and was preceded and induced by the deposition of new cementum on denuded roots. Resorption preceded deposition and periodontal ligament fiber orientation normalizes following osseous regeneration.

Marfino[6] in evaluating the repair of the dentogingival junction in dogs following flap and osseous surgery noted that gingival recession first became evident on the 51st day and that contrary to the observations of Linghorne and O'Connell microscopically no progressive coronal shift of the connective tissue and epithelial attachments were seen. An epithelial attachment was first noted at 23 days and averaged 1.50 mm in length. Healing in this study was functionally acceptable but with deformity.

In evaluating the repair sequence following mucogingival surgery

in dogs, Wilderman and coworkers[23] divided the repair process into three stages based on histologic findings. Phase I was the osteoclastic phase, lasting 2 to 10 days with peak activity between 5 to 10 days. Phase II was the osteoblastic phase lasting 10 to 28 days with peak osteoblastic activity seen between 21 to 28 days. The attachments of connective tissue and epithelium to tooth structure occur by the 21st day. Phase III is the phase of functional repair. This phase lasts 28 to 185 days during which bone maturation, cementoid formation, and periodontal ligament fiber orientation occur. Wilderman found that by 93 days the periodontal ligament space was restored, that septal bone completely regenerated and that radicular bone regenerated only by 50%, exhibiting functional repair with anatomic deformity.

West and Bloom[24] studied the wound healing in dogs following mucogingival surgery. Observations following complete and partial denudation were made. They found that where the bone was exposed complete and rapid bone resorption occurred. Cemental resorption was only occasionally seen. Healing occurred from the superior wound margin and epithelialization was seen at 21 days. Where the periosteum was left intact the buccal bone was only partially resorbed and healing was more rapid.

Staffileno and coworkers[25] histologically evaluated the healing of split thickness flaps in dogs. They noted epithelial regeneration occurred by 6 days with initial connective tissue differentiation. Osteoclastic and osteoblastic activity were consistent with Wilderman's

findings[23] occurring between 2 to 14 days and 6 to 21 days with peaks at 6 and 21 days, respectively. By 60 days a reorientation of cellular components and collagen fibers had occurred. Healing at 60 days was characterized by functional repair without anatomic deformity.

The effects of periosteal retention upon healing were further studied by Wilderman[26] again utilizing dogs as the experimental model. In this study the peak osteoclastic activity occurred between 4 to 6 days and the peak osteoblastic activity between 14 and 21 days. The functional orientation of the periodontal ligament fibers was noted between 21 to 28 days. Collagen fiber bundles were in evidence and functionally oriented at 90 days. The study showed that osseous resorption patterns varied with the thickness of the connective tissue remnant and the thickness of the bone. The healing pattern in this study as with Wilderman's 1960 study revealed a functional repair with an anatomic deformity.

Lobene and Glickman[27] studied the healing response of the alveolar bone to grinding with a diamond stone after full thickness flaps were reflected. They found that grinding bone resulted in more extensive bone loss, bone necrosis and delayed healing. Bone resoprtion was at its maximum at 28 days in this study and bone loss was as much as three times as great in the ground area as compared to the non-ground area - 0 to .5 mm compared to 0 to 1.7 mm crestal bone loss respectively.

Carranza and Carraro[28] utilizing full thickness and split thickness flap procedures in dogs found a statistically significant amount

of gingival recession in full thickness procedures. The loss of marginal bone and more apical positioning of the epithelial attachment were consistant with Wilderman's earlier findings. Glickman and coworkers[29] similarly found that removal of periosteum resulted in delayed healing, greater reduction of bone height, and healing with anatomic deformity.

In a study to delineate cellular activity in the repair of split thickness flaps raised and excised for secondary intention healing, Staffileno and coworkers[30] described three stages in the healing process. The stage of cellular mobilization and proliferation occurred between 0 and 48 hours. The stage of organization began at 4 days and ended at 21 days. Osteoclastic activity began at day 2 and peaked during this stage at day 4. Osteoblastic activity began at 7 days and coincided with Staffileno's osseous reconstruction stage. The osteoblastic stage peaked at 14 days and continued through 27 days. Epithelium completely covered the wound by 7 days. The healing pattern in this study revealed a complete functional repair with slight anatomical deformity of the dentogingival junction.

Hiatt and coworkers[31] utilizing full thickness flaps with full gingival retention on dogs noted rapid epithelial reattachment as the result of well adapted flap replacement. The minimal fibrin layer between flap and tooth served to prevent the apical downgrowth of the epithelium. Their study revealed evidence of connective tissue repair at 2 days, replacement of the fibrin clot by 2 weeks and by 4 weeks a

fairly well developed connective tissue attachment. Minimal bone loss was noted in this study as compared to the other dog studies with osseous regeneration occurring in all instances by one month. Hiatt et. al., felt that the retention of cementum coupled with properly prepared tooth surfaces aided in the reattachment of the connective tissue and was a factor in minimizing initial bone loss.

Caffesse[32] investigated the effects of reverse bevel flaps with osseous removal utilizing monkeys as the experimental model. Epithelial reattachment was observed at 9 days. Osteoclasia was noted to peak between 7 to 9 days. Caffesse speculated that after flap surgery the connective tissue regeneration precedes the epithelial regrowth due to the "stunning" effect the surgery has had upon the premitotic activity of the epithelial cells. The study showed that split flaps generally heal faster than full thickness flaps with initially less bone loss and loss of attachment.

Stallard and Hiatt[95] following full thickness mucoperiosteal flaps in dogs in which an attempt was made to remove all the cementum and some of the alveolar bone at the crest, histologically observed an induction capacity of retained mineralized fragments within the flap. At 3 weeks root resorption was evident on the planned root surfaces while an osteoid-like formation was observed on the cementum fragments. No deposition had occurred on the dentin fragments. By 4 weeks osteoid and cementoid had completely surrounded the dentin and cementum fragments. Additionally 2 to 4 mm of new bone formation was evident while portions of the newly formed periodontal ligament appeared functionally

oriented. At 4 months the epithelial attachment had completely regenerated. New cementum covered the exposed dentin. New bone had replaced the bone initially removed. Areas of ankylosis were also evident. At one year the dentin resorptive area was completely covered by new cementum. The authors state that "on analysis of results it was concluded that bone, cementum and dentin chips which remain in the wound following periodontal flap surgery serve as nidi for, or inducers of, new bone and cementum formation".

Henning[33] noted that the mitotic activity of the epithelial cells adjacent to the tooth surface was elevated for a period of 8 days or more following gingivectomy wounds in rats. Reattachment was shown to occur after the period of epithelialization at approximately 2 to 3 weeks. Henning states that epithelial cells secrete a cementing substance between themselves and the tooth but that time is required for the epithelial cell to reach a stage of organization where they can produce this cementing substance.

In a series of studies on the microvasculature of the healing periodontal wound, Kon et al.,[34] raised and replaced full thickness mucoperiosteal flaps in dogs. Their findings revealed an increased vascularization, inflammation, and replacement of the initial fibrin clot by young connective tissue cells by the 6th or 7th day. Between days 7 to 12 osteoclastic and osteoblastic activity predominated. The bone which was initially resorbed was completely rebuilt by 31 days. By the 38th day in this study all the tissues affected had regenerated.

There was some apical proliferation of the epithelial attachment. The dentogingival junction was effectively rebuilt by the 85th day. No resorptive bone lesions were in evidence although mild inflammatory cell infiltrates were noted.

2. Human Studies

Early researchers were fairly consistent in their recognition that a healthy cementum was necessary for reattachment. Noyes[35] as early as 1912 stated that "whenever the fibers have been stripped from the surface of the cementum, they can be reattached to it only by the formation of a new layer of cementum, building the fibers into it. The cells of the tissue must be in a normal and vitally active condition, and the surface of the root must be such that they can be in physiological contact with it".

McCall[36] states that although the exact mechanism of attachment to the cementum was a mystery, researchers were aware that the periodontal ligament fibers upon healing are oriented parallel to the root rather than perpendicular or at oblique angles to the root surface.

Workman[37] reflected mucoperiosteal flaps, replaced them and at four weeks noted "the two specimens give the appearance of never having been detached in so far as the relationship exists between the peridental membrane and the cementum is concerned. That is, there is no difference in the appearance between the detached specimen and the undetached specimen".

After root planning and soft tissue curettage, Schaffer and Zander[38]

reported that a new connective tissue and epithelial attachment were formed and that new cementum deposited upon the old cementum and dentin. They reported new periodontal ligament fibers embedded within the new cementum and that the new epithelial attachment was formed by cytoplasmic processes of the epithelial cells extending into the dentinal tubules.

Morris[39] created surgical pockets on human teeth marked for extraction for prosthetic reasons. Gingival and pocket markers were placed which penetrated the cementum. Morris found new cementum formation on old cementum and on dentin. In those areas where the dentin was not completely covered connective tissue healing occurred against both dentin and cementum. Connective tissue fibers were found parallel to the root surface until the 106th day when a general transverse pattern was noted. Morris concluded that the functional fiber orientation appeared to be solely dependent upon the duration of healing. Cementoid deposition was noted in the 56th day specimen on the root surface and in the pocket marker. In a subsequent study on the surgical detachment from non-vital teeth, Morris[40] found that reattachment and healing occurred against the cementum of non-vital teeth regardless of prior root preparation. The study, however, revealed that healing did not occur against the dentin in these non-vital teeth. The epithelium grew down past dentin where the dentin was continous with the gingival crevice and attached to cementum. Morris suggested as possible explanations for this finding that the lack of vitality may have affected fluid

exchange, that some "inductive principle" may have been lost, or that the medications used in root canal therapy affect the dentin's ability to be receptive to attachment.

Dedolph and Clark[41] raised and replaced full thickness mucoperiosteal flaps and noted that at three weeks the reformation of the epithelial attachment was complete, and that the connective tissue elements had been restored. The regeneration and rearrangement of free gingival periodontal fibers was seen at one week post operative. They were unable to distinguish the controls from the four week specimens.

Kohler and Ramfjord[8] conducted a clinical and histologic study of healing of mucoperiosteal flaps with no curettage of root surfaces. They found that healing occurred without any significant loss of periodontal attachment. No significant difference was found between the position of the free gingival margin, the gingival sulcus, or the alveolar crest before and after the procedure. The total loss of alveolar bone from the flap procedure alone was approximately .35 mm. The healing in this study was observed up to 196 days.

Morris[42] in a later study of healing related to extirpation of vital pulps determined that the presence of vital pulpal tissues seems to affect the location of the epithelial attachment. Although healing occurred in all cases treated the level of attachment showed a general loss in height of .5 to 5 mm. Morris[43] also studied the post surgical location of the epithelial attachment in vital teeth that had all exposed cementum removed. He found the location of the epithelial attachment varied with the depth of the cementum excavations. In shallow

excavations the epithelium attached at the apical border on dentin. In deeper excavations the epithelium bypassed the dentin and attached on cementum. Morris found that the connective tissue union at the first point of close contact between the periodontal and root tissues served as a barrier for further apical movement of the epithelial attachment.

In a subsequent study on the arrangement of periodontal ligament fibers postsurgically, Morris[97] found that counter to his findings in a previous study[39] the healing periodontal ligament fibers grow parallel to the root in an irregular meshwork of connective tissue fibers. Morris found no functional fiber orientation at 106 days and stated that "the re-orientation of these fibers to a normal direction is extremely slow and may never occur".

Donnenfeld and coworkers[44] conducted a clinical investigation of healing following apically positioned flaps. Generally the study revealed the procedure resulted in an increased width of attached gingiva, statistically significant bone loss, and an apical shift of the epithelial attachment. Specifically, the epithelial attachment showed an apical shift of .03 to 2.79 mm with a mean of .695 mm. There was a mean gain in the attached gingiva of 1.02 mm, and the alveolar bone showed a mean loss of .63 mm.

Friedman and Levine[45] described the status of information relating to the apically repositioned flap in 1964. They observed that the apically repositioned flap with or without osseous recontouring results in

either no bone loss or a negligible amount of permanent bone loss. The amount lost averaged .18 mm and was considered so small as to be clinically insignificant.

Pfeiffer's[46] histologic study of flap procedures revealed that with full thickness flaps osteoclastic activity is evident on the periosteal surface at 7 days and still very active at 14 days. More bone resorption was noted where thin facial bone existed initially. Full flaps resulted in osteoclasia and necrosis of the outer bone surface. Partial thickness flaps showed no osteoclasia with one exception where the periosteum was penetrated in the flap procedure.

Moskow[96] found calcified gingival inclusions in less than 25% of 400 specimens studied. Moskow states that gingival inclusions are a common occurrence during dental procedures and that they generally illicit only very minor foreign body responses unless they become incorporated with calculus or infected debris. He notes that many specimens show resorption on one side with active deposition of new hard substance on the other. A fibrous-like connective tissue capsule frequently surrounds the tooth fragments. Often the remnants may work their way through the gingiva and be lost.

Pennel[47] found that the crestal alveolar bone loss following flap and osseous surgery was insignificant when related to the total area of osseous support. The average reduction of alveolar crestal bone height was .54 mm.

Grant[48] found that osseous surgery often resulted in the sequestration of necrotic bone fragments. Osteoid formation was noted in one 30

day specimen. He states that it is often possible to see osteoclasts destroying the bone fragment in one area while contiguous osteoid deposition via osteoblasts is also evident in another. It was noted that oral epithelium at times invades the degenerating connective tissue and encircles the necrotic bone remnants. Expulsion or dissolution of the separated fragments was not frequently found nor was resorption followed by replacement. Grant states that some bone destruction following osseous surgery is unavoidable but that the extent is variable.

Healing after partial denudation was studied by Costich and Ramfjord[49]. Their finding showed bone resorption of much greater duration and severity than previously reported. Cemental resorption was reported for the first time, and was seen to occur at 3-3 1/2 weeks. Most specimens showed complete repair by 6 weeks. No well defined healing phases were evident. Ramfjord and Costich[50] conducted a subsequent study on healing involving partial thickness flaps. Their findings revealed that a severe inflammatory reaction resulted even when a periosteal covering remained to protect the bone. The bone resorption evidenced in this study was almost equal to that found in the denudation study. Ramfjord and Costich suggest that if it is not possible to replace the flap to cover the bone then a thick connective tissue covering should remain to protect the periosteum and bone.

Tavtigian[51] conducted a study to measure the height of the facial radicular alveolar crest after apically positioned flap surgery. The

average of the mean changes in the height of the radicular alveolar crestal bone was -.47 mm ± .143 mm. His findings suggest that crestal reduction will occur after apically positioning flaps.

Wilderman and coworkers[7] studied bone loss following osseous surgery. They classified the vestibular bone as thin, medium, and thick. The results showed that generally more crestal bone was lost in cases where thin bone existed initially. The least bone loss occurred where thick vestibular bone initially existed. The epithelial attachment was longer in all cases beginning at two weeks. This was possibly due to the inflammation present. Osteoblastic activity and bone repair peaked at 3 to 4 weeks after surgery. Osteoid was first formed on the periosteal surface of the alveolar bone at 3 weeks. Immature bone was replaced by an intermediate bone at 6 months and by mature bone by 18 months. The average loss of alveolar crestal bone was .8 mm. Maximum bone repair and almost complete anatomical restoration of the operated bone occurred where pre-operative bone was thick, cancellous and contained many marrow spaces. Cementoid was first formed in the surgically produced notch at 3 months, and below this point at approximately 2 months. Periodontal ligament fiber orientation was parallel to the long axis of the tooth until 5 to 6 months post operative.

The amount of alveolar crestal reduction following full and partial thickness flaps was further studied by Wood and coworkers.[53] Their results showed a statistically significant reduction in crestal

bone height for both procedures. The mean crestal bone loss for the full flap procedures was .62 mm with a range of .23 to 1.60 mm. The mean crestal bone loss for the split flap procedures was .98 mm with a range of .47 to 1.67 mm. The study showed greater crestal bone loss after partial thickness as compared to full thickness flaps. Wood et al., suggest that the results evidence the fact that the amount of crestal bone loss greatly depends upon the anatomy of preoperative supporting tissues. Teeth with thin radicular bone and teeth with thin connective tissue coverings tend to show greater crestal bone loss. Split thickness flaps performed over teeth with thin connective tissue coverings will yield greater bone loss than if full thickness flaps were utilized. They presumed that this relates to the loss of cellular viability due to interdiction of vascularity with resultant cell necrosis.

Levine and Stahl[54] report that connective tissue staining techniques demonstrate the presence of functionally oriented and attached fibers of the gingival complex three weeks after flap surgery, when fibers are left on cementum after reflection of a flap.

Stahl et al.[55] in a review of the then current literature on gingival repair state that regardless of the surgical modality, epithelialization will occur in 7-14 days and connective tissue organization and maturation will occur between 10-30 days. Stahl reports that complete healing of the connective tissue attachment can occur though the root surfaces may not have been curetted following flap reflection.

This process is called healing by scar. The apical migration of the epithelial attachment will occur if the collagen fibers are mechanically removed from the root surface. The apical migration is retarded by inflammation and collagen adhesions. In the instances where the collagen is not mechanically removed no new cementum is formed and the healing fibers align themselves parallel to the root. It is suggested that functional orientation of the periodontal ligament fibers may never reoccur.

In a review of the literature concerning cementum, Stahl[56] states that "most authors seem to suggest that cemental resorption must precede apposition and that cemental repair is seen most frequently in areas of cemental bays or nicks". Generally cellular rather than a cellular cementum fills these bays. New cemental formation has been reported as early as 6 weeks by Stahl.

C. Electron Microscopic Studies: The Nature of Attachment

Ussing[57] in an electron microscopic study on unerupted teeth noted an organic connection in the form of submicroscopic fibrils between the ameloblast and the enamel cuticle. Actual verification on human teeth was not possible due to the loss of enamel matrix during decalcification.

Stern[58] described the periodontal ligament fibers in rats as being composed of subunits or fibrils, some less than 100Å in length with no periodicity. As they insert into the cementum, the periodontal fibrils are arranged perpendicular to the tooth surface. Stern indicates

that even when these fibrils run parallel or tangential to the cemental surface they turn before inserting and enter the cementum at approximately a right angle. Other angles of insertion were infrequently found.

Listgarten[3] studied the dentogingival junction in humans and found an attachment apparatus of epithelium to calcified structure consisting of hemidesmosomes and a basement lamina which connects the epithelium to the tooth or it cuticles. This attachment apparatus was remarkably similar to that seen at the junction of any epithelium-connective tissue interface. The basement lamina was measured at 400 to 1200Å with the average approximately 800Å consisting of a lamina densa and lamina lucida measuring approximately 400Å each. Listgarten identified two cuticles at the epithelial attachment. He named these cuticle A and cuticle B.

Schroeder and Theilade[59] found the mean thickness of attached gingiva at the level of the epithelial attachment to be approximately .15 to .3 mm. The basement lamina was found to be 340 to 570Å thick and separated from the cytoplasmic membrane of the epithelial basal cells by the lamina lucida, 240 to 430Å thick. Hemidesmosomal connections were found between the basal cells and the basal lamina.

Ito and coworkers[60] identified three electron dense layers between the enamel and epithelial attachment. The type I layer was the most electron dense and was found next to the epithelial cells. It measured .5 microns in width. This layer was homogenous and connected the

epithelial cells by hemidesmosomes. The type II layer was the middle layer, less electron dense and measuring approximately .1 to .2 microns wide. Type III layer was found adjacent to enamel. It appeared to be finely granular and measured 1 micron in width. Where the epithelial attachment occurred on cementum only the type I layer was observed. A zone 500Å in width appeared between the type I layer and the epithelial cells. The connections of the epithelial cells were via hemidesmosomes.

Schroeder[2] in a later study on humans measured the average thickness of the junctional epithelial cells to be 12 to 18 cells thick, and parallel to the tooth surface. Schroeder termed the epithelial-enamel junction the epithelial attachment lamina (EAL). The EAL was a complex of organic layers between the epithelial cell surface and the enamel. The EAL consisted of two structurally and histochemically different layers EAL-1 and EAL-2. The EAL-1 was found to always cover the surface of the epithelial cells and be continuous with the epithelial intercellular substance. EAL-1 was moderately electron dense, slightly fibrillar and measured 940-1540Å. The epithelial cells presented their hemidesmosomal connections to this surface. EAL-2 was interposed between EAL-1 and the enamel matrix. This structure was not always apparent. EAL-2 was electron dense, fibrillar, striated and measured 940 to 7130Å. Schroeder combined the afribillar cementum layer and the dental cuticle into this EAL-2 classification.

In order to clarify Listgarten, Ito, and Schroeder's classifications the following chart is presented:

Structure	Listgarten (1966)	Schroeder (1969)	Ito (1967)
Basement Lamina	Basement Lamina	EAL-1	clear zone
Afibrillar Cementum	Type A Cuticle		type II layer (superficial)
		EAL-2	type III layer (deep)
Dental Cuticle	Type B Cuticle		type I layer

Frank and coworkers[61] conducted an electron microscopic study of gingival reattachment after flap surgery. Their findings showed that the ultrastructure of the reformed epithelial attachment was similar to that observed prior to surgery. The epithelial cells were separated from the lamina propria by a distinct basement membrane consisting of a lamina lucida and lamina densa. Along the dentinal surface an extracellular space of 400-1200A containing an amorphous substance was noted. Hemidesmosomes face this extracellular space the entire length of the epithelial cell surface.

Taylor and Campbell[62] surgically detached the gingival epithelium from the tooth by inserting a special spatula. Healing utilizing both light and electron microscopes was studied. They noted well formed hemidesmosomal attachment of the apical 1/2 to 3/4 of the epithelium at 3 days. The 5 day specimen showed complete reattachment. Taylor and Campbell found the presence of a basal lamina with two strata comparable to the lamina lucida and the lamina densa. They suggest this basal lamina to be of epithelial origin because no underlying connective tissue is found in the region of the epithelial attachment.

D. Attachment, Inflammation, Epithelial and connective tissue changes.

1. Attachment

Gottlieb[63,64,4,65,66] proposed a concept for the development of the dentogingival junction in which he stated that after completion of enamel deposition, the inner enamel epithelium or ameloblasts produce a cuticle, the primary enamel cuticle. Following the production of this cuticle the ameloblasts were thought to degenerate and disappear. At the time of eruption the cells of the outer enamel epithelium come in contact with the primary enamel cuticle. Gottlieb felt that the cells of the outer enamel epithelium transformed to squamous epithelial cells and that they produced a keratinized layer which became structurally united with the primary enamel cuticle. This product of the squamous epithelial cells was termed the secondary enamel cuticle and it served as the origin of the epithelial attachment following the degeneration and loss of cellular layers of the developing dental organ.

Weski[68] stated shortly after Gottlieb's 1921 publications that the gingival sulcus represents an intraepithelial split. He called this split a retrocuticular fissure of the epithelium and stated that as the tooth erupts and pierces the oral epithelium the split appears in the epithelial layer. Weski felt that the majority of the epithelial cells kept their connection with the basal layer of the epithelium and only a few cells remain attached to the cuticle of the enamel.

Becks[69] in conducting a study of humans stated that "when the epithelium of the mouth becomes fused with the enamel epithelium and

the removal of necrotic cell remnants follows, the degeneration of the enamel epithelium progresses apically and the mouth epithelium proliferates downward to cover the defect in the surface...concurrently, the cuticula dentis is left intact on the surface of the teeth". "This means that the normal pocket is not formed between the cuticula dentis and the deeper layer of the enamel epithelium or by an intra-epithelial split, but between the surface of the mouth epithelium, which now represents a part of the pocket epithelium and the enamel epithelium, which is more or less degenerated. The bottom of the pocket proceeds apically by this progressive degeneration of the enamel epithelium." In the presence of injury or inflammation a deepening of the pocket also occurs.

Gottlieb[70] attempted to explain the continued apically growth of the epithelial attachment by the concept of cementopathia. The continuous deposition of new cementum inside the epithelial attachment forms the barrier against the apical growth of the epithelial attachment. When the cementum layer becomes calcified and ages, without a new layer being deposited on its surface, its effectiveness against apically growth ceases. Gottlieb explains such occurences as gingival recession, pocket formation, pathologic wandering of teeth, and passive eruption by the concept of cementopathia.

Aisenberg and Aisenberg[71] introduced a fourth concept for pocket formation. They state that epithelial projections migrate apically between the existing fiber bundles of the periodontal membrane before detachment of these fibers from cementum. The epithelial projections extend between and around the existing fiber bundles a short distance

away from cementum and in strands of varying thickness. The authors state that since proliferating epithelial projections are always observed where epithelial lined tissues are involved in the inflammatory process they should be considered normal extensions of the gingival epithelium.

Waerhaug[67] by inserting thin steel blades into the sulcus down to the cemento-enamel junction hypothesized that no epithelial attachment of an organic nature existed. Waerhaug felt the term epithelial cuff described the relationship of the gingival tissue to the enamel. There have been controversial results by various researchers trying to duplicate Waerhaug's findings. In any event the light microscope appears to be an incompetent tool for such judgements since the epithelial attachment is beyond the range of resolution of the light microscope.

Butcher[72] studied the surface structure of teeth from Rhesus monkeys following extraction and subsequent reimplantation. He observed that "a sulcus or crevice forms as an intraepithelial split in the enamel epithelium". The cause of the split was uncertain and the depth of the sulcus varied from shallow to deep. Butcher gave recognition to the existence of primary and secondary enamel cuticles. He found no cellular extensions of the secondary cuticle into the primary cuticle or enamel, though he did identify their existance between the primary cuticle and enamel. Butcher identified the secondary cuticle as the keratinized product of the superficial layer of cells belonging to the

enamel epithelium.

Toto and Sicher[73] studied the jaws of rats and mice as well as gingival specimen of rats, mice, and humans to determine the nature of the epithelial attachment. They found a neutral mucopolysaccharide at the basement membrane, intercellularly, and as a cuticle on the dental surface of the attached epithelium. This neutral mucopolysaccharide substance is elaborated by the epithelial cells and renewed as the epithelial cells undergo mitosis and renewal. It serves as an effective membrane between the epithelium and the tooth and acts as a cementing substance.

Wertheimer[74] utilizing various staining techniques attempted to determine the reactivity similarities between apical cuticles, secondary dental cuticles, and hyaline bodies. He found that although the derivation, function and composition of these three structures was in doubt there was a consistency in their reactivity to various stains and reactions employed. Wertheimer suggests that the epithelium most likely plays a role in the formation of these structures.

Loe[66] attempting to bolster the concept of Waehaug regarding the mode of attachment of epithelium to calcified tooth surface stated "the most convincing evidence against the existence of structural continuity and in favor of the concept of the dentogingival junctions as a contact relationship is derived from the study of the dynamic processes taking place in this area. The continuous loss at the surface and the renewal of the epithelium imply that the union between the surface cells and the enamel would have to be continously re-established irrespective of

whether the attachment is mediated by a secondary cuticle on in some other way." In summarizing his apologea on the morphology, chemistry and physiology of the dentogingival junction Loe further states that "following the atrophy and disappearance of the ameloblasts, the epithelium facing the tooth surface is not in structural continuity with it but is kept in close contact with it by the stickiness of the intercellular substance of the superficial cells and tonus exerted by the blood pressure and the connective tissue fibers of the marginal gingiva. This relationship is adequately expressed by the term epithelial cuff."

2. Inflammation, Epithelial and Connective Tissue Changes.

Goldman[75] in a study on humans of the changes in the pattern of the gingival fibers in the presence of disease or inflammation found notable architectural alterations. The gingival crestal fibers were replaced by dense inflammatory infiltrates. The inflammatory cells dispersed between the collagen bundles creating fragmentation. The fiber bundles were generally destroyed in midsection with the cemental portion remaining in tact for some period. The inflammatory cells, primarily identified as plasma cells and lymphocytes, eventually replaced the connective tissue in the corium permitting apical proliferation of the junctional epithelium with pocket formation as well as proliferation of the epithelial rete pegs into the connective tissue corium. Goldman found that epithelial migration ceased where connective tissue remnants connected to the cementum provided a barrier. Generally, with increases in the state of inflammation concomitant fiber

destruction and replacement by inflammatory cells occurred. In a subsequent publication Goldman[76] states that the transseptal fibers of the periodontal membrane provide a ligamentous-like barrier between adjacent teeth to prohibit extensive apical migration. In states of repair where portions of the transseptal fiber arrangement had previously been destroyed by inflammation the reformation of transseptal fiber groups are not regarded as new fiber group formations but as a union of previously existing periodontal membrane fibers.

Wassermann[77] conducted a study in Sprague Dawley rats which supported the thesis that connective tissue fibrillogenesis was a function of the fibroblasts. Primary fibrils rather than collagenous fibrils have an intimate developmental relationship with the fibroblast. The tight fitting mantle which surrounds the cells and the absence of well defined cell borders of the fibroblasts suggested to Wassermann that the primary fibrils along with other cytoplasmic components constituted a cortical zone of the cell where intercellular growth occurred followed by extracellular detachment into the ground substance. Fusion of these primary fibrils for the formation, growth and maturation of collagenous fibrils occurred within the ground substance.

Grant and Orban[78] suggest that the initial penetration of the bacterial toxins is via the epithelial attachment. They found in a periodontitis study, that the pocket epithelium became altered by an increase in size of intercellular spaces encouraging the ingress of the bacterial toxins and the concomitant egress of polymorphonuclear leukocytes as a

defense mechanism. Subsequent alterations in the subepithelial connective tissue occurred with the dense infiltration of plasma cells and lymphocytes. The connective tissue fiber bundles were destroyed which made imminent the apical proliferation of the epithelial attachment. The epithelium terminated where dense connective tissue fibers were still embedded into the cementum.

In a review of repair systems Ratcliff[79] offers three possible explanations as to why cementum, which has been pathologically exposed via apical migration of the epithelial attachment, fails to permit new attachment. He states that the molecular bonding adhesion potential of epithelial cells to the pathologically exposed cementum is reduced by the lack of organic components or collagen fibrils to reform a strong mucopolysaccharide bond. The increased mineral content or the lowered organic component of the exposed cementum may prohibit the new attachment. Secondly, Ratcliff suggests that proteolytic enzymes retained within the porous cementium after exposure to pocket microbial flora would lyse the mucopolysaccharides elaborated by the young proliferating epithelial cells thus preventing reattachment. Thirdly, he suggests the possibility of toxins which have penetrated the porous cementium initiating antigen-antibody reactions and thus interfering with healing and prohibiting attachment.

Stern[80] studied collagen solubility of human gingiva and found that in the presence of inflammation the degradation of pre-existing mature collagen fibers was accompanied by a shift in the percentage of collagen which was being synthesized and organized on a subfibrillar

level. Stern felt this might help explain the finding of collagen degradation and epithelial proliferation associated with gingival inflammation. Thus the increase in soluble collagen may be due to partial degradation of pre-existing insoluble collagen or due to an alteration in the maturation pattern of collagen synthesized in the presence of inflammation.

Fullmer and coworkers[81] found that pure epithelial cells in culture and variably inflammed gingival connective tissue free of epithelium were able to produce the enzyme collagenase in culture. Collagen is the predominant structural protein of the periodontal ligament, alveolar bone, and cementum; therefore, collagenase is capable of degrading most of the periodontal tissues. Fullmer suggests that collagenase may be responsible for the normal connective tissue turnover of collagen and the intensified destruction seen in periodontal disease.

Stanton and coworkers[82] in a study of collagen restoration during healing projected the time for complete collagen restoration at 49 days following wounding via gingivectomy. A productive phase of collagen repair was noted to last approximately two weeks and this proceeded the actual collagen reparative phase. Stanton and coworkers found that the level of collagen noted immediately after the removal of the inflammed tissue via gingivectomy was more than 50% greater than that found in 6 day specimens and slightly less at 14 days. Strong collagen recovery was noted at 21 days and 28 days although the 28 day specimen revealed collagen levels slightly less than the 0 day specimen.

Toto and Gargiulo[83] histologically studied the alteration of the epithelium and connective tissue in the presence of inflammation and noted that the inflammed gingiva had lost its acid mucopolysaccharide intercellular cementing substance as well as its desmosomes connections. Edema and polymorphonuclear leukocyte infiltrates were evident. The lamina propria contained thin walled capillaries, collagen fibers which had lost their acid mucopolysaccharide coating, and replacement of degraded collagen fibers by perivascular plasma cells. Alveolar bone loss occurred by both endosteal and periosteal resorption. Collagen fibers within the marrow spaces were noted to unravel and disappear. The neutral polysaccharide of the epithelial attachment was lost.

Ten Cate and Deporter[84] conducted an electron microscopic examination of fibroblasts within the periodontal ligament of functioning lower first molars of mice. The study revealed the presence of membrane-bound intracytoplasmic profiles containing banded collagen. The study suggests that the fibroblast serves as the cellular basis for both connective tissue turnover as well as remodeling and that the distinction between the two functions may not be great.

Grant and Bernick[85] utilizing thick sections to provide a three dimensional perspective, studied the nature of epithelial rest cells in miniature swine. They found that a continuum may exist between the cells of the reduced enamel epithelium and the epithelial rest cells of Malassez. This finding was best demonstrated in unerupted or newly erupted teeth. It was not discernable in older, functional, disease

free teeth may be due to the density of the connective tissue bundles or due to loss of continuity via cell degeneration. The authors found apically projecting proliferating cords from the epithelial attachment which "seemed to be continuous with the epithelial rest". They suggest that the confluence may be initially present, lost, then reestablished during inflammation and thus may be a factor in the apical progression of the epithelial attachment and subsequent pocket formation.

Polson, Kennedy, and Zander[86] created periodontitis in squirrel monkeys and subjected them to thermal injury. They noted that at 6 months the junctional epithelium was apically positioned on the dementum, a cell-rich collagen-poor connective tissue area existed beneath the epithelium and that loss of alveolar bone was still eivdent. Recovery had not occurred.

Polson[87] utilized the same ligation technique to induce periodontitis in squirrel monkeys and then subject them to mechanical trauma. The mechanical trauma created by a wooden wedge driven between two teeth produced an area of necrosis which was found immediately beneath the cell-rich collagen-poor area caused by the marginal periodontitis subjacent to the junctional epithelium. The lesion extended from the level of the alveolar crest to the upper half of the periodontal ligament. At three weeks the necrotic area was replaced by a highly cellular loosely arranged connective tissue. The periodontal ligament space had increased dimensionally through alveolar bone resorption. At 8 weeks new bone formation was evident. The width of periodontal ligament, cellularity and orientation of fibers was similar in nature to the periodontal ligament

of a tooth which had not undergone trauma but had an existing periodontitis for the same duration.

Listgarten[88] found epithelial cell rests in approximately 20% of sections from albino mice jaw block sections. The rest cells were located in the coronal half of the periodontal ligament in a 3:1 ratio to the apical half. The rest cells were invariable in close proximity to the cemental surface. The cells possessed hemidesmosomes and a basal lamina to the surrounding connective tissues as would be expected of typical epithelial-connective tissue junctions. The individual cells possessed desmosomal, gap and tight junctions along their borders with adjacent epithelial cells.

Attstrom and coworkers[89] found that in clinically healthy beagle dogs the normal gingival tissues possesses small numbers of isolated inflammatory cells beneath and within the junctional epithelium. The transmigration of leukocytes was a constant finding which persists independent of the presence or absence of an inflammatory infiltrate. These findings are fairly consistent with the histologic picture of gingiva in the clinically healthy human, where the connective tissue adjacent to the base of the gingival sulcus always shows some sign of chronic inflammatory cell infiltration.[90,91]

Schroeder[92] noted a remarkably rapid and complete breakdown of collagen in beagle dogs with the development of acute exudation and inflammatory cellular infiltrates. The collagen loss was 60% in some areas. The pathologic mechanism responsible for this rapid breakdown is thought to be of an enzyme nature which works through the influx of

hydrolytic substances from plague growing in the sulcus or through enzymes released from host cellular elements.

Aleo and coworkers[93] found that roots of extracted periodontally involved teeth which had been treated with 45% phenol in H_2O at 60°C for 1 hour then washed with 70% ethanol when placed with cultured human gingival fibroblasts displayed reattachment. Likewise extracted periodontally involved teeth which had their cementum mechanically removed also demonstrated reattachment in a fibroblast culture. The phenol and curettement apparently remove the lipopolysaccharide, endotoxin, which becomes embedded in the porous cementum and serves to prevent attachment.

Novaes and coworkers[94] in a discussion of the development of periodontal clefts note that in the presence of the constant inflammation that exists in the human gingiva various resorptive and proliferative reactions occur. The inflammatory exudates spread apically through the gingival connective tissues but also laterally toward the outer aspect of the gingiva and alveolar mucosa. Collagen and matrical resorption are mediated via hydrolytic enzyme activity. As the connective tissue is destroyed, the pocket epithelium proliferates and migrates to fill the voids created by the loss of the connective tissue. Eventually, anastomosis occurs between the pocket and gingival epithelium as the intervening connective tissue is lost. This process though relatively slow can lead to cleft formation and gingival recession.

Grant and coworkers[12] utilizing marmosets as a model studied the leukocyte migration through the junctional epithelium, the proliferation

of the junctional epithelium and sulcular epithelium, the area and density of inflammatory cell concentrations in the gingival corium, vascular proliferation, the area of collagen fiber alteration and loss, and the amount of alveolar bone loss. They found no correlation between the alveolar bone loss and the other parameters.

CHAPTER III

MATERIAL AND METHODS

Patients whose teeth were condemed for periodontal or prosthetic reasons were invited to volunteer for this study. The experimental procedures were limited to teeth with apparently healthy gingival tissues or to areas with shallow pockets. An effort was made to select teeth that had extruded or where alveolectomy was prescribed, in order to avoid creating deformities in denture bearing tissues.

Four adult patients, two male and two females, ranging in age from 42 to 63 years were selected. A thorough examination of the periodontium was performed on each patient, and each patient was determined by medical history and examination to be in sound clinical health. Five specimens obtained from these four patients were utilized in this study. The specimens consisted of three mandibular molar teeth, one maxillary canine and one mandibular canine.

The experimental surgeries performed in this study attempted to duplicate specific clinical periodontal surgical procedures. In each case the procedure was modified by making grooves or notches as landmarks in the tooth surface to assist in histologic interpretation. Appropriately these grooves recorded 1) the presurgical level of the gingival margin, 2) the presurgical depth of the sulcus or pocket, 3) the level of the alveolar crest upon the reflection of a gingivomucoperiosteal flap, and

4) the post surgical level of the alveolar crest where osteoectomy was performed (See diagrams 1 and 2).

The following surgical procedures were studied:

1) Apically positioned flaps: full thickness mucoperiosteal flaps were raised and repositioned apically to cover the alveolar crestal bone.
2) Apically positioned flaps with osteoectomy: full thickness mucoperiosteal flaps were raised, alveolar crestal bone was removed with a surgical curette, and the flap was repositioned apically to cover the alveolar crestal bone.

For each procedure a horizontal notch was made with a diamond stone on the labial surface of the experimental tooth at the level of the gingival crest. Measurements were recorded from the gingival crest to the bottom of the gingival crevice or pocket utilizing a dull periodontal probe prior to the administration of anesthesia. Upon reflection of the full thickness mucoperiosteal flap a second horizontal notch was made with a diamond stone on the labial surface of the tooth indicating the bottom of the gingival crevice previously recorded. For surgical procedure 1, the surgical curette marked the height of the alveolar crest, and in surgical procedure 2, the surgical curette was used to mark the height of the alveolar crest, to remove approximately 1 mm of alveolar bone in a vertical dimension and then to mark the new height of the alveolar crest. In each procedure interrupted 4-0 black silk sutures were utilized to reposition the gingival margin to cover the

alveolar bone. Orban's surgical periodontal pack was placed to protect the wound and removed at one week. No instructions were given and no attention was paid to the patient's oral hygiene.

Block sections were removed at 60, 90, and 120 days postoperatively utilizing a technic described by Kohler and Ramfjord.[8] During the section removal, care was taken to include the labial gingiva, underlying alveolar process, periodontal ligament, and the tooth as an intact unit. In order to avoid damage on block removal the vertical incisions for the experimental area were moved mesially and distally to include the adjacent teeth where possible.

The specimens were prepared for microscopic observation by fixing in 10% formalin, dehydrated, embedded in celloidin, decalcified in nitric acid-formalin, and processed for nitro-cellulose embedding in the routine manner. The specimens were sectioned at 10-15 micron intervals for microscopic morphometric measurements and stained with hematoxylin and eosin and Mallory's connective tissue stain.

A 10 mm square grid was calibrated at 10x magnification for morphometric measurements. A conversion factor of .029 was used to convert units to millimeters. To provide uniformity and standardization one observer utilizing the same microscope recorded all measurements.

No attempt was made to access the severity of inflammation by stereometric cell count using the Weibel method[9,10] as proposed by Schroeder[11] because it was found by Grant[12] that the principle of randomnicity upon which the Weibel technique is based was in fact lacking. Grant determined that the inflammatory process was focal, directional, and

chronologically sequential. In addition the chronological sequence of inflammation may differ between different specimens. Severity of inflammation was instead related to the distribution of the round cells present and the evidence of collagen loss. Zachrisson[13], Oliver, Holm-Pedersen and Loe[14], and Angelopoulos[15] have presented methods of accessing round cell inflammatory infiltrates. Each method makes a totally subjective analysis of the specimens studied. An objective analysis of round cell infiltrates though desirable is difficult to achieve. In this study the severity of inflammation was evaluated as mild, moderate, or severe depending upon the concentration of round cell infiltrates seen on each specimen and the corresponding appearance of collagen loss. Although recognition is given to the fact that the degree of collagen loss will generally vary directly with the quantity of inflammatory cells present, no definitive statement can be made in accessing areas that appear to be cell poor and collagen poor, as compared to areas which are cell rich and collager poor, and as reflections of the status of inflammation. Mild inflammation is characterized by sparse distribution of inflammatory cells, generally seen perivascularly, with little collagen loss evident. Moderate inflammation is characterized by moderately dense accummulations of inflammatory cells in isolated areas with sparse distribution noted elsewhere. Areas of collagen dissolution are evident although some normal fiber organization is present. Severe inflammation is characterized by dense aggregation of inflammatory cells throughout the area. Extensive areas of collagen loss are evident, marked by large areas of dissolution, no organization nor functional orientation of

existing fibers.

In order to more accurately access the status of inflammation in various sections of the same specimen, the dentogingival junction was compartmentalized into three zones and individual accessments of inflammation based on the previously stated criteria were made. Zone I (see diagram 3) is bordered apically by the apical extent of the functional epithelium, coronally by the tooth and the outer oral epithelium, and buccal-lingually it comprises the inner one-third of the gingival corium. The apically border for zone 2 is determined by dividing the connective tissue attachment in half as measured from the apical extent of the junctional epithelium to the alveolar crest. The coronal and lateral borders for zone 2 are the apical border of zone I, the outer oral epithelium, the tooth, and the middle one-third of the gingival corium, respectively. Zone 3 is bounded by the apically border of zone 2 coronally, the alveolar crest apically and the outer oral epithelium, the tooth, and outer one-third of the gingival corium, laterally. The zones correspond to the spread of inflammation as observed in microscopic specimens by Grant. Correlations between the severity of inflammation and variations in the morphometric analysis will be made.

For each of the five specimens utilized in this study, mean values and ranges in millimeters will be determined for the sulcus depth, epithelial attachment, connective tissue attachment, bone removed and bone resorbed. Additionally the standard deviation will be determined for the epithelial and connective tissue attachment. The data will be compared to the values obtained by Gargiulo and coworkers to determine if the

mean values are within the range found by Gargiulo and if a significant correlation exists between the presurgical and postsurgical dimensions of the dentogingival junction.

Furthermore an accessment of the degree of inflammation in the postsurgical specimens will be made and correlated by the specimen age and surgical procedure to the histologic observations of the healing process.

CHAPTER IV

OBSERVATIONS

A. Morphometric Analysis:

A total of five cases were utilized in this study. Cases 1 and 2, consisting of 24 and 19 sections respectively, present the healing response following full thickness flap reflection and replacement with no osteoectomy performed. Cases 3, 4, and 5, consisting of 21, 13, and 21 sections respectively, present the healing response following full thickness flap reflection and replacement with osteoectomy of the alveolar crestal bone performed. Mean values are presented for the four or five parameters investigated depending upon the particular surgical procedure. Standard deviations are presented for the epithelial and connective tissue attachments values only. A dimensional analysis of the attachment apparatus of the dentogingival junction is of primary concern. Mean values will be compared to the ranges presented in Gargiulo's phase IV analysis (Table 1).

1. Case 1: Female patient, age 53, mandibular right first molar. The section was removed at 90 days. Table 2 provides the mean values for the sulcus depth (A-B), epithelial attachment (B-C), connective tissue attachment (C-D), and bone resorption (E-D). No osteoectomy was performed.

2. Case 2: Female patient, age 42, mandibular right first molar. The section was removed at 120 days. Table 3 provides the mean value determinations. No osteoectomy was performed.
3. Case 3: Male patient, age 50, mandibular left first molar. The section was removed at 60 days. Table 4 presents the mean value determinations. Osteoectomy was performed and is represented by symbol E-F. Bone resorption is represented by symbol F-D.
4. Case 4: Male patient, age 63, mandibular right cuspid. The section was removed at 120 days. Osteoectomy was performed. Table 5 provides the data.
5. Case 5: Same patient as in case 4. Maxillary right cuspid. The section was removed at 120 days. Osteoectomy was performed Table 6 provides the data.

Table 7 represents a compilation of the data from the five cases and provides a comparison of the mean average values to the mean average values of Gargiulo's phase IV analysis.

B. Assessment of Inflammation

Table 8 presents a compilation of the total number of sections in each case for which assessments of inflammation based upon the compartmentalization of the gingiva have been made. Combinations of various inflammatory patterns, when not observed, have been excluded from the table. Table 9 presents the frequency by percentage in which severe, moderate, or mild inflammation is found in compartments 1,2, and 3

respectively.

C. Histologic Observation

Marked inflammation was evident in the great majority of sections studied. Epithelial proliferation, connective tissue dissolution with inflammatory cell infiltration, edema and vascular dilation and osteoblastic and osteoclastic activity of the alveolar crestal bone were generally uniform observations. The more specific histologic observations are indicated below as an analysis of figures 1 through 14.

Figure 1: 90 day section. No osteoectomy was performed. Excessive vascularity of the gingival crest is noted. In some instances the gingival vessels are only one or two cell layers removed from the gingival sulcus. Calculus formation is evident in the surgically created notch. The epithelial attachment was torn in sectioning. The portion of compartments 1 and 2, which are shown, are severely inflammed.

Figure 2: 90 day section. No osteoectomy was performed. The osteoblastic response at the alveolar crest is noted with young osteophytic bone and osteoid formation. Periodontal ligament fibers appear to be functionally oriented though still immature as noted by the excessive cellularity. Reversal lines mark the areas from which new bone formation occurred. Epithelial rests are noted near the cementum surface.

Figure 3: 120 day section. No osteoectomy was performed. Osteoblastic activity is evident on the periodontal ligament side of the

alveolar bone. Osteoblasts line the bone surface on this side. Crestal alveolar bone regeneration is apparent. The bone is a highly fibrous woven bone. Cementoid deposition is evident in the surgically created notch. Cemental fragments are surrounded by dense connective tissue. Some cementoid or osteoid deposition is evident. Periodontal ligament fibers are functionally oriented. Reversal lines mark the amount of osseous regeneration. Some osteoclastic activity is still evident on the periosteal surface of the alveolar bone.

Figure 4: 120 day section. No osteoectomy was performed. Osteoblastic activity evident on periodontal ligament side of the alveolar bone. Crestal alveolar bone regeneration is marked by reversal line. Periodontal ligament fibers are seen radiating from the alveolar bone and are functionally oriented. Dento-alveolar (dento-periosteal) periodental ligament fibers are noted. Cementoid deposition is evident in the surgically created notch as are cemental fragments with osteoid or cementoid deposition. Due to the vascularity in the periodontal ligament space it is difficult to trace the entire length of the horizontal fibers. Mild inflammation is noted in compartment three.

Figure 5: 120 day section. No osteoectomy was performed. The section shows severe inflammation in compartment 1 with an extensive

collagen free area. This suggests that the loss of the collagen fiber barrier is concomitant with the apical proliferation and elongation of the junctional epithelium. Marked vascularity of the gingival crest is noted as is the thickness of the outer oral epithelium with elongated epithelial proliferations into the inflammed lamina propria. Dense surface keratinization is evident. The remaining dentogingival fibers appear to have some functional orientation. The inflammatory state in compartment 2 is classified as severe. Plaque and calculus formation are apparent in abundance.

Figure 6: 120 day section. No osteoectomy was performed. The junctional epithelium ends where dense dentogingival fibers are embedded in cementum. Compartments 1 and 2 show inflammation.

Figure 7: 120 day section. No osteoectomy was performed. An example is seen of severe, severe, and mild inflammation in compartments 1, 2, and 3 respectively. Very long junctional epithelium is noted.

Figure 8: 120 day section. No osteoectomy was performed. Section depicts extensive vascularity at gingival crest. Severe inflammation in compartments 1 and 2, with little or no evidence of collagen remnants. Confluence of junctional epithelium with a proliferating strand from the outer oral epithelium. The gingival margin is not keratinized. A vacuolated cellular population is seen. Elongation of the epithelial

attachment is evident. There appears to be an attachment of the sulcular epithelium to calculus.

Figure 9: 120 day section. Osteoectomy was performed. Proliferation of the junctional epithelium into the collagen poor zone of the gingiva corium is seen. The epithelial attachment terminates at the coronal border of the second surgical notch. Elongated epithelial rete pegs and general thickening of the outer oral epithelium is noted. The surface epithelium is heavily keratinized. The junctional epithelium is difficult to trace. There appears to be an interruption of the attachment by connective tissue fibers attached to the cementum coronally to the first surgical notch. The status of the inflammation in compartments 1 and 2 is classified as moderate and moderate, respectively.

Figure 10: 60 day section. Osteoectomy was performed. Repair of dentogingival fibers is noted. Functional orientation of periodontal ligament fibers is noted. Cemental fragment is seen, however, cementoid formation is not apparent in this section. Minimal osteoid formation is noted on the periodontal ligament side of the alveolar crest while osteoclastic activity is apparent on the periosteal surface. Scattered inflammatory cells are noted. The gingival collagen fibers do not appear functionally oriented. The inflammation in compartment 3 is classified as mild.

Figure 11: 60 day section. Osteoectomy was performed. Alveolar crest appears flat or almost concave with active osteoclastic activity noted. Osteoblasts are lining the periodontal ligament surface of the alveolar bone. Osteoid is apparent though not abundantly. The periodontal surface of the alveolar bone shows minimal new bone formation. Vascular channel extends from the gingival corium into the periodontal ligament space. Periodontal ligament fibers are interrupted by vascular channels.

Figure 12: 120 day section. Osteoectomy was performed. Osteoblasts are in evidence lining the periodontal ligament side of the alveolar bone. Osteoid formation and new bone formation are evident. The periosteal surface reveals resorptive bays undergoing active osteoclasia. The periodontal ligament fibers appear highly cellular and functionally oriented. Reversal lines outline the area of osseous regeneration on the periodontal ligament surface of the alveolar bone. Osteoid and osteoblastic activity are apparent in the endosteal marrow space.

Figure 13: 120 day section. Osteoectomy was performed. An example of severe, moderate, and mild inflammation in compartments 1,2, and 3, respectively, is noted. Sequestered cemental fragments can be seen. The extent of the osteoblastic activity on the periodontal ligament side of the alveolar bone can be

discerned. Heavy keratinization and numerous collagen poor bones are evident.

Figure 14: 120 day section. Osteoectomy was performed. Endosteal bone apposition is noted within the marrow spaces of the alveolar bone. Osteoblastic activity is apparent on the periodontal ligament side. Osteoclastic activity is evident on the periosteal surface. Epithelial rest cells are noted in the periodontal ligament space.

CHAPTER V

DISCUSSION

The results of the morphometric analysis of this study provide for some interesting comparisons with the work of Gargiulo. Statistical correlations cannot be drawn between the dimensional variations of the dentogingival junction as they appear in the normal healthy adult and as they appear postsurgically in the adult periodontal patient. Comparisons, however, can be made between the morphometric relationships of the dentogingival junction as they appear in phase IV of passive tooth exposure (eruption)(table 1) and the postsurgical specimens which upon healing resemble phase IV of passive tooth exposure in that in both instances the epithelial and connective tissue attachments of the dentogingival junction are located apical to the cemento-enamel-junction.

The mean values for the sulcus depth, epithelial and connective attachments as determined in this study fall within the range of values provided in table 1. The depth of the sulcus in postsurgical specimens is subject to variation. The range of values noted in the five cases presented is .182 to .695 mm. This approximately one-half millimeter difference can be accounted for as a normal variation due to flap replacement, suturing techniques, and pack placement procedures. Further, some shrinkage may have occurred due to formalin fixation and loss of fluid in the treatment with ascending alcohol solutions ranging from 70% to 100%.

A point of interest, however, is the sulcus depth values noted in cases 4 and 5 as a possible reflection of the status of inflammation. A moderate inflammatory state was found in compartment 1 in approximately 77% of the sections for case 4, and in approximately 90% of the sections in case 5. These values compare to 21%, 0%, and 0% for cases 1, 2, and 3 respectively.

Cases 1 and 2 had no osseous surgery performed. The connective tissue attachments compare favorably between 90 and 120 days. There was an almost total regeneration of the resorbed crestal alveolar bone between this time interval. This finding is supported by various researchers[31,34,45], however; other researchers[28,29,23,8,44,47,51] have found that full thickness flap reflection resulted in a more significant amount of permanent alveolar crestal bone loss. The epithelial attachment in case 1 is approximately .5 mm greater in length than in case 2. The inflammation noted in compartments 1 and 2 of case 2 was severe in approximately 85% of the sections observed. The inflammation noted in compartment 1 of case 1 was severe in 80% of the sections; however, moderate or mild inflammation was observed 100% of the time in compartment 2 of case 1. It may well be that the severity of the inflammation in compartment 2 of case 2 has permitted the elongation of the epithelial attachment to occur,[75,78] however, if such were the case, it does not appear that the epithelial attachment elongation has been at the expense of the connective tissue attachment. More likely the variations of a comparative nature between separate specimens are normal. This assumption would be supported by the range variations provided in the phase IV analysis (table 1).

Cases 3, 4, and 5 have all had osteoectomy performed. The time interval between 60 and 120 days appears to be an active period. Morphometrically, minimal alveolar crestal bone resorption appears to have occurred by 60 days; however, between 60 and 120 days marked resorption of the alveolar crestal bone has occurred. This falls within Wilderman's functional repair phase[23] which lasted between 28 and 185 days. Wilderman's 1970[52] study revealed an average alveolar crestal bone loss of .8 mm in human subjects studied up to 6 months. The greatest bone loss occurred on teeth with initially thin roots. The findings of .649 and .813 mm bone loss in cases 4 and 5, respectively, is consistent with Wilderman's findings. The teeth surgerized in cases 4 and 5 were canine teeth which due to their natural prominence in the dental arch would be expected to have a thin labial plate of alveolar bone and which thus would be expected to have an increased amount of postsurgical resorption.

The connective tissue component for both the osseous and non-osseous cases demonstrated noteworthy standardization and showed a significant correlation to the values shown in the phase IV analysis. The epithelial component of the sulcular and junctional epithelium showed less standardization and was subject to more variation. The collective values of the epithelial and connective tissue components provide a dimensional consistency to the dentogingival junction which lends to an accurate assessment of postsurgical healing.

The fact that no attention was given to the state of the patient's oral hygiene in this study is a factor which must be considered in

assessing the observations. It is well known that histologic specimens of clinically healthy human gingiva show some degree of chronic inflammation subjacent to the junctional epithelium and gingival sulcus. The severe states of inflammation may certainly have affected our demensional findings; however, it can be said that in the presence of severe inflammation the dentogingival junction postsurgically assumes a functional morphometric relationship which appears consistent with the phase IV analysis of the disease free human.

Histologic observations reveal cementoid formation in the 90 and 120 days sections; however, this observation was not apparent in the 60 day sections. Although it is generally agreed upon that cementoid formation precedes cementum maturation, various studies[23,39,52,95] have revealed a wide chronological variation for initial cementoid formation. Osteoid formation was evident in the 60, 90 and 120 day sections. This observation is consistent with studies previously reported. As mentioned, the period between 60 and 120 days appears to be active from a morphometric standpoint. Histologically, the 60 day section (Figure 11) reveals a flattened, almost concave crestal arrangement of the alveolar bone. Little osteoid formation is evident on the periodontal ligament side of the alveolar bone. Although osteoblasts are evident; conversely, on the periosteal surface marked osteoclastic activity is noticeable. The 120 day sections (Figures 12 and 13) in contradistinction show the crestal alveolar bone to be undergoing osteoblastic and osteoclastic remodeling in an attempt to reestablish the rounded physiologic contouring which existed initially.

For the osseous and non-osseous surgical cases observed, a functional orientation of periodontal ligament fibers appeared initially at 60 and 90 days, respectively (Figures 10 and 2). It can be hypothesized that had a 60 day section of a nonosseous surgical case been prepared and studied, the fiber orientation of the periodontal ligament would also have been functionally oriented. This finding is contrary to the findings of Morris[97] and Wilderman[7].

Possible mechanisms for gingival recession are evident in figures 8 and 9. Figure 9 reveals a moderately inflammed gingival corium which has a broad collagen poor zone. The junctional epithelium has proliferated into the gingival corium and is subjacent to the outer oral epithelium. Confluence of these epithelial structures though not evident in this figure may likely have occurred in subsequent sections. Figure 8 reveals a cell rich collagen poor gingival corium in which confluence of the outer oral and the junctional epithelium has occurred. As mentioned by Novaes[94] this lateral spread of the epithelium with subsequent anastomosis may lead to gingival cleft formation or gingival recession. The anastomosis of the outer oral and junctional epithelium may serve to interdict the vascularity to portions of the gingival crestal and sulcular tissues thus leading to cell death and eventual loss of these cells into the gingival sulcus.

The degree of surface keratinization evident in figures depicting the gingival tissues is of interest in that the tissues show heavy keratinization in the presence of severe and moderate inflammation. Previous

studies [98] [52] have revealed that a reduction in gingival keratinization is associated with increasing gingival inflammation.

The morphometric findings in this study provide insight into the nature of the healed periodontal tissues following surgical intervention. The data shows that when mucoperiosteal flaps are reflected osseous changes via osteoclastic activity will occur in the crestal alveolar bone. The data further shows that the destructive techniques, currently employed in an attempt to eliminate pathologic pockets and reestablish the parabolic osseous contours, which existed in the disease free adult, are not predictable procedures. It is evident that extensive resorption occurs to the crestal alveolar tissues following osteoectomy procedures and that a definite crestal deformity results from these procedures. The morphometric data reveals that a definite and rather predictable dimensional relationship exists between the tissues of the dentogingival junction. Following periodontal surgery, apparently regardless of the status of the gingival inflammation, the connective tissue attachment assumes a definite dimensional state. Variations in the epithelial attachment are apparent and as stated previously this component of the dentogingival junction is subject to greater variations. The question of the mechanism of the reattachment of the tissues of the dentogingival junction to the cementum is often raised. Whether or not a true connective tissue attachment occurs or whether the attachment is solely via an elongated epithelial attachment is often questioned. Although greater variation is seen in the epithelial component, in no instances did an increased epithelial attachment dimension

appear to occur at the expense of the connective tissue attachment. The data suggests that an accurate assessment of the height of the alveolar bone in the healed surgerized periodontal patient can be made by applying the dimensional values for the dentogingival junction obtained in this study to an accurate determination of the bottom of the gingival sulcus. These data have application in the presurgerized periodontal patient for projection of the consequences of traumatic osseous surgery can be made.

Although subsequent studies of this nature would be desirable to establish the possible sequential alterations of the tissues of the dentogingival junction over a 15 to 180 day period, for example, the inherent difficulties of working with humans makes the likelihood of such an undertaking doubtful.

CHAPTER VI

CONCLUSIONS

1. Full thickness mucoperiosteal flap reflection with osteoectomy results in additional loss of crestal alveolar bone by postsurgical osteoclastic resorption between 60 and 120 days. Healing of the alveolar crest with deformity is observed.
2. Dimensional similarities are apparent although statistical correlations of the dimensions of the dentogingival junction cannot be made between disease free adults and the postsurgical results of the adult periodontal patients.
3. The morphometric analysis of the dentogingival junction in postperiodontal surgery cases reveals noteworthy standardization of the connective tissue component and shows significant correlation to the values in phase IV analysis of passive tooth exposure (passive eruption).
4. Despite the presence of severe and moderate inflammation the connective tissue component of the dentogingival junction appears to heal postsurgically in a predictable manner.
5. The epithelial component of the dentogingival junction showed less standardization and was subject to more dimensional variation than the connective tissue component.
6. Full thickness mucoperiosteal flap reflection without osteoectomy results in crestal alveolar bone alterations via osteoclastic activity

although significant recovery is noted by 120 days.

7. More meaningful assessment and description of the pattern of inflammation and its progression is achieved by the compartmentalization method utilized in this study.

CHAPTER VII

SUMMARY

A study was undertaken to microscopically determine the morphometric dimensions of the dentogingival junction following various periodontal surgical modalities.

Four adult periodontal patients served as the experimental models. Two patients providing two block sections had full thickness mucoperiosteal flaps reflected and replaced to cover the alveolar bone. Two other patients providing three block sections had full thickness mucoperiosteal flaps reflected, osteoectomy performed, and the flaps replaced to cover the alveolar bone. The block sections were obtained at 90 and 120 days for the non-osteoectomy cases, and 60 and 120 days for the osteoectomy cases. The sections were embedded in celloidin, decalcified, stained with H & E and sectioned at 10-15 microns providing a total of 98 specimens for morphometric analysis. Presurgical measurements of pocket depth were obtained and surgical landmarks were made to mark the presurgical height of the gingival crest, the bottom of the gingival sulcus, the height of the alveolar crest, and the new height of the alveolar crest following ostectomy. No attention was paid to the patient's oral hygiene in this study.

The results show that following periodontal flap surgery morphometric analysis reveals the dimensional variations of the components of

the dentogingival junction to fall within the range of values revealed in Gargiulo's 1961 analysis of disease free humans. The connective tissue component of the dentogingival junction shows noteworthy standardization and appears to correlate significantly to the values revealed in the phase IV analysis of passive eruption. The epithelial component of the dentogingival junction showed less standardization and was subject to greater variation.

A method of assessing the status of inflammation by compartmentalization of the gingival tissues was proposed.

REFERENCES

1. Gargiulo, A.W., Wentz, F.M., and Orban, B.: Dimensions and Relations of the Dentogingival Junction in Humans. J. Periodont., 32:261, 1961.

2. Schroeder, H.E.: Ultrastructure of the Junctional Epithelium of the Human Gingiva. Helv. Odont. Acta., 13:65, 1969.

3. Listgarten, M.A.: Electron Microscopic Study of the Gingivo-Dental Junction of Man. Am. J. Anat., 119:147, 1966.

4. Schroeder, H.E. and Listgarten, M.A.: Fine Structure of the Developing Epithelial Attachment of Human Teeth. In Vol. 2, Monographs in Developmental Biology, New York: S. Karger, 1971.

5. Morris, M.: Healing of Human Periodontal Tissues Following Surgical Detachment From Vital Teeth: The Position of the Epithelial Attachment. J. Periodont., 32:108, 1961.

6. Marfino, N., Orban, B., and Wentz, F.M: Repair of the Dentogingival Junction Following Surgical Intervention. J. Periodont., 30:180, 1959.

7. Wilderman, M., Pennel, K., King, K., and Barron, J.: Histogenesis of Repair Following Osseous Surgery. J. Periodont., 41:551, 1970.

8. Kohler, C.A. and Ramfjord, S.P.: Healing of Gingival Mucoperiosteal Flaps. Oral Surg., Oral Med., and Oral Path., 13:89, 1960.

9. Weibel, E.R., Kistler, G.S., and Scherle, W.F.: Practical Stereological Methods for Morphometric Cytology. J. Cell Biology, 30:23, 1966.

10. Weibel, E.R.: Stereological Principles for Morphometry in Electron Microscopic Cytology. Int. Rev. Cytol., 26:235, 1969.

11. Schroeder, H.E. and Munzel-Pedrazzdi, S.: Morphometric Analysis Comparing Junctional and Oral Epithelium of Normal Human Gingiva. Helv. Odont. Acta., 14:53, 1970.

12. Grant, D., Bernick, B.M., and Levy, B.M.: Histopathologic Changes in Osseous, Collagenous, Vascular and Epithelial Compartments in Gingival Inflammation. Abstract 786 Speical Issue B,; J. Dent. Res., 55:258, 1976.

13. Zachrisson, B.: A Histological Study of Experimental Gingivitis in Man. J. Perio. Res., 3:293, 1968.

14. Oliver, R.C., Holm-Pederen, P., and Loe, H.: The Correlation between Clinical Scoring, Exudate Measurements and Mircoscopic Evaluation of Inflammation in the Gingiva. J. Periodont., 46:201, 1969.

15. Angelopoulos, A.P.: Studies of Mast Cells in the Human Gingiva, II, Topographical Distribution. J. Perio. Res., 8:314, 1973.

16. Skillen, W.S. and Lundquist, G.R.: An Experimental Study of Peridental Membrane Reattachment in Healthy and Pathologic Tissues. J.A.D.A., 24:175, 1937.

17. Beube, F.E.: A Study on Reattachment of the Supporting Structures of the Teeth. J. Periodont., 18:55, 1947.

18. Bordon, S.M.: Histological Study of Healing Following Detachment of Tissue as is Commonly Carried Out in the Vertical Incision For the Surgical Removal of Teeth. J. Can. D.A., 14:510, 1948.

19. Beube, F.E.: The Problem of Reattachment. J. Periodont., 31:310, 1960.

20. Linghorne, W.J. and O'Connell, D.C.: Studies in the Regeneration and Reattachment of the Supporting Structures of the Teeth. I. Soft Tissue Reattachment. J. Dent. Res., 29:419, 1950.

21. Linghorne, W.J. and O'Connell, D.C.: Studies in the Regeneration and Reattachment of the Supporting Structure of the Teeth. II. Regeneration of Alveolar Process. J. Dent. Res., 30:604, 1951.

22. Linghorne, W.J.: Studies in the Reattachment and Regeneration of The Supporting Structures of the Teeth. IV. Regeneration in Epithelized Pockets Following the Organization of a Blood Clot. J. Dent. Res., 36:4, 1957.

23. Wilderman, M., Wentz, F., and Orban, B.: Histogenesis of Repair After Mucogingival Surgery. J. Periodont., 31:283, 1960.

24. West, T.L. and Bloom, A.: A Histologic Study of Wound Healing Following Mucogingival Surgery. (Abstract). J. Dent. Res., 40:675, 1961.

25. Staffileno, H., Wentz, F., and Orban, B.: Histologic Study of Healing of Split Thickness Flap Surgery in Dogs. J. Periodont., 33:56, 1962.

26. Wilderman, M.N.: Repair After a Periosteal Retention Procedure. J. Periodont., 34:487, 1963.

27. Lobene, R., and Glickman, I.: The Response of Alveolar Bone to Grinding with Rotary Diamond Stones. J. Periodont., 34:105, 1963.

28. Carranza, F.A., and Carraro, J.J.: Effect of Removal of Periosteum on Postoperative Results of Mucogingival Surgery. J. Periodont. 34:223, 1963.

29. Glickman, I., Smulow, J.B., O'Brien, T., and Tannen, R.: Healing of the Periodontium Following Mucogingival Surgery. Oral Surg., Oral Med., and Oral Path., 16:530, 1963.

30. Staffileno, H., Levy, S., and Gargiulo, A.: Histologic Study of Cellular Mobilization and Repair Following a Periosteal Retention Operation Via Split Thickness Mucogingival Flap Surgery. J. Periodont., 37:117, 1966.

31. Hiatt, W.H., Stallard, R.E., Butler, E.D., and Badgett, B.: Repair Following Mucoperiosteal Flap Surgery With Full Lingual Retention. J. Periodont., 39:11, 1968.

32. Caffesse, R.G., Ramfjord, S.P., Nasjleti, C.E.: Reverse Bevel Periodontal Flaps in Monkeys. J. Periodont., 39:219, 1968.

33. Henning, F.R.: Epithelial Mitotic Activity After Gingivectomy. J. Perio. Res., 4:319, 1969.

34. Kon, S., Novaes, A.B., Ruben, M.P., and Goldman, H.M.: Visualization of the Microvasculature of the Healing Periodontal Wound. IV. Mucogingival Surgery: Full Thickness Flap. J. Periodont., 40:441, 1969.

35. Noyes, F.B.: A Textbook of Dental Histology and Embryology: Philadelphia, Lea and Febiger, 1912.

36. McCall, J.O.: An Improved Method of Inducing Reattachment of the Gingival Tissue "in Periodontoclasia", Dental Items of Interest, 68:344, 1926.

37. Workman, C.M.: Studies in Reattachment of the Gingivae. M.S. Thesis, Northwestern University, 1947.

38. Schaffer, E.M., and Zander, H.A.: Histological Evidence of Reattachment of Periodontal Pockets, Paradent., 7:101, 1953.

39. Morris, M.L.: The Reattachment of Human Periodontal Tissues Following Surgical Detachment: A Clinical and Histological Study, J. Periodent., 24:220, 1953.

40. Morris, M.L.: Healing of Human Periodontal Tissues Following Surgical Detachment From Non-Vital Teeth: J. Periodont. 28:222, 1957.

41. Dedolph, T.H., and Clark, H.B.: A Histological Study of Mucoperiosteal Flap Healing. J. Oral Surg., 16:367, 1958.

42. Morris, M.L.: Healing of Human Periodontal Tissues Following Surgical Detachment and Extirpation of Vital Pulps, J. Periodont., 31:23, 1960.

43. Morris, M.L.: Healing of Human Periodontal Tissues Following Surgical Detachment From Vital Teeth: The Position of the Epithelial Attachment. P. Periodont., 32:108, 1961.

44. Donnenfeld, O.W., Marks, R.M., and Blackman, I.: The apically Repositioned Flap-A Clinical Study. J. Periodont., 35:381, 1964.

45. Friedman, N., and Levine, H.L.: Mucogingival Surgery: Current Status. J. Periodont., 35:5, 1964.

46. Pfeiffer, J.S.: The Reaction of Alveolar Bone to Flap Procedures In Man. Periodontics, 3:135, 1965.

47. Pennel, B., King, D., Wilderman, M., and Barron, J.: Repair of the Alveolar Process Following Osseous Surgery. J. Periodont., 38:426, 1967.

48. Grant, D.A.: Experimental Periodontal Surgery: Sequestration of Alveolar Bone. J. Periodont., 38:409, 1967.

49. Costich, E.R., and Ramfjord, S.P.: Healing After Partial Denudation of the Alveolar Process. J. Periodont., 39:127, 1968.

50. Ramfjord, S.P., and Costich, E.R.: Healing After Exposure of Periosteum on the Alveolar Process. J. Periodont., 39:199, 1968.

51. Tartigian, R.: The Height of the Facial Radicular Alveolar Crest Following Apically Positioned Flap Operations. J. Periodont., 41:412, 1970.

52. Lainson, P.A., and MacKenzie, I.C.: An Examination of the Cytology of Uninflamed and Inflamed Gingiva Using a Filter Imprint Technique. J. Periodont., 47:477, 1976.

53. Wood, D.L., Hoag, P.M., Donnenfeld, O.W., Rosenfeld, D.D.: Alveolar Crest Reduction Following Full and Partial Thickness Flaps. J. Periodont., 43:141, 1972.

54. Levine, H.L. and Stahl, S.S.: Repair Following Periodontal Flap Surgery with the Retention of the Gingival Fibers. J. Periodont., 43:99, 1972.

55. Stahl, S.S., Slavkin, H.D., Yamada, L., and Levine, S.: Speculations About Gingival Repair. J. Periodont., 43:395, 1972.

56. Stahl, S.S.: The Nature of Healthy and Diseased Root Surfaces. J. Periodont., 46:156, 1975.

57. Ussing, M.J.: The Development of the Epithelial Attachment. Acta Odont. Scanc., 13:123, 1955.

58. Stern, I.B.: An Electron Microscopic Study of the Cementum, Sharpey's Fibers and Periodontal Ligament in the Rat Incisor. Am. J. Anat., 115:377, 1964.

59. Schroeder, H.E. and Theilade, J.: Electron Microscopy of Normal Human Gingival Epithelium. J.Perio Res., 1:95, 1966.

60. Ito, H., Enomoto, S., and Kobayashi, K.: Electron Microscopic Study of the Human Epithelial Attachment. Bull. Tokyo Med. Dent. Univ., 14:267, 1967.

61. Frank, R., Fiore-Donno, G., Cimasoni, G., and Ogilvie, A.: Gingival Reattachment After Surgery in Man: An Electron Microscopic Study. J. Periodont., 43:597, 1972.

62. Taylor, A.C. and Campbell, M.M.: Reattachment of Gingival Epithelium to the Tooth. J. Periodont., 43:281, 1972.

63. Gottlieb, B.: Der Epithelansatz am Zahme. Otsch Mschr Zahnheilk, 39:142, 1921a.

64. Gottlieb, B.: Atiologie und Prophylaxe der Zahn Karies. Z Stomatol., 19:129, 1921b.

65. Listgarten, M.A.: Structure of Surface Coatings on Teeth. A Review. J. Periodont., 47:139, 1976.

66. Loe, H.: The Structure and Physiology of the Dento-Gingival Junction. Structural and Chemical Organization of Teeth, Vol II. Edited by A.E.W. Miles. Academic Press, New York, 1967.

67. Waerhaug, J.: The Gingival Pocket. Odont. Tidskr., Co. Supp.1, 1952.

68. Weski, D.: Die Chronisch Margin Alem Entzundungen des Alveolarfortsatzes mit besonderer Berucksichtigung der Alveolar Pyorrhoe. Vrtljschr. f. Zahnheilk, 38:1, 1922.

69. Becks, H.: Normal and Pathologic Pocket Formation. JADA, 16:2167, 1929.

70. Gottlieb, B.: The New Concept of Periodontoclasia. J. Periodont., 1:7, 1946.

71. Aisenberg, M.S., and Aisenberg, A.D.: A New Concept of Pocket Formation. Oral Surg., Oral Med., and Oral Path, 1:1047, 1948.

72. Butcher, E.O.: The Cuticles, Epithelial Attachment and Reattachment in the Incisors of the Rhesus Monkey. Brit. Dent. J., 94:197, 1953.

73. Toto, P.D. and Sicher, H.: The Epithelial Attachment. Periodontics, 2:154, 1964.

74. Wertheimer, F.W.: A Histologic Comparison of Apical Cuticles, Secondary Dental Cuticles and Hyaline Bodies. J. Periodont., 37:91, 1966.

75. Goldman, H.M.: Histologic Topographic Changes of Inflammatory Origin in the Gingival Fibers. J. Periodont., 23:104, 1952.

76. Goldman, H.M.: The Behaviour of Transseptal Fibers in Periodontal Disease. J. Dent. Res., 36:249, 1957.

77. Wassermann, F.: Fibrilogenesis in the Regenerating Rat Tendon with Special Reference to Growth and Composition of the Collagenous Fibril. Am. J. Anat., 94:399, 1954.

78. Grant, D.A. and Orban, F.: Leukocytes in the Epithelial Attachment. J. Periodont., 31:87, 1960.

79. Ratcliff, P.A.: An Analysis of Repair Systems in Periodontal Therapy. Periodontal Abstracts, 14:57, 1966.

80. Stern, B.: Collagen Solubility of Normal and Inflammed Human Gingiva. Periodontics. 5:167, 1967.

81. Fullmer, H.M, Gibson, W.A., Lazarus, G.S., Bladen, H.A., and Whedon, K.A.: The Origin of Collagenase in Periodontal Tissues of Man. J. Dent. Res., 48:646, 1969.

82. Stanton, G., Levy, M., and Stahl, S.S.: Collagen Restoration in Healing Human Gingiva. J. Dent. Res., 48:27, 1969.

83. Toto, P. and Gargiulo, A.: Epithelial and Connective Tissue Changes in Periodontitis. J. Periodont., 41:587, 1970.

84. Ten Cate, A.R. and Deporter, D.A.: The Role of the Fibroblast in Collagen Turnover in the Functioning Periodontal Ligament of the Mouse. Arch. Oral Biol., 19:339, 1974.

85. Grant, D.A., and Bernick, S.: A Possible Continuity Between Epithelial Rests and Epithelial Attachment in Miniature Swine. J. Periodont., 40:87, 1969.

86. Polson, A.M., Kennedy, J.E., and Zander, H.A.: Trauma and Progression of Marginal Periodontitis in Squirrel Monkeys. I. Co-destructive Factor of Periodontitis and Thermally-Produced Injury. J. Perio. Res., 9:100, 1974.

87. Polson, A.M.: Trauma and Progression of Marginal Periodontitis in Squirrel Monkeys. II. Co-destructive Factors of Periodontitis and Mechanically-Produced Injury. J. Perio. Res., 9:108, 1974.

88. Listgarten, M.: Cells Rests in the Periodontal Ligament of Mouse Molars. J. Periodont., 10:197, 1975.

89. Attstrom, R., Graf-DeBeer, M., and Schroeder, H.E.: Animal and Histologic Characteristics of Normal Gingiva in Dogs. J. Perio Res., 10:115, 1975.

90. Grant, D.A., Stern, I.B., and Everett, F.G.: Orban's Periodontics. 4th ed. The C.V. Mosby Company, Saint Louis, 1972.

91. Goldman, H.M. and Cohen, D.W.: Periodontal Therapy. 5th. Ed. The C.V. Mosby Company, Saint Louis, 1973.

92. Schroeder, H.E., Graf-De Beer, M., and Ahstrom, R.: Initial Gingivitis in Dogs. J. Perio Res., 10:128, 1975.

93. Aleo, J.J., De Renzis, F.A., and Farber, P.A.: In Vitro Attachment of Human Gingival Fibroblasts to Root Surfaces. J. Periodont., 46:639, 1975.

94. Novaes, A.B., Ruben, M.P., Kon, S., Goldman, H.M., and Novaes, A.B. jr.: The Development of the Periodontal Cleft. J. Periodont., 46:701, 1975.

95. Stallard, R., and Hiatt, W.: The Induction of New Bone and Cementum Formation. I. Retention of Mineralized Fragments within the Flap. J. Periodont., 39:273, 1968.

96. Moskow, B.S.: Calcified Gingival Inclusions. Periodontics. 3:115, 1965.

97. Morris, M.L.: Healing of Human Periodontal Tissues Following Surgical Detachment: The Arrangement of the Fibers of the Periodontal Space. Periodontics. 1:118, 1963.

98. Weiss, M.D., Weinmann, J.P., and Meyer, J.: Degree of Keratinization and Glycogen Content of the noninflamed and Inflamed Gingiva and Oral Mucosa. J. Periodont. 30:208, 1959.

APPENDIX

Diagrams

Tables

Figures

Representation of Landmarks used for Morphometric Analysis

in non-osteoectomy Cases

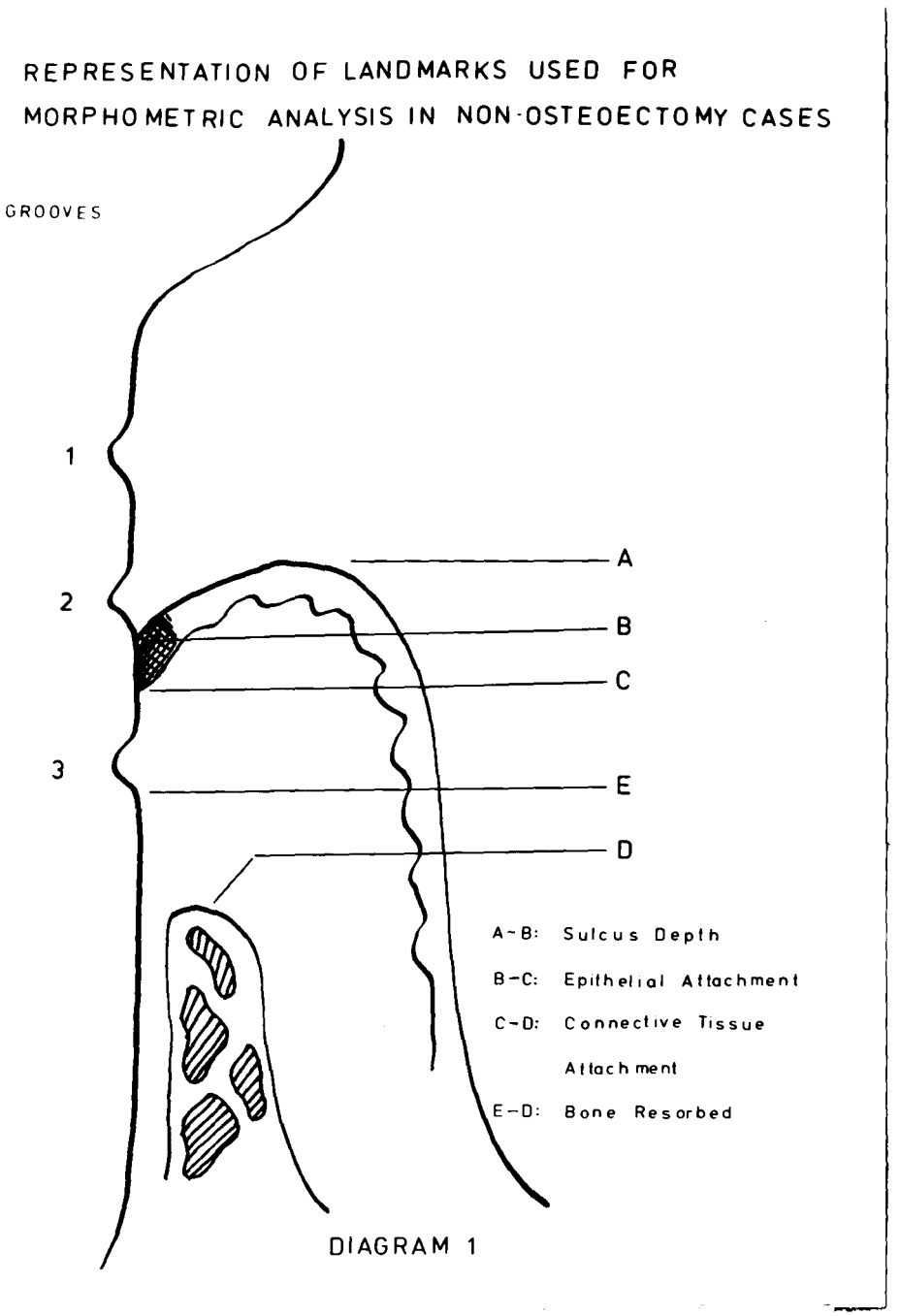

DIAGRAM 1

Representation of Landmarks used for Morphometric Analysis in Osteoectomy Cases

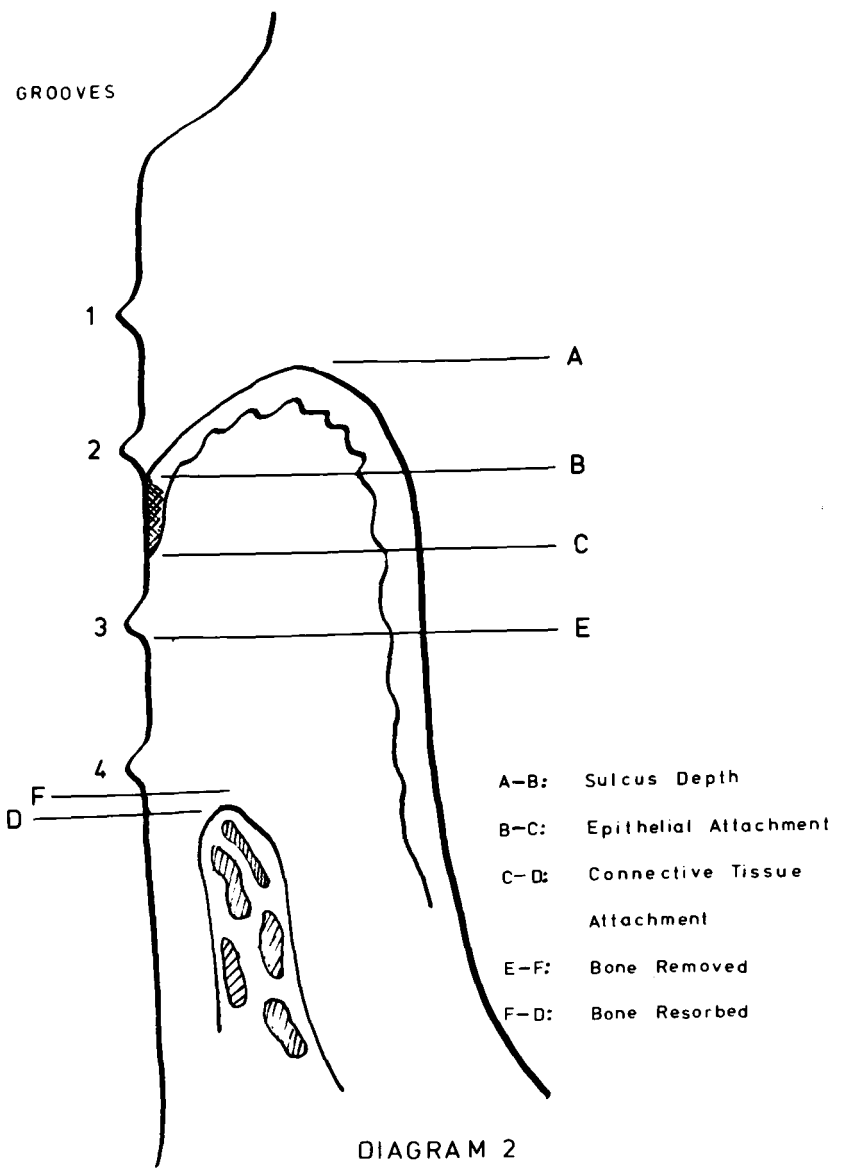

DIAGRAM 2

Compartmentalization of Gingival Tissue For Assessment of Inflammation

COMPARTMENTALIZATION OF GINGIVAL TISSUE FOR ASSESSMENT OF INFLAMMATION

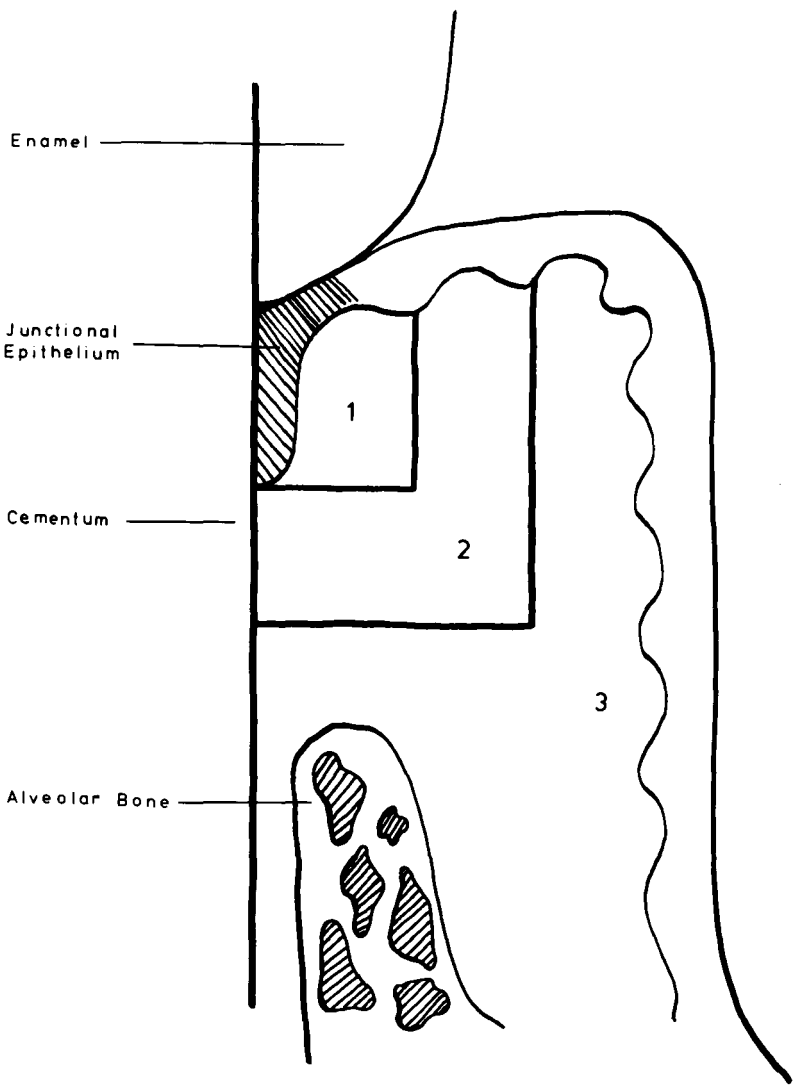

DIAGRAM 3

Table 1

Mean Values of the Components of the Dentogingival Junction in Phase IV of Passive Eruption

	Measurement	Range (mm)	Mean Average
A.	Sulcus depth	0.00 to 2.25	1.76
B.	Attached epithelium	0.08 to 2.65	0.71
C.	Apical point of epithelium attached below cemento-enamel junction	0.39 to 6.08	1.41
D.	Bottom of sulcus from cemento enamel junction	-0.03 to 5.84	-1.14
E.	Cemento enamel junction to alveolar bone	1.10 to 10.88	2.81
F.	Deepest point of epithelial attachment to alveolar bone	0.00 to 6.52	1.06

Table 2

Case 1 - Morphometric Analysis - No osteoectomy was performed

Slide	A-B	B-C	C-D	E-F	E-D
1	.377	.29	1.218		.174
2	.29	.29	1.247		.174
3	.377	.348	1.102		.232
4	.58	.377	1.015		.436
5	.377	.377	1.16		.145
6	.406	.406	1.102		.319
7	.406	.377	1.073		.348
8	.493	.377	.957		.377
9	.522	.406	.957		.377
10	.493	.406	.957		.377
11	.522	.667	.696		.493
12	.638	.493	.754		.493
13	.667	.58	.667		.464
14	.319	.348	1.247		.232
15	.29	.29	1.334		.203
16	.348	.348	1.131		.232
17	.406	.377	1.015		.29
18	.493	.406	.957		.406
19	.406	.319	1.16		.203
20	.667	.493	.754		.493
21	.609	.348	.812		.348
22	.348	.348	1.247		.174
23	.638	.464	.783		.493
24	.377	.406	1.015		.29
Total 24	11.05	9.54	24.36		7.77
Mean	.460	.396	1.015		.324
Range	.29 - .667	.29 - .667	.667 - 1.334		.145 - .493
1 S.D.		±.0894	±.1918		

note: all values are in millimeters

Table 2a

Case 1: Mean values of the components of the dentogingival junction

	Measurement	Range (mm)	Mean Average (mm)
A.	Sulcus depth (A-B)	.29 - .667	.460
B.	Attached epithelium (B-C)	.29 - .667	.396
C.	Connective tissue attachment (C-D)	.667 - 1.334	1.015
D.	Osseous resorption (E-D)	.145 - .493	.324

Table 3

Case 2 - Morphometric Analysis - No osteoectomy was performed

Slide	Sulcus (A-B)	Epithelial Attachment (B-C)	Connective Tissue Attachment (C-D)	Bone Removed (E-F)	Bone Resorbed (E-D)
1	.609	1.073	.812		.087
2	.406	.928	.957		.087
3	.464	1.073	1.102		.145
4	.522	.638	1.16		.058
5	.58	1.073	1.218		.174
6	.29	.696	1.218		-.058
7	.29	1.102	.928		.058
8	.609	.812	.957		.087
9	.609	.812	.957		.087
10	.29	.754	1.218		.058
11	.58	.986	.812		.116
12	.493	.841	1.015		.087
13	.436	1.131	1.218		.145
14	.377	.667	1.218		-.174
15	.436	.87	1.015		.058
16	.377	1.16	1.131		.174
17	* -	-	-		-
18	.638	1.015	.783		.116
19	.348	.725	1.189		-.174
20	.406	.696	1.102		-.203
Total 19	8.760	17.052	20.011		.812
Mean	.461	.897	1.053		.043
Range	.29 - .638	.638 - 1.16	.783 - 1.218		.058 to -.174
1 S.D.		±.1749	±.1513		

* Specimen torn in preparation, not included in study.

Note: All values are in millimeters

Table 3a

Case 2: Mean values of the components of the dentogingival junction

	Measurement	Range (mm)	Mean Average (mm)
A.	Sulcus depth (A-B)	.29 - .638	.461
B.	Attached epithelium (B-C)	.638 - 1.16	.897
C.	Connective tissue attachment (C-D)	.783 - 1.218	1.053
D.	Osseous resorption (E-D)	.058 to -.174	.043

Table 4

Case 3 - Morphometric analysis - osteoectomy was performed

Slide	A-B	B-C	C-D	E-F	F-D
1	.696	.638	.406	.436	.087
2	.667	.58	.406	.436	.087
3	.754	.638	.348	.406	.087
4	.638	.696	.377	.348	.029
5	.638	.783	.464	.406	.087
6	.667	.203	.754	.638	.232
7	.812	.377	.928	.406	.058
8	.667	.29	.667	.58	.116
9 *	-	-	-	-	-
10	.667		.522	.348	.058
11 *	-	-	-	-	-
12	.667	.232	.812	.609	.203
13	.812	.493	.436	.377	.058
14	.725	.493	.377	.436	.058
15	.696	.493	.551	.377	.058
16	.638	.436	.696	.377	.058
17	.812	.377	.58	.406	.058
18	.58	.609	.725	.406	.058
19	.725	.436	.58	.377	.058
20	.696	.406	.754	.406	.058
21	.667	.145	.841	.638	.203
22	.696	.232	.841	.638	.261
23	.667	.174	.841	.638	.232
Total 21	14.59	9.31	12.90	9.69	2.204
Mean	.695	.443	.615	.461	.105
Range	.58 - .812	.145 - .783	.348 - .928	.348 - .638	.029 - .261
1 S.D.		±.1830	±.1869		

* Specimen torn in preparation, not included in study.

Note: All values are in millimeters

Table 4a

Case 3: Mean values of the components of the dentogingival junction

	Measurement	Range (mm)	Mean Average (mm)
A.	Sulcus depth (A-B)	.58 - .812	.695
B.	Attached epithelium (B-C)	.145 - .783	.443
C.	Connective tissue attachment (C-D)	.348 - .928	.615
D.	Bone removed (E-F)	.348 - .638	.461
E.	Bone resorbed (F-D)	.029 - .261	.105

Table 5

Case 4: Morphometric Analysis - osteoectomy was performed

Slide	A-B	B-C	C-D	E-F	F-D
1	.116	.464	.783	1.45	.522
2	.377	.145	1.247	1.682	.609
3	* -	-	-	-	-
4	.174	.203	1.247	1.508	.667
5	.203	.377	1.218	1.624	.667
6	.261	.087	1.363	1.537	.696
7	.261	.145	1.421	1.566	.725
8	.145	.174	1.131	1.479	.638
9	.174	.203	1.189	1.508	.667
10	.145	.203	1.015	1.421	.638
11	.174	.174	1.131	1.363	.696
12	.116	.203	1.073	1.421	.58
13	.145	.232	.986	1.392	.609
14	.203	.116	1.392	1.566	.725
Total 13	2.494	2.726	15.20	19.52	8.439
Mean	.192	.210	1.17	1.50	.649
Range	.116-.377	.087-.464	.783-.1.421	1.363-1.682	.522-.725
1 S.D.		±.1034	±.1789		

* Specimen torn in preparation, not used in study

Note: All values are in millimeters

Table 5a

Case 4: Mean values of the components of the dentogingival junction

	Measurement	Range (mm)	Mean Average (mm)
A.	Sulcus depth (A-B)	.116 - .377	.192
B.	Attached Epithelium (B-C)	.087 - .464	.210
C.	Connective tissue attachment (C-D)	.783 - 1.421	1.17
D.	Bone removed (E-F)	1.363 - 1.682	1.50
E.	Bone resorbed (F-D)	.522 - .725	.649

Table 6

Case 5: Morphometric Analysis - osteoectomy was performed

Slide	A-B	B-C	C-D	E-F	F-D
1	.116	.29	1.044	1.073	.87
2	.174	.087	1.073	.986	.638
3	.116	.203	1.131	1.363	.667
4	.087	.29	1.105	.928	.58
5	* -	-	-	-	-
6	.145	.145	1.044	1.305	.58
7	.116	.232	1.044	.957	.58
8	.116	.174	1.247	1.073	.428
9	.261	.232	1.363	.841	.986
10	.116	.087	1.247	1.073	.928
11	.116	.261	1.102	1.363	.638
12	.145	.116	1.131	1.247	.928
13	.145	.087	1.305	1.073	.928
14	.145	.087	1.073	1.044	.667
15	.116	.087	1.276	1.044	.928
16	.145	.116	1.073	1.044	.812
17	.406	.058	1.131	.87	.377
18	.464	.116	1.131	.493	1.276
19	.261	.174	1.508	.841	.986
20	.145	.087	1.247	1.073	.87
21	.203	.232	1.595	.87	.957
22	.29	.174	1.015	.812	.957
Total 21	3.828	3.335	24.795	21.373	17.081
Mean	.182	.159	1.181	1.018	.813
Range	.087-.464	.058-.29	1.015-1.595	.812-1.363	.377-1.276
1 S.D.		±.0229	±.160		

* Specimen torn in preparation, not used in study
NOTE: all values are in millimeters

Table 6a

Case 5: Mean values of the components of the dentogingival junction

	Measurement	Range (mm)	Mean Average (mm)
A.	Sulcus depth (A-B)	.087 - .464	.182
B.	Attached epithelium (B-C)	.058 - .29	.159
C.	Connective tissue attachment (C-D)	1.015 - 1.595	1.181
D.	Bone removed (E-F)	.812 - 1.363	1.018
E.	Bone resorbed (F-D)	.377 - 1.276	.813

Table 7. Compilation of data and comparison to phase IV analysis

Case	A-B	B-C	C-D	E-F	E-D (F-D)	B-C + C-D
1 (90 days)	.460	.396	1.015		.324	1.411
2 (120 days)	.461	.897	1.053		.043	1.950
3 (60 days)	.695	.443	.615	.461	.105	1.058
4 (120 days)	.192	.210	1.17	1.50	.649	1.38
5 (120 dyas)	.182	.159	1.181	1.018	.813	1.340
mean average values	.398 *(.4155)	.421 (.428)	1.007 (.9925)	.993 .993	.1835 (Avg. cases 1 & 2) .522 (Avg. cases 3,4,5)	1.428 1.421
mean avg. values and ranges for phase IV Analysis (1)	1.76 000-2.25	0.71 0.08-2.65	1.06 0.00-6.52			

* ( ):mean average for the study of 98 total sections

Table 8. Assessment of Inflammation by Gingiva Compartments

Case	Compartment 1	Severe	Severe	Severe	Moderate	Moderate	Total
	2	Severe	Severe	Moderate	Moderate	Mild	
	3	Mild	Moderate	Mild	Mild	Mild	
1				19	3	2	24
2		10	6	3			19
3		12	5	4			21
4				3	9	1	13
5				2	19		21
Total		22	11	31	31	3	98

Table 9. Degree of Inflammation by Gingival Compartment

Compartment	Degree of Inflammation	Frequency
1	Severe	65.3%
	Moderate	34.7%
	Mild	0
2	Severe	33.7%
	Moderate	63.3%
	Mild	3%
3	Severe	0
	Moderate	11.2%
	Mild	88.8%

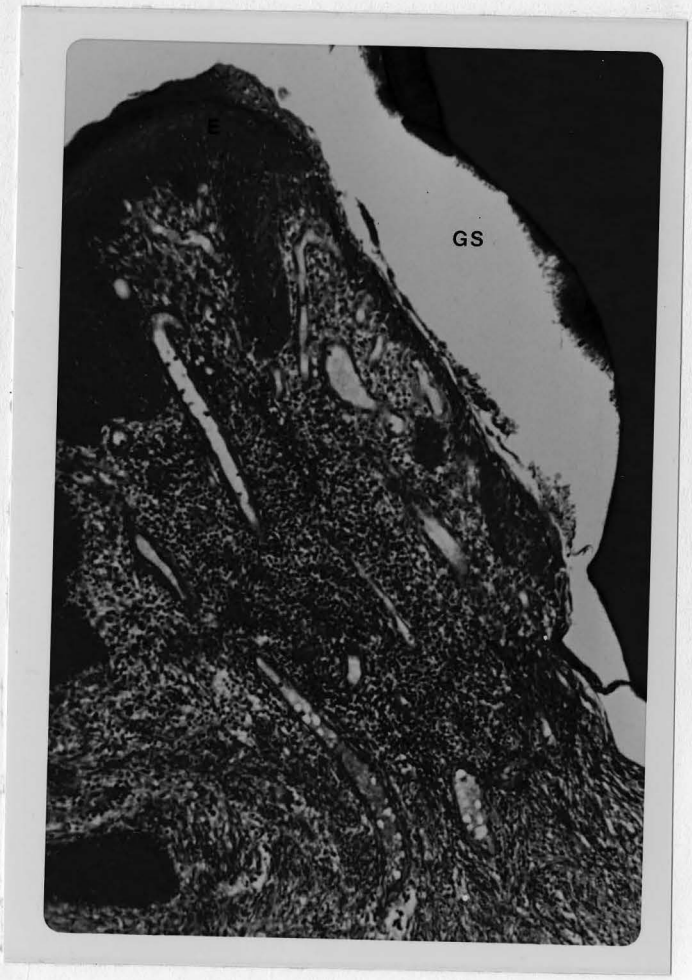

Figure 1: 90 day section with no osteoectomy performed. Note the excessive vascularity of the gingival crest.

E: epithelium
GS: gingival sulcus

Figure 2: 90 day section with no osteoectomy performed. The osteoblastic response at the alveolar crest is noted.

PL: periodontal ligament
OS: osteoid
OB: osteophytic bone
RL: reversal line

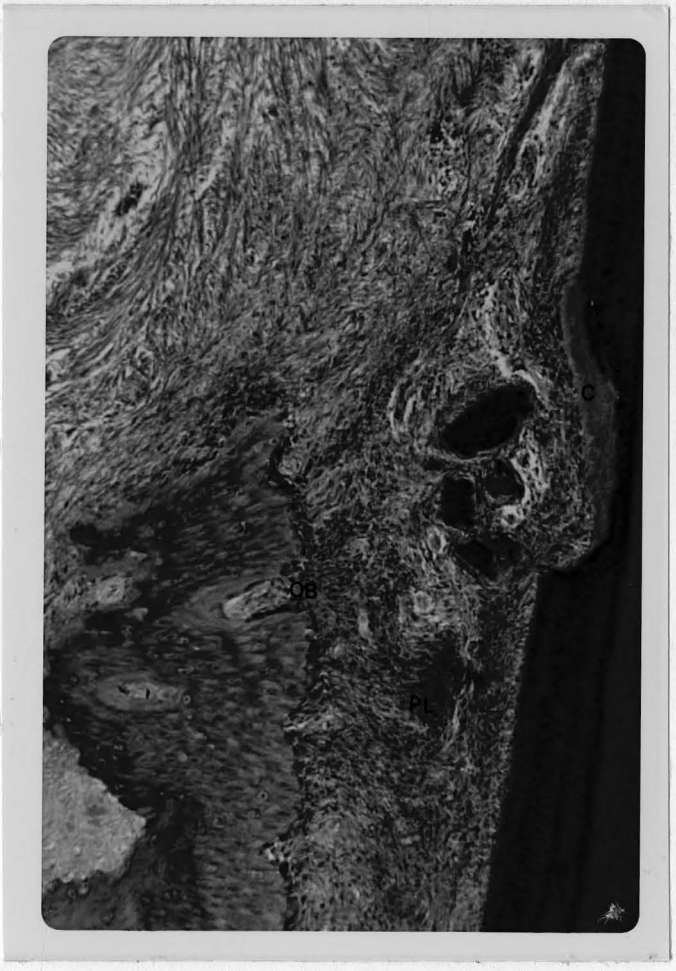

Figure 3: 120 day section with no osteoectomy performed.

PL: periodontal ligament
C: cementoid deposition
OB: osteoblasts

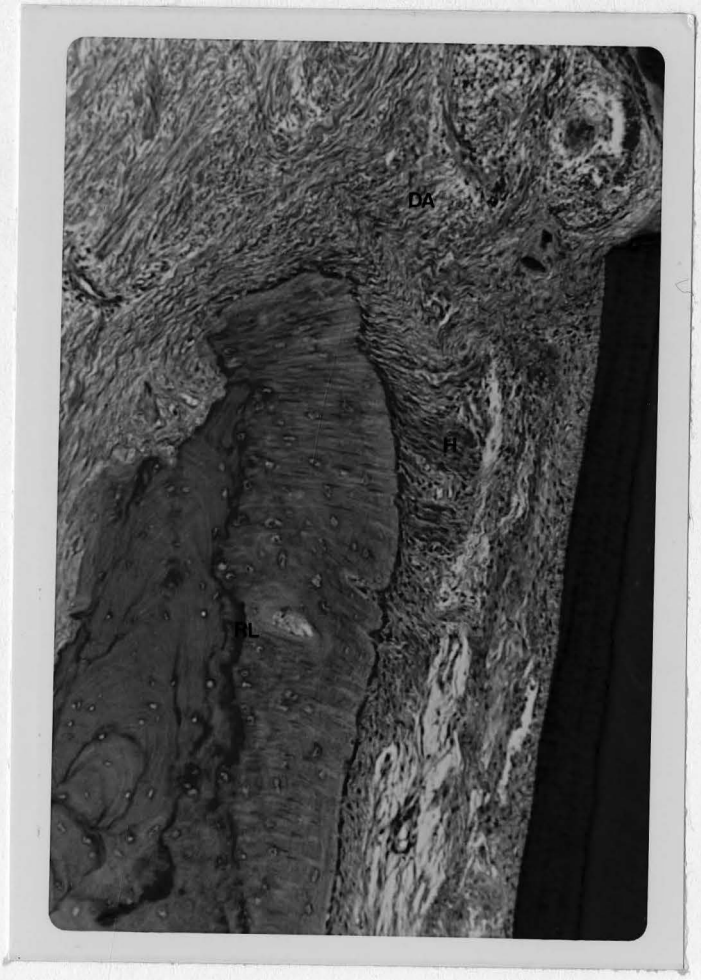

Figure 4: 120 day section. No osteoectomy performed.

RL: reversal line
DA: Dento-alveolar periodontal ligament fibers
H: horizontal periodontal ligament fibers

Figure 5: 120 day section. No osteoectomy was performed. Note severe inflammation in compartment I.

CF: collagen free area
AP: apical proliferating junctional epithelium
D: dentin
C: cementum

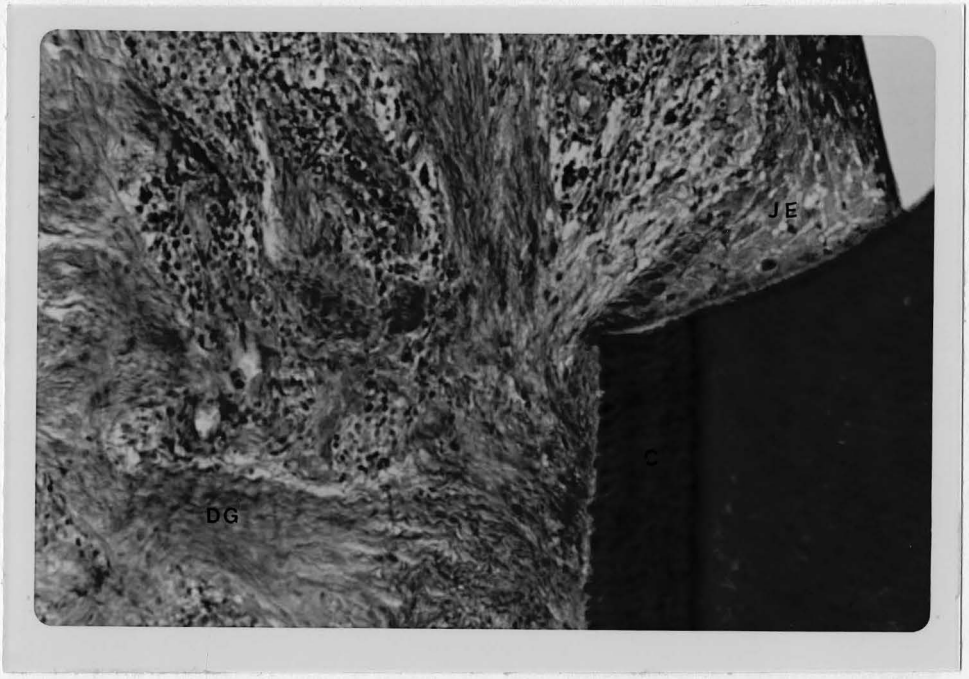

Figure 6: 120 day section. No osteoectomy was performed. Note termination of junctional epithelium where dentogingival fibers are embedded in cementum.

JE: junctional epithelium
DG: dentogingival fibers
C: cementum

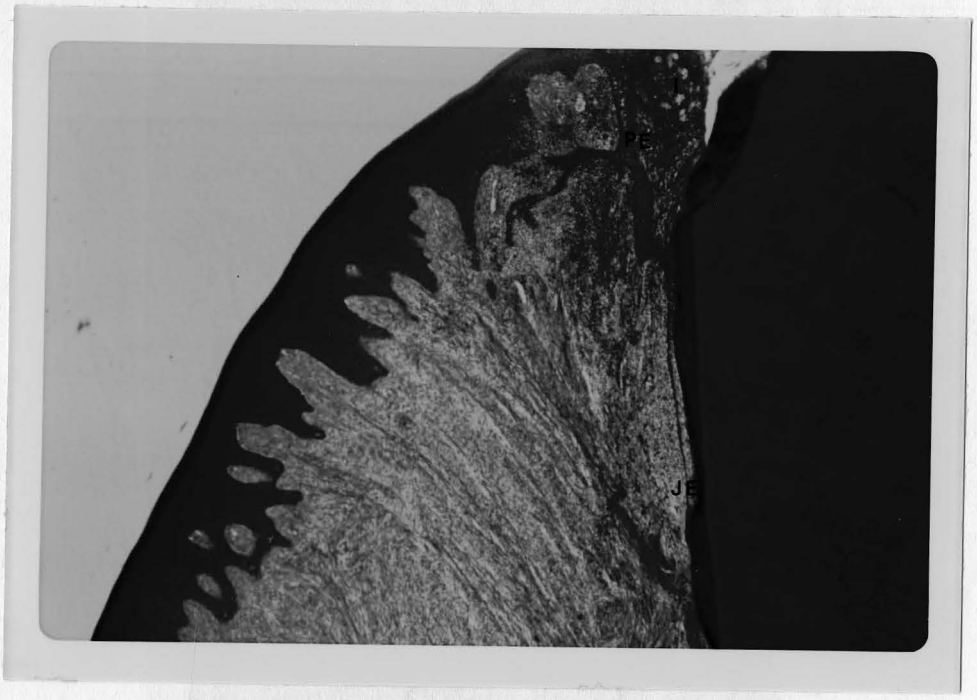

Figure 7: 120 day section. No osteoectomy was performed. Note apparent fusion of proliferating epithelium, elongated junctional epithelium and severe inflammation.

PE: proliferating epithelium
JE: junctional epithelium
I: severe inflammation

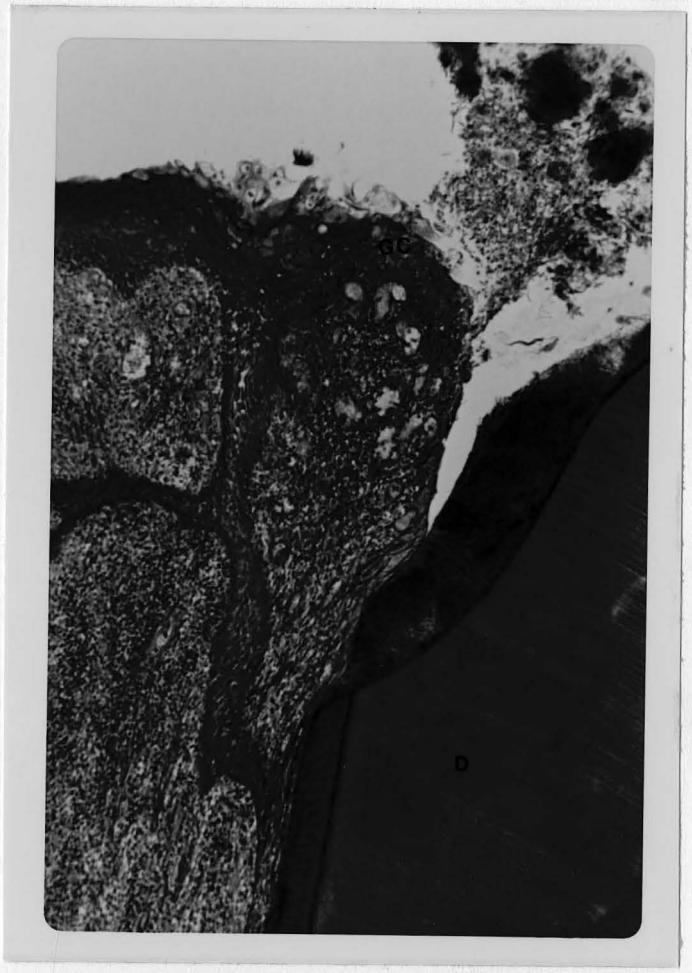

Figure 8: 120 day section. No osteoectomy was performed. Note excessive vascularity at gingival crest and proliferation at outer oral and junctional epithelium.

CA: calculus
D: dentin
GC: gingival crest

Figure 9: 120 day section. Osteoectomy was performed. Note proliferation of junctional epithelium into the collagen poor zone of the gingiva corium.

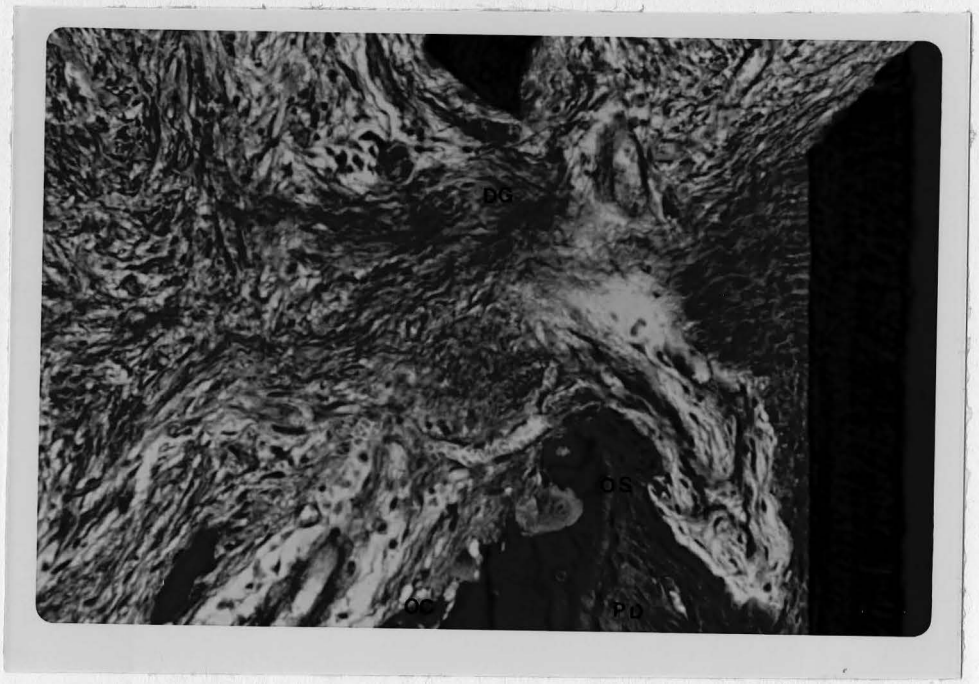

Figure 10: 60 day section. Osteoectomy was performed. Note repair of dento-gingival fibers above alveolar crest.

DG: dento-gingival fibers
OC: osteoclast
OS: osteoid formation
PD: periodontal ligament
CF: cemental fragment

Figure 11: 60 day section. Osteoectomy was performed. Note vascular channel projecting into periodontal ligament space and osteoclastic activity at alveolar crest.

OS: osteoclast
VC: vascular channel
PD: periodontal ligament space

Figure 12: 120 day section. Osteoectomy was performed. Note osteoclastic activity on periosteal surface and osteoblastic activity on periodontal ligament surface.

PA: periosteal surface
O: osteoid
PD: periodontal ligament
RL: reversal line

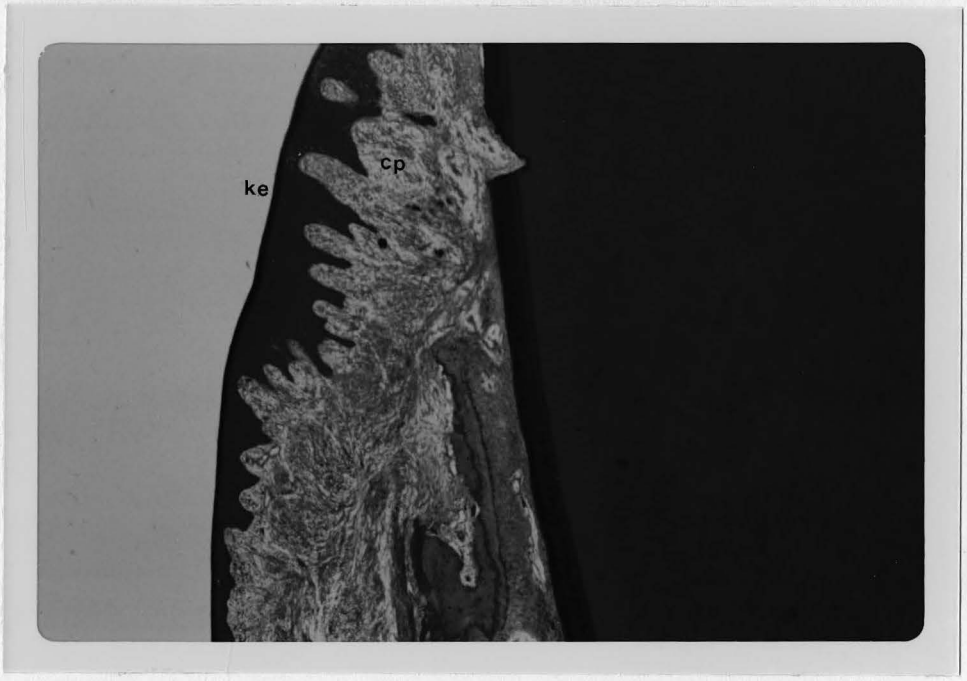

Figure 13: 120 day section. Osteoectomy was performed. Note heavy keratinization and numerous collagen poor zone.

CP: collagen poor zone
KE: keratinized epithelium

Figure 14: 120 day section. Osteoectomy was performed. Endosteal bone apposition is seen.

EB: endosteal bone apposition
OC: osteoclastic activity
OB: osteoblastic activity
PD: periodontal ligament space

APPROVAL SHEET

The thesis/dissertation submitted by Doctor Richard J. Rizzo has been read and approved by the following committee:

Doctor Anthony W. Gargiulo, Director
Clinical Professor and Chairman,
Periodontics, Loyola

Doctor Patrick D. Toto
Professor and Chairman,
General and Oral Pathology, Loyola

Doctor Hal D. McReynolds
Associate Professor,
Histology, Loyola

The final copies have been examined by the director of the thesis/dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis/dissertation is now given final approval by the Committee with reference to content and form.

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

3-31-79
Date

Director's Signature