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A HISTOLOGIC STUDY OF HEALING
FOLLOWING RE-ENTRY SURGERY IN DOGS

by

James M. Giblin

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

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CHAPTER I
INTRODUCTION

Periodontal surgical techniques are constantly being modified as more information becomes available, through research, to guide the clinician in his procedures.

One of the more recent modifications is the split thickness mucosal flap. This surgical procedure basically consists of preparation of a mucosal flap with retention of the periosteal connective tissue upon the alveolar bone surface and results in a minimized post-operative alveolar bone loss.

It is reported in the literature that wounds undergoing reinjury show a decreased post-operative healing time and that the ultimate tensile strength of these reinjured wounds is increased.

This pilot investigation was undertaken to study histologically the post-operative healing sequence following a surgical re-entry procedure utilizing split thickness mucosal flaps.

The combination of the split thickness mucosal flap technique with the re-entry procedure, at the time of maximum post-operative cellular activity, is studied to determine the effect upon the healing sequence following the second surgical procedure.

CHAPTER II

REVIEW OF THE LITERATURE

I. Introduction

Many oral surgical procedures are performed which require access to anatomical structures beneath the oral mucosa. These procedures most often are facilitated by preparation of a mucoperiosteal flap for exposure of the underlying structures with increased accessibility for surgical correction of underlying pathologic deformities. Following completion of the operation, the flaps are generally reapposed and sutured in position to aid in the subsequent post-operative healing.

In recent years considerable investigation has been performed to determine that method or methods of flap preparation which will allow adequate accessibility for performance of anticipated surgical procedures with emphasis being placed on minimal trauma to the surgical area. This minimized trauma is then reflected in the ability of the area to respond post-operatively by healing to approximate preoperative levels.

What these investigators have searched for is a method of gaining access to underlying anatomical structures that will allow adequate access for accomplishment of anticipated surgical procedures with minimal trauma to the surgical area and with predictable post-operative healing results.

A review of the literature reveals many studies relative to the histology of healing of mucoperiosteal and mucosal flaps. Some of these studies were accomplished on human subjects and other investigations were carried out utilizing experimental animals, primarily dogs, rabbits and

rats. Some studies were conducted on the clinical aspects of healing while others combined both the clinical and histologic healing phases.

II. Clinical Studies

Several studies were conducted on the clinical aspects of the healing of mucoperiosteal flaps. The term mucoperiosteal flap refers to a surgical flap of tissue consisting of one-half of the papillary gingiva, as well as the marginal, attached and unattached vestibular alveolar mucosa with the underlying periosteum. This is severed from the necks of the teeth and the alveolar bone by two vertical incisions at the mesial and distal borders of the flap. The only variation in this flap design was due to some authorities who showed a reluctance to sever the marginal gingiva from the teeth. In those cases, a horizontal incision was made below the margin of the gingiva to connect with the two vertical incisions, thus leaving the marginal gingiva intact.

Dingman (1947) conducted several studies of healing of mucoperiosteal flaps on humans. He stated that it was perfectly permissible to detach the gingival tissue from around the necks of the teeth. When these flaps were carefully replaced, they reattached, and in seven to ten days it was clinically impossible to detect that the gingival attachments had been disturbed.

Corn (1962) in a study of human subjects to determine a method of vestibular fornix deepening that would afford minimal associated loss of alveolar crest bone proposed that the periosteal covering be retained to the maximum with a horizontal surgical incision perforating the periosteum at the depth of the extension. He postulated that the resultant scarring would present a barrier to the shallowing of the vestibule. Clinical results

substantiated this theory and it was concluded by this researcher that a conservation of alveolar bone is effected by retention of the periosteal covering. It was further concluded that periosteal retention facilitates healing and affords a more predictable surgical result.

Bohannon (1962) conducted a series of clinical studies utilizing 27 human subjects to investigate the various surgical techniques utilized in vestibular depth alteration and to determine the amount of retained alteration post-operatively. His method of measurement utilized reontgenographic cephalometry for evaluation purposes, using lead shot placed in the depth of the vestibular fornix. In this way he was able to follow post-operatively the healing pattern of the soft tissue level with respect to underlying bony landmarks. He found that by removal of the periosteum and the adherent fibrous tissues and by exposing completely the alveolar plate of bone during the vestibule extension, the mean gain post-operatively was approximately 45% of the original extension dimension. When the extension was accomplished with retention of the periosteum, the mean gain post-operatively was approximately 13% of the original extension dimension. Although the predictability of the operation in which the periosteum was completely removed was greater, the associated post-operative pain and increased healing time indicated that retention of the periosteum is to be desired.

Carranza and Carraro (1963) presented an investigation utilizing four adult dogs as experimental animals to study quantitatively the periodontal response to bone denudation in mucogingival surgery. Basically, their method consisted of creating bilateral wounds, retaining the periosteum on

one side and eliminating periosteum and attached gingiva on the other side.

The effect of the removal of periosteum on the position of the gingiva and the mucogingival line was studied by measurements from a fixed point in the crown, one, two and three months post-operatively. It was found that mucogingival surgery including removal of periosteum produces a much greater gingival recession than the same operation without removing it.

Much attention in the healing studies of mucoperiosteal flaps was focused on the attachment of the gingiva to the tooth.

It should be noted that several different views exist on the relationship between the crevicular epithelium and the enamel and cementum or both. Orban (1952) stated that there is an organic union of the epithelium in attachment to the tooth. Waerhaug (1952) feels that his investigations prove that it is not possible to distinguish microscopically between tissue attachment and intimate tissue contact. Stahl (1962) in his study of reattachment of epithelium and connective tissue following gingival injury in rats states that it appears that the epithelium adheres to the tooth wall through cytoplasmic adaptation to the microscopic irregularities in the tooth surface. This cytoplasmic adaptation is later changed to a keratin-like structure adhering to the roughnesses of the tooth surface. The first interpretations that an organic union exists is the best supported and most widely accepted view. However, with the increased and expanded usage of the electron microscope, new investigations into this relationship have been initiated.

III. Histologic Studies

Related Reference Studies

Reports by Botsford (1941) indicate that the gain in tensile strength of a resutured wound is regularly greater than the rate of gain in a primary wound. Utilizing 77 adult guinea pigs and 11 adult dogs as experimental subjects, he created bilaterally symmetrical wounds, allowed them to heal, and then reopened them for resuturing. Following resuturing he tested the tensile strength of the resutured wounds against a series of initial control incisions. Possible factors involved in such an effect were proposed as follows: The tensile strength gain in the resutured wound was brought about by continuation of the local process of fibroblastic regeneration after resuturing without the necessity for return to the initial phases of healing or the progression of systemic effects set in motion by the primary stress of wounding.

Orban and Archer (1945) conducted a study to obtain fundamental information on the healing of wounds following the elimination of gingival pockets by gingivectomy in human subjects. Gingivectomy was performed on mandibular teeth and tissue was then removed at two day intervals to study progressively the healing of the wound site. The wound was allowed to heal without application of a protective dressing. The behavior of the blood clot was noted and the progress of re-epithelization of the wound was observed. The epithelization of the wound was completed fourteen days following the operation with increased consolidation of the wound.

Bernier and Kaplan (1947) conducted a study of the repair of gingival tissue in humans after surgical intervention utilizing a periodontal dressing post-operatively for ten days. They utilized block sections for post-operative histologic studies. Their findings reflected a wound

covered by fairly well developed stratified squamous epithelium after six days with attachment of the alveolar crest fibers to the alveolar bone and an essentially normal periodontal membrane. Sixteen day specimens showed mature epithelium with newly developed rete pegs and fibrous connective tissue that had become markedly collagenous, probably partly as a result of scar formation. These results, they felt, indicated that the use of a pack on the exposed tissue surfaces after a surgical operation facilitated the healing process.

Workman (1947), upon removing and studying block sections of surgically detached tissue from the maxilla and mandible in humans, concluded that four weeks post-operatively the relationship between the tooth and periodontal ligament was restored to its preoperative status.

Svoboda (1947) operated mucoperiosteal flaps in two human subjects and concluded that the area was completely healed, clinically, in twenty-one days. Further histologic study, however, revealed that thirty-one days were required for complete repair.

Borden (1948), by a series of measurements from a study of mucoperiosteal flaps in dogs and humans, reported that a reattachment of the detached tissue to the tooth surface does take place. However, he observed that the depth of the gingival sulci around the operated teeth decreased due to a recession of the gingival tissue. Also, a decided v-shaped loss of tissue existed at the gingival extremity of the vertical incision; the extent of the defect varied inversely with the thickness of the incised tissue. From his clinical observations, the author concluded that a surgical flap, whenever possible, be reflected so as not to involve the

marginal gingiva of the adjacent teeth. The histologic examination showed that: (1) connective tissue fibers of the periodontal membrane will reattach themselves to the cementum; (2) granulation tissue formed in the periodontal space with subsequent fibrosis at the site of separation; (3) new cementum, bone, and connective tissue fibers were not essential for reattachment.

Sandblom (1949) proposed a systemic healing-promoting factor as being responsible for the increased rate of healing he found in wounds made after a given time at a distance from a primary wound site. Utilizing rabbits as experimental subjects, he prepared two incisions on the back of each experimental animal. After exactly five days the wounds were excised and measured for tensile strength. Fifteen days after the initial wounds had been made, two new incisions were prepared on the opposite side of the body of the same experimental animal strictly symmetrical with the first incisions. After five days the secondary incisions were excised and measured for tensile strength. A comparison of five-day tensile strength measurements showed the secondary wound to be appreciably stronger. He concluded that if symmetrical incisions are made successively in the skin of rabbits, the secondary wounds heal more rapidly than the primary. This increased rate of healing he attributed to a systemic healing-promoting factor which appeared within five and one-half days after injury. It increased rapidly to a maximum between the first and fourth weeks post-operatively. During this time the secondary wounds were 30-40% stronger than the primary wounds.

This systemic concept was disputed by Taffel et al (1951) in their experimental study on the effect of trauma on wound healing in which they conducted similar studies on approximately 200 white rats. The systemic

concept was further disproved by Sovlov and Dunphy (1954) in their study of the mechanisms of wound healing in a comparison study of preliminary local and distant incisions. Wounds of the abdomen made fifteen days after wounds on the back, in 48 male Hishaw rats, did not show any greater tensile strength on the third day than three-day abdominal wounds of a control group on which no previous wounding had been made. Fifteen-day-old abdominal wounds that were opened and resutured revealed a significant increase in tensile strength on the third day after being resutured.

Grant and Ivancie (1957) determined the differences in the replacement tissue after gingival repositioning operations in fourteen human subjects. Their procedure consisted of deepening the mandibular labial vestibule and freeing the overlying muscle fibers and connective tissue from the periosteum. This tissue was removed slightly further than was desired for the end result to allow for some reattachment at the base. All cases were covered with heavy surgical pack and allowed to heal. Biopsy specimens were obtained thirty days post-operatively. Control specimens were selected from areas immediately adjacent to the surgical sites for comparison on an individual basis. The alveolar mucosa with its elastic fibers was replaced with collagenous connective tissue in eight months. They found epithelial ridge formation, keratinization and orientation of the mature collagen fiber bundles. However, the result was that this new tissue was less differentiated and considered to be functionally immature gingiva.

A histologic study of mucoperiosteal flap healing in six human subjects conducted by Dedolph and Clark (1958) showed that the epithelial attachment was restored to its preoperative status in twenty-one days. Within the same

time period, the periodontal fiber bundles and the other connective tissue elements were restored and an inflammatory response at that time was mild or absent. The clinical appearance of the flap areas was indistinguishable from that of the control specimen at the end of this three week period.

Douglas (1958) conducted an experimental surgery study to determine if acceleration of wound healing could be produced on a localized basis by preliminary wounding. Incised wounds were made in the lumbodorsal aponeurosis of rabbits and after varying periods of time they were exposed, opened, and resutured. At the time, fresh incised wounds were made on the opposite side and resutured. After an interval, the tensile strength of the resutured wounds was tested against that of the fresh wounds and the mean tensile strength of the resutured wounds was greater than that of the fresh wounds at all time intervals. The maximal acceleration effect was seen when the interval between the wounding and resuturing was twenty-one days.

Arnold and Hatchett (1962) conducted a comparative investigation of two mucogingival surgical methods wherein the "gingival replacement" and "repositioning of the attached gingiva" operations were compared, utilizing dogs as experimental subjects. Clinically, the wounds healed to approximate pre-operative dimensions with the "repositioning of attached gingiva" operation yielding approximately twice the post-operative attached gingival zone as did the "gingival replacement" type operation. The authors believed that the tissues destroyed tended to be replaced by the same tissues that border or surround the wound. In the case of the "gingival replacement" operation, the majority of the bordering tissue was alveolar mucosa with a relative small amount of attached gingiva. Both tissues proliferated but the results

were primarily alveolar mucosa. On the other hand, the "reposition" operation left a wound bordered primarily by attached gingiva with the result of more attached gingiva in the post-operative healing. These results are in direct accord with Friedman (1957) and Ariando and Tyrrell (1957).

Recent Studies Immediately Pertinent to Experiment

A study by Mittelman (1958) on primary wound healing of a 1.5 mm. stab wound in the attached gingiva of humans showed that epithelial regeneration is more rapid than connective tissue regeneration. Even though the wound site was covered by epithelium in twenty-four hours, inflammation subsisted in the connective tissue as long as seventy-two hours.

A study of ten young adult dogs on which 6 x 7 mm. of crestal bone was removed in a flap operation was done by Marfino (1958). A medicated pack was placed between the flap and the tooth and removed after three weeks. Results showed a new connective attachment of 1.25 to 2.50 mm. Atrophy of the gingiva decreased the depth of the post-operative pocket by one-third. A small amount of new bone was observed at the alveolar crest but without total regeneration of the original morphology.

Mucogingival surgery by Wilderman (1959) consisted of exposing the alveolar bone in dogs by a gingival reposition operation. The results showed a deficiency of repair of the vestibular bone so that the fibrous connective tissue attachment increased to compensate for this lack of bone. Also, the epithelial attachment would not reattach at the cemento-enamel junction. Instead, reattachment occurred in a more apical position on the root surface. The author concluded that the dento-gingival junction exhibited a functional repair but with an anatomical deformity.

Kohler and Ramfjord (1960) investigated clinically and histologically the healing of surgical mucoperiosteal gingival flaps in fifteen human subjects, with special emphasis on avoidance of preoperative injury or curettage of the root surfaces. Findings reflected that surgical mucoperiosteal flaps separating the gingiva from the teeth healed without any significant loss of periodontal attachment and that the presence or absence of presurgical inflammation or variations in occlusal forces did not influence the post-operative healing.

Staffileno (1961), utilizing dogs as experimental animals, conducted studies of the specific reaction of bone tissue, connective tissue and epithelium in the split thickness mucosal flap operation. The split thickness mucosal flap operation consisted of two vestibular incisions, one mesial to the first premolar and the other distal to the fourth premolar. In preparing the flap, the attempt was made to strip the complete epithelial attachment, split the fibrous connective tissue attachment to the tooth without exposing the root, and continue the incision to the unattached vestibular alveolar mucosa without exposing the vestibular plate of bone. The histologic study of this type of flap operation revealed no anatomic deformity after split thickness mucosal surgery with the dimensional measurement of the distance of the alveolar crest to epithelial attachment and the length of fibrous connective tissue attachment remaining at preoperative control levels.

Stahl (1962) studied the effects of repeated injuries on gingival healing in rats. The initial injury was accomplished by utilizing a No. 6 round bur to drill a cavity into the mesial wall of the tooth with simul-

taneous injury to the mesial gingiva. After a twelve day healing period, the healed mesial gingiva was scraped to the level of the alveolar crest, thus constituting a repeated injury. Histologic findings revealed that the repeated injury epithelized in five days as compared to twelve days following the initial injury. The study also indicated that reinjury of a healed gingival wound did not affect the ultimate healing potential and attachment level in normal adult rats.

Klingsberg and Butcher (1963) investigated the epithelial function in periodontal repair in rats by a comparison study of the healing sequences in three reproducible wound types with variable epithelial and connective tissue involvement. Their findings show that removal of the epithelium produces rapid fibroblastic karyolysis and periosteal atrophy. As indicated by the time required for healing and the extent of associated bone loss, removal of the epithelium constitutes as severe a periodontal insult as removal of the entire mucoperiosteum. They further concluded that the rapid and complete repair of flap wounds as compared with the other wounds appears to be associated with the maintenance of the epithelial continuity.

Pfeifer (1963) utilizing eight human subjects conducted a study of the growth of gingival tissue over denuded bone in an attempt to determine the source of the young proliferative tissue that covers the bone and forms the new attached gingiva. He noted that the source of new connective tissue was primarily the periodontal ligament. Further, he noted that function, after dressing removal, altered healing and through stimulation caused a plural potential differentiation to attached gingiva. He concluded that proliferative tissue attached to the root surface of the tooth and formed an

attached epithelial cuff. He noted, also, that the amount of osteoclastic activity following mucogingival surgery varies with the amount of exposed bone and the method by which the exposed bone is protected.

Wilderman (1963) conducted an experiment to study histologically the repair of the oral mucosa and the dentogingival junction in dogs. A measured mucogingival flap consisting of the gingival epithelium and a portion of the underlying connective tissue was reflected and then excised. The epithelial attachment and a portion of the gingival fibers attached to the tooth were also removed. No exposure of bone was evident and the wound was covered by a layer of connective tissue of varied thickness. He found that osteoclastic activity began in the crestal area immediately and reached a peak between the four and six day post-operative periods. In contrast to the crestal area, the osteoclastic activity in an area below the crestal area that was notched during the experimental procedure continued to be present twenty-eight days post-operatively. He further found that epithelial migration from the wound edges began early and completely covered the wound in ten days. Young connective tissue originated and proliferated from the tissues at the sites of bone resorption. These sites he noted as being the periodontal ligament, the marrow spaces, Haversian canals, vestibular periosteum and exposed connective tissues. The greatest bone formation was reported as occurring in the crestal area between fourteen and twenty-one days post-operatively.

CHAPTER III
MATERIALS AND METHODS

This investigation was conducted on five adult dogs with completely erupted permanent teeth. These experimental animals were approximately two years of age.

The experimental procedure was conducted in three phases;

Phase I, or the initial surgical entry, consisted of surgical preparation of a mucosal flap and the reposition of this flap. (Plate II, Figures 3-4).

Phase II, or the intermediate healing, consisted of a twenty-one day healing period following reposition of the mucosal flap.

Phase III, or the surgical re-entry, consisted of surgical preparation of a mucosal flap in the same site as in Phase I following an identical technique. This flap was then reapposed and the experimental animals sacrificed at various time intervals to allow a study of the healing process.

Surgical procedures were performed under general anesthesia utilizing one cc. of sodium nembutal (25 mg/cc) solution per kilogram of animal weight administered intravenously.

Surgery was performed on the animals involving the border of free gingiva, attached gingiva, and the alveolar mucosa in the buccal region of the premolar teeth in the maxillae and the mandible (Plate I, Figure 1).

The surgical techniques were identical in all respects and involved one-half of the mouth at a time. Ipsilateral maxillary and mandibular quadrants were operated during one procedure to insure adequate experimental data for comparative purposes.

A notch was prepared preoperatively with a rotary diamond instrument in the buccal enamel surface of the crown of each tooth, at the level of the free gingival margin. This notch was utilized as a fixed clinical reference in determination of the preoperative level of the free gingiva on the prepared histologic slides.

The maxillary surgical procedure in Phase I consisted of two vertical incisions which were made from the free gingival margin into the vestibular alveolar mucosa. One incision was placed mesially to the first premolar while the other was made distal to the third premolar. These vertical incisions penetrated the fibrous periosteum.

The mandibular surgical procedure in Phase I consisted of two vertical incisions which were made from the free gingival margin into the vestibular alveolar mucosa. One incision was placed mesially to the second premolar while the other was made distal to the fourth premolar. These vertical incisions penetrated the fibrous periosteum.

These procedures provided the lateral outlines of the maxillary and mandibular mucosal flaps. These flaps were then delicately dissected in the deep layers of the lamina propria from the underlying tissues, in a plane parallel to the long axis of the tooth, in such a manner as to leave a portion of the connective tissue remaining intact on the tooth surface and on the periosteum of the buccal alveolar bone while the mobile side

of the dissection became what will hereinafter be referred to as a split thickness mucosal flap (Plate II, Figure 3). The clinical base of the flap was established in the vestibular alveolar mucosa. This split thickness mucosal flap then consisted of one-half of the interproximal or papillary gingiva, as well as marginal, attached and unattached vestibular alveolar mucosa. The flap size was approximately 15 mm. by 45 mm. Once these flaps were operated, they were reapposed to their original position and fixed with 000 black silk suture (Plate II, Figure 4).

This then completed the surgical procedure of Phase I.

Following reapposing and fixation of the split thickness mucosal flap, the area of initial surgical entry was allowed to heal for a twenty-one day period.

This completed Phase II or intermediate healing period of the procedure.

The surgical site in Phase III, or surgical re-entry, was dictated by the initial surgical entry procedure previously outlined. The original surgical incisions were still discernible on the buccal gingival surface and another split thickness mucosal flap was surgically prepared following identical surgical procedures as utilized in the initial surgical entry of Phase I.

This split thickness mucosal flap was then reapposed and fixed with 000 black silk suture.

Post-operative medications immediately following each surgical procedure consisted of 600,000 units of penicillin administered intramuscularly. This daily dosage was continued for two additional days

post-operatively.

The diet for the animals during the experimental period consisted of milk and eggs the first day post-operatively; milk-softened Pard and eggs the next two days post-operatively; and milk-softened Pard the remainder of the time.

Kodachrome clinical photographs were taken of the experimental areas at various times to record clinical impressions.

Surgically operated areas were allowed to heal and the experimental animals were sacrificed at predetermined intervals to obtain specimens for progressive histologic study of the healing processes. The animals were sacrificed by an overdose of sodium nembutal solution administered intravenously.

Post-re-entry sacrifice times of the experimental animals were:

- zero hours (Plate II, Figure 4),
- two days (Plate IV, Figure 7),
- six days (Plate VI, Figure 12),
- fourteen days (Plate VII, Figure 15)
- twenty-one days (Plate IX, Figure 19) and
- sixty days (Plate XI, Figure 24).

Following sacrifice of the experimental animals, the maxillae and mandible were immediately removed, washed in clear water, and immersed and allowed to remain in 10% formalin solution for a two-week period.

Control specimens were taken from unoperated areas of one animal (Plate I, Figure 1).

Histologic specimens were obtained by the following methods:

Fixation - 10% neutral formalin solution.

Decalcification - large quantities of a 5% aqueous nitric acid solution observed every two days until completely decalcified. Specimen washed in running water for twenty-four hours, then neutralized 10% formalin solution to which an excess of calcium or magnesium carbonate had been added, again washed in water for twenty-four to forty-eight hours.

Dehydration - 75% alcohol (twenty-four hours)

95% alcohol (twenty-four hours)

100% alcohol (twenty-four hours)

Ether-alcohol - half and half (twenty-four hours)

Embedding - celloidin

thin - one week

medium - one week

thick - one week

Sectioning - serially - oral-vestibular sections

Staining - hemotoxylin-eosin

Gomori's trichrome

Silver impregnation

The maxillary second premolar and the mandibular third premolar areas were considered typical central wound areas for histologic examination.

(Plate I, Figure 1).

Interproximal, radicular and interradicular bucco-lingual sections were prepared from these typical areas for histologic evaluation.

CHAPTER IV

1. MACROSCOPIC FINDINGS OF HEALING OF SPLIT THICKNESS MUCOSAL FLAP

A split thickness mucosal flap measuring 15 mm. by 45 mm. was elevated and reflected from the vestibular plate of alveolar bone in the area of the mandibular and maxillary premolar teeth (Plate II, Figure 3). The periosteal connective tissue covering remained on the buccal surface of the alveolar bone. After surgery, the split thickness mucosal flap was reapposed and fixed in its original position with 000 black silk suture (Plate II, Figure 4). The gingival border of the split thickness mucosal flap was sutured to the fixed half of the papillary portion of the lingual attached gingiva. A clot formed on the wound periphery resulting in primary closure with minimal hemorrhage.

This initial surgical injury was allowed to heal for a period of twenty-one days. At the end of this healing period, the split thickness mucosal flap area appeared, clinically, to be healed except for the scar formations in the area of the laterally placed vertical relief incisions. The tissues gave a clinical impression of being normal in every other respect. There was never any change in the character of pigmentation of the tissue.

After this twenty-one day healing period, a second split thickness mucosal flap was elevated, reflected, and reapposed in an identical manner to the first surgical procedure. Following fixation of this second split thickness mucosal flap, clinical observations were made to record macroscopic impressions of the progression of healing of the second split thickness mucosal flap procedure.

After the second surgical procedure, the split thickness mucosal flap was reapposed to its original position and fixed with 000 black silk sutures. The gingival border of the split thickness mucosal flap was sutured to the fixed half of the papillary portion of the lingual attached gingiva. A clot formed on the wound periphery and resulted in a primary closure with minimal hemorrhage.

In two days the split thickness mucosal flap (Plate IV, Figure 7) appeared swollen with the areas of the vertical incision being clearly discernible as a bright red line. The gingival border of the split thickness mucosal flap reflected a rolled edematous edge which folded over and into the incision.

At six days (Plate VI, Figure 12) the vertical incision areas were still discernible by a linear depression in that area. Clinical observation during this period indicated that the flap was firmly attached to the underlying connective tissues.

Within the fourteen day (Plate VII, Figure 15) post-operative period, the flap area appeared healed except for the scar formations which were visible as the original borders of the split thickness mucosal flap. The tissues gave a clinical impression of being normal in every other respect.

In the twenty-one day animal (Plate IX, Figure 19) and sixty day animal (Plate XI, Figure 24), the split thickness mucosal flap areas appeared the same as the control specimen with the exception of the scar formation noted in the area of the lateral vertical relief incisions.

2. MICROSCOPIC FINDINGS OF HEALING OF SPLIT THICKNESS MUCOSAL FLAP

I. Initial or Preparatory Stage 0-2 Days

- A. Bleeding and Clotting 0 Hour
- B. Initial Flap Fusion (Anchoring Clot) 0-48 Hours
- C. Inflammation 0 Hour

II. Productive Stage 2-6 Days

- A. Epithelial Regeneration
 - 1. Epithelial Bridging
 - 2. Epithelial Proliferation and Maturation
 - 3. Epithelial Proliferation and Maturation - Summary
- B. Connective Tissue Production
 - 1. Connective Tissue Fusion
 - 2. Connective Tissue Proliferation
- C. Inflammation

III. Reconstructive Stage 0-60 Days

- A. Reconstruction of Connective Tissue
 - 1. Continued Connective Tissue Proliferation 11-21 Days
 - 2. Connective Tissue Maturation - Summary
- B. Reconstruction of Bone 0-60 Days
 - 1. Osteoclasia and Osteogenesis
 - 2. Summary

2. MICROSCOPIC FINDINGS OF HEALING OF SPLIT THICKNESS MUCOSAL FLAP

I. Initial or Preparatory Stage 0-2 Days

A. Bleeding and Clotting 0 Hour

At zero hour the specimen of the operated animal showed a split thickness mucosal flap in which the incision had extended through the surface epithelium and into the lamina propria so that approximately one-half of the lamina propria remained as a bed of connective tissue (Plate III, Figure 5) on the buccal aspect of the alveolar bone. That portion of the lamina propria and epithelium which remained attached to the periodontium and to the periosteum overlying the vestibular plate of the alveolar bone formed the fixed, or stump, portion of the operative site. This portion of residual tissue included a portion of the severed attached epithelial cuff which remained as a tissue remnant attached to the tooth surface. A thin clot was noted between the flap and the fixed, or stump, portion of the residual tissue (Plate III, Figure 5).

B. Initial Flap Fusion (Anchoring Clot) 0-48 Hours

Within the short time interval following preparation of the second split thickness mucosal flap and sacrifice of the experimental animal, an early fusion of the split thickness mucosal flap and the stump with the opposing connective tissues was mediated by a clot. The clot consisted of a fibrinous stroma into which was emeshed many polymorphonuclear leukocytes and erythrocytes. Bacteria and an exudate and transudate, as a result of tissue injury, also were present in this area at this time.

This matrix of clotted elements occupied the incision area, acting as a wound seal in fusing the split thickness mucosal flap with the remaining connective tissue layers by means of the fibrinous network.

C. Inflammation 0 Hour

An inflammatory response was noted in the zero hour specimen immediately following the surgical preparation of the second split thickness mucosal flap. Primarily, there was an acute influx of polymorphonuclear leukocytes into the formed clot.

Observation of the lamina propria immediately below the epithelium in the gingival zone of the flap (Plate XII, Figure 27) showed dilated capillaries congested with blood elements. The central portion of the capillaries were occupied by erythrocytes while the periphery showed a margination of the polymorphonuclear leukocytes.

The connective tissue of the gingival zone of the flap contained many extravasated polymorphonuclear leukocytes.

The supra-alveolar and alveolar zones (Plate XII, Figure 27) of the split thickness mucosal flap showed dilated capillaries filled with blood cells including polymorphonuclear leukocytes. The number of polymorphonuclear leukocytes noted in the connective tissue in these areas was less than the number of polymorphonuclear leukocytes noted in the connective tissue of the gingival zone of the split thickness mucosal flap, indicating the most intense inflammatory response to have occurred initially in the gingival zone of the split thickness mucosal flap.

The connective tissue adjacent to the periosteum and periodontium contained capillaries that were dilated, but there was only an occasional

polymorphonuclear leukocyte to be found in the extravascular areas.

The coronal portion of the periodontal ligament in the zero hour specimen manifested dilated capillaries with only slight extravasation of polymorphonuclear leukocytes.

II. Productive Stage 2-6 Days

A. Epithelial Regeneration

1. Epithelial Bridging

Forty-eight hours following surgical preparation of the second split thickness mucosal flap, a bridging of the epithelial area of the incision had been effected (Plate IV, Figure 8). This bridging was completed below the original epithelial surface within the incision itself. From both sides of the incision the epithelium was decreasing in dimension from the basal cell layer to the outer keratin layer (Plate IV, Figure 9). It may be described as a slipping or stretching of the cells over one another to effectively close the incision over the clot. The epithelium migrated downward along the lateral walls of the incision and joined within the incision itself. At the junction, the epithelium was one to two cells in thickness with no distinctly discernible continuous basal cell layer.

The epithelial cells above the junction of the new migrating epithelium were loosely arranged and undergoing hydropic degeneration with intracellular and intercellular edema.

The six day specimen reflected advanced epithelial regeneration. The epithelial bridging was completed and the basal cell layer was continuous along the entire incision area. There was a distinct lack of cellular continuity in the superficial epithelial layers as a result of intracellular

and intercellular edema.

2. Epithelial Proliferation and Maturation

After the epithelium had bridged the incision area at the forty-eight hour interval, there was a continuous proliferation of epithelial cells to quantitatively restore the epithelium in that area to normal dimensions.

Mitosis was observed in the six day specimens. Intracellular and intercellular edema were present with some cells that appeared to have undergone hydropic degeneration. Regeneration of the basal cell layer and prickle cell layers was observed. Slight surface defects were noted in the epithelial surface at the six day interval. The thickness of the epithelium was increased in the area of the incision. Normal epithelial ridges were not present at this time.

The fourteen day specimens showed limited mitotic figures in the prickle cell layer. Surface defects were still noted in the form of cellular remnants and intracellular edema of the epithelial cells. Normal epithelial ridges were not present in the area of the incision and an increased thickness of the epithelium was seen in the form of an elongated projection of epithelium into the former incision (Plate VII, Figure 16). In the twenty-one and sixty day specimens, the epithelial proliferation had restored the gingival epithelium to preoperative thickness and morphology (Plate IX, Figure 20).

3. Epithelial Proliferation and Maturation - Summary

Specimen Interval
Following Re-entry
(Notations in parenthesis
indicate number of days
since initial surgery)

Epithelial Repair Status

A. Zero Day (21)

1. Epithelium not continuous.
 2. Incision area filled with clot.
-

B. Two Day (23)

1. Epithelium bridged incision.
 2. Basal cell layer not continuous.
 3. Surface epithelial cells undergo intra-cellular and intercellular edema.
 4. Surface epithelium undergoes hydropic degeneration.
 5. Epithelium in incision one to two cells in thickness.
-

C. Six Day (27)

1. Basal cell layer continuous.
2. Mitosis observed in epithelium.
3. Intracellular and intercellular edema present.
4. Hydropic degeneration present.
5. Thickness of epithelium increased in excess of preoperative dimension.
6. Slight surface defects present.
7. Epithelial ridges absent.

3. Epithelial Proliferation and Maturation - Summary (continued)

Specimen Interval
Following Re-entry
(Notations in parenthesis
indicate number of days
since initial surgery)

Epithelial Repair Status

-
- | | |
|----------------------|--|
| D. Fourteen Day (35) | <ol style="list-style-type: none"> 1. Intracellular and intercellular edema present. 2. Hydropic degeneration present. 3. Mitosis observed in epithelium. 4. Epithelial thickness in excess of preoperative dimension. 5. Epithelial ridges absent. |
|----------------------|--|
-
- | | |
|------------------------|--|
| E. Twenty-one Day (42) | <ol style="list-style-type: none"> 1. Epithelium returned to normal thickness and morphology. |
|------------------------|--|
-
- | | |
|-------------------|--|
| F. Sixty Day (81) | <ol style="list-style-type: none"> 1. Epithelium returned to normal thickness and morphology. |
|-------------------|--|
-

B. Connective Tissue Production

1. Connective Tissue Fusion

The two day specimen showed that a fusion had occurred between the split thickness mucosal flap and the adjacent connective tissues (Plate IV, Figure 8). The gingival zone was seen to be fixed by a matrix that showed multidirectional layering of a fibrinous coagulum, fusing the split thickness mucosal flap to the fixed connective tissue of the stump portion of the

surgical site. The entire fibrinous coagulum area was heavily infiltrated by polymorphonuclear leukocytes.

The lateral areas of the incision reflected proliferating vascular endothelial cells and young immature fibroblasts projecting into the fibrin network of the clot.

The areas adjacent to the incision consisted of loose connective tissue bundles infiltrated heavily with a cellular infiltrate of acute inflammatory cells (Plate IV, Figure 9). Budding capillaries were noted along the incision periphery.

At the two day interval the process of organization of the clot had already begun. The space occupied by the clot was a discontinuity in the tissues, and it was into this clot that the young reparative tissues grew from the periphery, penetrating and replacing it. This granulation tissue consisted of young budding capillary blood vessels, proliferating fibroblasts, and phagocytes.

2. Connective Tissue Proliferation

After forty-eight hours, connective tissue proliferation was noted in the periosteum of the vestibular alveolar bone. The periosteal connective tissue showed intense proliferation with differentiation of many fibroblasts, formation of new blood vessels and many undifferentiated mesenchymal cells (Plate V, Figure 11).

Connective tissue proliferation at six days was considerably more advanced than was noted at forty-eight hours. The area of incision of the gingival zone of the split thickness mucosal flap (Plate VI, Figure 13) was filled with immature young loose connective tissue consisting of numerous

young fibroblasts, disoriented collagen fibers and some polymorphonuclear leukocytes. Undifferentiated mesenchymal cells were noted in the area of capillaries.

In the supra-alveolar zone of the incision area the connective tissue had not advanced as much in its repair as was noted in the gingival zone. A graduated rate of repair relative to the depth of incision was observed; that is, the deeper the penetration into the incision, the lesser the degree of connective tissue production noted. Many fibroblasts were seen in the supra-alveolar zone, along with some histiocytes. The formation of new collagen bundles lagged behind that of the gingival zone. The tissue noted in the supra-alveolar and alveolar zones at six days was characteristic of granulation tissue.

The incision area was still clearly discernible at the six day time interval along its entire length. This discernibility was based on the inability of the fibers, the cells, and the intercellular substance to accept a high degree of stain, the increased cellularity of the area and the orientation of the capillaries and collagen fibers in a direction parallel to the vestibular surface of the alveolar bone. This was contrasted to the normal surrounding connective tissue fibers which in general were oriented perpendicular to the vestibular surface of the alveolar bone.

C. Inflammation 0 Hour

In the forty-eight hour specimens, all zones of the split thickness mucosal flap showed dilated capillaries congested with blood elements. Intense infiltration of polymorphonuclear leukocytes was noted in all zones of the connective tissues of the split thickness mucosal flap and in the

lamina propria of the fixed portion of the operative side adjacent to the tooth surface and the periosteal layer of the vestibular alveolar bone. The periosteal tissues adjacent to the vestibular alveolar bone reflected some dilation and congestion of capillaries with slight infiltration of polymorphonuclear leukocytes. The periodontal ligament space was normal.

The six day specimens showed the gingival, the supra-alveolar and the alveolar zones with capillaries that appeared near normal. These three zones and the adjacent periosteal connective tissue layer of the vestibular alveolar bone contained a moderate infiltration of polymorphonuclear leukocytes and macrophages.

III. Reconstructive Stage 0-60 Days

A. Reconstruction of Connective Tissue

1. Continued Connective Tissue Proliferation 14-21 Days

Connective tissue proliferation had continued and the fourteen day specimens (Plate VIII, Figure 17) exhibited, in the gingival zone, connective tissue that reflected orientation of mature collagen fiber bundles similar to the adjacent connective tissue collagen bundles. The incision was still discernible, however, due to the increased fibroblast-fiber ratio.

The supra-alveolar and alveolar zones showed even less maturation in that the fibroblasts and fibers were oriented in a direction parallel to the vestibular surface of the alveolar bone as contrasted to the normal surrounding connective tissue fibers which were generally oriented in a perpendicular plane to the vestibular surface of the alveolar bone. A much higher cellularity existed in the incision area than in the adjacent tissues.

At twenty-one days the connective tissue in the supra-alveolar and

alveolar zones of the incision (Plate IX, Figure 20) was distinguishable from the normal adjacent connective tissues. This difference was based on the remaining high degree of cellularity and the disorientation of the connective tissue collagen fibers within the incision site.

The sixty day specimens showed connective tissues in the incision area which appeared normal (Plate XI, Figure 25). Collagen fiber bundles were dense and well oriented to the adjacent tissue and the cellular fiber ratio was indistinguishable from that of the adjacent tissues.

2. Connective Tissue Maturation - Summary 6-60 Days

Specimen Interval Following Re-entry (Notations in parenthesis indicate number of days since initial surgery)	Connective Tissue Maturation
A. Six Day (27)	<ol style="list-style-type: none"> 1. Young fibroblasts - grossly disorganized. 2. Fiber bundles disorganized with no pattern of placement. 3. Young immature collagen fibers. 4. High fibroblast-fiber ratio. 5. Incision area clearly discernible. 6. Undifferentiated mesenchymal cells in area of capillaries.
B. Fourteen Day (35)	<ol style="list-style-type: none"> 1. Young fibroblasts - organization of fibers and capillaries beginning in direction parallel to vestibular surface of alveolar bone. 2. Gingival zone reflects highest degree of maturation of connective tissue.

2. Connective Tissue Maturation - Summary 6-60 Days (continued)

Specimen Interval
Following Re-entry
(Notations in parenthesis
indicate number of days
since initial surgery)

Connective Tissue Maturation

3. Supra-alveolar and alveolar zones reflect lesser degree of maturation of connective tissue.
4. Fibroblast-fiber ratio less than six day specimen.
5. Incision still discernible.
6. Undifferentiated mesenchymal cells in area of capillaries.

C. Twenty-one Day (42)

1. Fibroblasts still not mature.
2. Fibroblast-fiber ratio lowered - near normal.
3. Collagen fiber bundles accept wavy appearance.
4. Islands of high ratio fibroblast-fiber content remain.
5. Incision area discernible.

D. Sixty Day (81)

1. Mature fibroblasts.
2. Fibroblast-fiber ratio normal.
3. Collagen bundles oriented to normal.
4. Considered mature connective tissue.

B. Reconstruction of Bone 0-60 Days

1. Osteoclasia and Osteogenesis

A description of specimens representative of each experimental time

SCHOOL OF

interval is presented to adequately portray the chronological developments observed in the reconstruction of the vestibular alveolar bone adjacent to the incision area of the second split thickness mucosal flap.

a. Zero Hour Specimens

At zero hours following the preparation of the second split thickness mucosal flap, the vestibular surface of the alveolar bone showed a resorption pattern (Plate III, Figure 5) that had penetrated the vestibular plate through the outer circumferential lamellae into the interstitial and Haversian lamellae. Severe undermining resorption was seen in the alveolar crest area with the original crest height being maintained. The resorption pattern corresponded in depth to the depth of the incision.

Osteoblasts (Plate III, Figure 6) were observed along the extent of the resorbed vestibular alveolar surface. Deposition of osteoid tissue was clearly seen at this time delineated by a reversal line on the resorbed alveolar surface. An occasional osteoclast was noted.

Cellular activity was noted in the cambium layer of the adjacent periosteal connective tissue.

b. Two Day Specimens

Osteoblasts were noted along the extent of the vestibular alveolar surface (Plate V, Figure 10) and in adjacent marrow spaces with the associated deposition of osteoid tissue. Osteoclasts in Howship's lacunae were noted on the alveolar surface (Plate V, Figure 11).

The cambium layer of the adjacent periosteum reflected intense fibroblastic production with an associated dense arrangement of collagenous fibers making up the outer periosteal layer (Plate V, Figure 11).

c. Six Day Specimens

Six days following preparation of the second split thickness mucosal flap, a large number of multinucleated giant cells (Plate VI, Figure 14), or osteoclasts, were observed along the extent of the vestibular alveolar plate. Dispersed between these cells was noted remnants of the original osteoid appositional layer.

As a result of the noted increased osteoclastic activity, the vestibular alveolar crestal area showed a loss in height due to resorption. Further resorption was noted along the vestibular alveolar surface. Osteoblastic activity at six days was diminished as compared to that noted at the zero and two day intervals.

The adjacent periosteum showed an arrangement of large dense collagenous fiber bundles (Plate VI, Figure 14).

d. Fourteen Day Specimens

By the fourteenth day following preparation of the second split thickness mucosal flap, the osteoblastic activity (Plate VIII, Figure 17) had increased to be seen along the entire vestibular alveolar surface and in the adjacent narrow spaces (Plate VIII, Figure 18). Minimal osteoid deposition was noted at this time.

Osteoclastic activity had declined from its maximum, as seen at six days, and only an occasional osteoclast was noted.

Cellular activity in the adjacent periosteum was noted to have increased markedly at this time with a dense protective fibroperiosteum resultant.

e. Twenty-one Day Specimens

Osteoblastic activity with a continuous osteoid layer was observed

over the extent of the resorbed area of the vestibular alveolar surface extending around the alveolar crest and into the periodontal ligament area (Plate X, Figure 21-23).

Osteoclastic activity was minimal with only an occasional osteoclast noted.

The highly cellular, dense collagenous fibroperiosteum noted in the fourteen day specimens was present.

f. Sixty Day Specimens

A continuous layer of lamellated bone (Plate XI, Figure 25) was noted on the vestibular alveolar and crestal surfaces at this time. A reversal line was discernible clearly delineating this appositional layer. Osteoblasts were noted along the entire vestibular surface adjacent to this new bone.

2. Reconstruction of Bone - Summary

Specimen Interval
Following Re-entry
(Notations in parenthesis
indicate number of days
since initial surgery)

Description

A. Zero Day (21)

1. Scalloped alveolar surface due to resorption.
2. Osteoblasts observed over extent of vestibular alveolar surface.
3. Osteoid layer present.
4. Osteoclastic activity minimal.
5. Adjacent periosteum shows cellular proliferation.

2. Reconstruction of Bone - Summary (continued)

Specimen Interval Following Re-entry (Notations in parenthesis indicate number of days since initial surgery)	Description
B. Two Day (23)	<ol style="list-style-type: none"> 1. Osteoblasts observed over extent of vestibular alveolar surface. 2. Osteoid layer present. 3. Osteoclastic activity minimal. 4. Cellular proliferation noted in adjacent periosteum.
C. Six Day (27)	<ol style="list-style-type: none"> 1. Osteoclastic activity maximal. 2. Osteoblastic activity minimal. 3. Remnants of osteoid layer remain. 4. Dense collagenous fibroperiosteum noted.
D. Fourteen Day (35)	<ol style="list-style-type: none"> 1. Osteoclastic activity decreased. 2. Osteoblastic activity increased. 3. Minimal osteoid deposition noted. 4. Fibroperiosteum present.
E. Twenty-one Day (42)	<ol style="list-style-type: none"> 1. Osteoclastic activity minimal. 2. Osteoblastic activity maximal. 3. Osteoid deposition noted. 4. Fibroperiosteum present.
F. Sixty Day (61)	<ol style="list-style-type: none"> 1. Lamellated bone deposition noted. 2. Osteoblastic activity persists. 3. Osteoclastic activity minimal. 4. Fibroperiosteum present.

CHAPTER V

DISCUSSION

A. Introduction

This investigation was conducted to study histologically the effect of re-injury upon the sequence of healing of a surgical wound.

The original surgical wound, a split thickness mucosal flap, was prepared, reapposed, fixed, and allowed to heal twenty-one days. The re-injury or as referred to in this study, the surgical re-entry, was prepared in the same area, reapposed, fixed and the experimental animals sacrificed at specified time intervals following the surgical re-entry to obtain sections for a histologic chronology of the healing sequence following the re-entry.

The re-entry interval of twenty-one days was selected on the basis of previous investigations of wound healing. Staffileno (1961) indicated that major osteoblastic activity occurred during the twenty-one day period following initial surgery. Wilderman (1959) reported greatest bone formation to have occurred from twenty to twenty-eight days post-operatively and Douglas (1958) reported that maximal acceleration effect between wounding and resuturing was noted at the twenty-one day interval. It was proposed that the period of maximum osteoblastic activity following the initial surgical entry should be utilized in an attempt to capitalize upon this major cellular activity.

One concept of wound healing proposes that the repeat injury does not have to undergo a period of wound debridement or reorganization and thus a more rapid and extensive healing sequence will result.

On this basis, following surgery there is a mobilization and follow up of cellular activity, a portion of which results in the formation of osteoid

tissue over the vestibular and crestal bone. Following this osteoid tissue deposition, it was proposed that re-entry surgery could be accomplished with lessened alveolar bone resorption by benefiting from the advanced cellular activity and the protective, resorption-resistant, osteoid tissue deposited on the alveolar bone surface.

An investigation to study histologically the repair of alveolar bone, connective tissue, and epithelium following preparation of a split thickness mucosal flap was completed by Staffileno (1961). His study involved preparation of a split thickness mucosal flap with reapposition and a post-operative histologic healing sequence study. His summary of findings was as follows:

"The early hours showed a fusion of the flap with the remaining fixed connective tissue by means of a fibrin coagulum. Inflammation took place immediately and continued through six days. Reserve cells had proliferated at this time and continued to proliferate throughout the healing period.

"After forty-eight hours the epithelium responded by increased activity to repair the wound defect. In six days the epithelial regeneration was accomplished. At the same time, the connective tissue was highly active and differentiation of many cells occurred. Lymphocytes, phagocytes, fibroblasts, osteoclasts, osteoblasts, and endothelial cells were numerous and mobilized to bring about repair of this split thickness flap.

"Osteoclastic resorption was exhibited most during the six days post-operative time. After that their activity diminished until their complete disappearance between fourteen and twenty-one days.

"Osteoblastic activity was observed in the marrow spaces two days after surgery. The result was the formation of osteophytic bone to compensate for the loss on the vestibular plate side. However, the intense osteoblastic bone formation was evident at twenty-one days in the vestibular plate and crestal areas.

"In sixty days all tissues had repaired themselves and the result showed no anatomic deformity. All healing of the tissues was restored to a preoperative status."

This investigation, on the other hand, superimposes one injury upon another with an intermediate healing period interposed between injuries. The general healing patterns of the two investigations correspond with noted modifications in time increments and quantitative results.

B. Epithelium

The epithelium following the re-entry surgery at the zero hour time interval reflected an incision through the epithelial layer into the lamina propria of the connective tissue. The incision edges showed frayed or ragged epithelial cells bordering the cut edges and folding over slightly into the incision proper. By the forty-eight hour time interval, the epithelium had by a migratory and sliding action into, over, and through the clot, effected a joining of opposite epithelial layers to create a more or less permanent covering for the wound. This was characteristic and occurred despite a lack of complete organization of the underlying tissues. This seal, or covering, though only one or two cells in thickness indicates a purposeful body response for protection of the underlying structures.

This rapid rate of epithelial repair noted following the re-entry

surgery at forty-eight hours corresponds to findings reported in a re-injury study conducted by Stahl (1962). In this study he utilized Long Evans strain of rats for experimental animals.

The rats received gingival and tooth injury at the mesial wall of the maxillary left and right first molars by utilizing a No. 6 round bur to drill a cavity into the mesial wall of the teeth with simultaneous mesial gingival injury. Twelve days following the initial injury, the wound was found to be epithelized with a new gingival papilla present at the mesio-gingival margin. The healed site was then scraped to the level of the alveolar crest, thus creating a re-injury. Following this re-injury, epithelization was noted at five days with epithelium adhering to the cut dentinal wall and tooth surface against which it healed.

This showed a marked decrease in time of epithelization comparing the five days following the re-injury to the twelve days required for epithelization following the initial injury.

Following completion of this initial epithelial seal or covering, epithelization progressed toward the eventual goal of restoring the epithelium to preoperative status. This in effect is a quantitative restoration to regain the lost dimensional cellular relationships. The epithelial layer was restored to preoperative dimensions by the fourteenth day post-operatively but, as noted in the findings, normal epithelial morphology was not present, in that abnormally shaped epithelial extensions persisted into the incision area with noted surface defects.

Mitosis occurred in the epithelium only after the cell layers had increased in thickness (six days) and it is obvious that the productivity

and thickening of the cell layers is more physical than proliferative due to the fact that mitosis was not apparent until the cell numbers had increased. It is also obvious that there was no accelerated mitotic activity in the adjacent cells to cause the noted migration of epithelial cells to seal the incision. At no time were mitotic figures present in the epithelial layers in other than limited numbers.

The epithelial attachment in fourteen days had regenerated in form and position relative to its preoperative status. There was no anatomical deformity after re-entry surgery and the dimension of the distance of the alveolar crest to epithelial attachment and the length of epithelial attachment remained the same as in the control specimens.

Comparatively, this epithelial repair is more rapid than that reported by Staffileno. Return to preoperative maturity and morphology on the other hand was comparable to that reported by Staffileno in that a preoperative status was attained in this investigation twenty-one days post-operatively. It can be noted then, from this investigation, that epithelization following a second injury resulted in a more rapid epithelial repair to the incision area.

C. Inflammation

Inflammation was noted in the clot and surrounding connective tissues immediately following preparation of the second split thickness mucosal flap. Primarily this was characterized by congested and dilated capillaries, margination of polymorphonuclear leukocytes, and infiltration of the surrounding tissues by extravasated acute inflammatory cells. This acute inflammatory cell infiltration was most pronounced in the gingival zone of

the split thickness mucosal flap and clot area and was for all practical consideration absent in the connective tissues attached to the vestibular surface of the alveolar bone. This acute inflammatory process persisted in the forty-eight hour specimens with spread of the inflammation to all zones of the split thickness mucosal flap, connective tissue fixed to the vestibular surface of alveolar bone, and into the crestal area of the periodontal ligament space.

Six days following preparation of the second split thickness mucosal flap the inflammatory process had subsided and was noted only in the connective tissues of the fixed portion of the surgical site adjacent to the vestibular alveolar bone. From this six day time interval the inflammatory processes decreased to near normal with chronic inflammation being noted in the gingival zone of the mucosa only in later specimens.

These findings were in direct accord with those noted by Staffileno in his investigation. A correlative progression of the inflammatory process from the gingival areas to the deeper incision areas was noted with subsiding of this process at the six day interval in most areas and with persisting chronic inflammation being noted in the gingival areas of all subsequent specimens.

D. Connective Tissue

1. Role of the Undifferentiated Mesenchymal Cell.

The role of the undifferentiated mesenchymal cell in repair must be discussed in order to adequately portray its most important role in this phenomena.

In the reproduction of connective tissue, a source of new connective

tissue producing cells must be available in order to accomplish reproduction. Toto and Abati (1963) have stated that it is evident that differentiated cells do not have the capacity to undergo mitotic division. Without this capacity it is impossible for them to participate in the formation of granulation tissue, or, subsequently, connective tissue since granulation tissue is the precursor to connective tissue repair and formation.

As different cells are needed for granulation tissue production, only the undifferentiated mesenchymal cells or, as they refer to the, undifferentiated reserve connective tissue cells, are available to meet this need. Therefore, they must be able not only to proliferate but also to differentiate into the cells needed. Differentiated cells such as fibroblasts do not have this capacity to undergo mitotic division or proliferate; therefore, all such cells must arise from undifferentiated mesenchymal cells.

As these undifferentiated mesenchymal cells increased, they began to differentiate into specific cell types. Initially, they were noted to differentiate into osteoclasts, along the outer vestibular alveolar plate, and osteoblasts in the marrow spaces (0-6 days).

Concurrently, in the incision area and the periosteum there was differentiation to fibroblasts (0-6 days).

There was always a general overlapping of activities of osteoclasts, osteoblasts and fibroblasts during the repair periods.

2. Connective Tissue Proliferation and Maturation.

a. Incision Area

The connective tissue proliferation and maturation in the area of the incision progressed comparable to that healing pattern presented by

Staffileno in his investigation of healing of a split thickness mucosal flap. The re-injury or surgical re-entry does not appear to have altered the normal connective tissue reparative progression.

As the acute inflammatory process began to subside there was noted an increased production of undifferentiated mesenchymal cells in the perivascular spaces. Thereafter, the production of these cells continued and contributed to the regeneration and repair of the connective tissue. These new undifferentiated mesenchymal cells act as parent cells, which differentiate into fibroblasts.

At the six day level of repair this increased production was characterized by an incision area filled with connective tissue of high cellularity with immature disoriented fibrils of small dimensions. Incomplete organization of fiber bundles was noted generalized throughout the incision area.

In the fourteen and twenty-one day post-operative specimens there was a lessened cellular-fiber ratio as the repair process progressed. The collagen fiber bundles became more organized and oriented to meet the functional demands.

The connective tissues were not considered to have matured to a state considered normal until the sixty day post-operative time interval. At that time the cellular fiber ratio was normal, normally oriented, and mature fiber bundles were noted throughout the incision area.

The connective tissue attachment showed no dimensional change after re-entry surgery.

It has been shown by some investigators that resutured or re-injured wounds result in a higher tensile strength following healing (Sovlov and

Dumphy, 1954 - Douglas, 1958).

This investigation did not show any decrease in connective tissue healing time or any advantage to be gained in the connective tissue repair from a histologic standpoint. Incrementally, the healing pattern followed that presented by Staffileno after a single injury. Therefore, it must be observed that no gains in connective tissue repair were noted following re-entry.

b. Periosteal Area.

The periosteal connective tissue area presented a pattern of tissue proliferation and maturation differing from that noted in the incision area. The periosteal area, as previously described, remained intact on the vestibular surface of alveolar bone following preparation of the second split thickness mucosal flap.

Following the initial and second injuries, intense cellular activity was noted occurring in the cambium layer of this periosteal connective tissue zone with mitotic figures noted. Resultant from this mitosis and increased fibroblastic activity there were noted larger, denser fiber bundles in the fibrous layer of the periosteum. This zone of extremely dense connective tissue is thought to have afforded the vestibular alveolar bone surface further resistance to resorption.

E. Osteoclasia and Osteogenesis

The bone tissue was not exposed in split thickness mucosal surgery, but was always protected by the retained periosteal tissue covering; however, osteoclastic resorption was noted. This resorption was noted to a minimum degree in the narrow spaces with the major portion of the resorption taking place in the area of the circumferential lamellae of the vestibular alveolar plate.

Resultant from the initial surgery, the vestibular alveolar surface presented a scalloped pattern reflecting intense arrested osteoclastic activity prior to re-entry. This resorptive pattern had, in the time intervals up to re-entry, penetrated the outer circumferential lamellae and encroached into the outer Haversian systems. Osteoclastic activity during the zero hour specimen following re-entry had declined and osteogenesis had begun. This osteoblastic activity resulted in the formation, by the time of re-entry, of an osteoid layer upon the vestibular alveolar surface and alveolar crest areas. So, up to the re-entry period, the vestibular alveolar surface as a result of the initial surgery had undergone intense osteoclasia and osteogenesis with deposition of osteoid tissue.

At twenty-one days following the initial surgical entry, a surgical re-entry was performed. As a result of the trauma of this second injury, a new sequence of alveolar resorption and repair was initiated.

Maximum osteoclastic resorption was once again noted in the six day specimens. This resorption was in a lesser degree than that noted following the initial entry, probably as a result of resistance to resorption afforded by the presence of the dense fibroperiosteum and the resorption resistant osteoid tissue layer apposed on these surfaces following the initial surgical entry.

In the six day specimens osteoblastic activity was noted in the marrow spaces probably as a compensatory action to strengthen the vestibular bone from within to offset the losses due to the osteoclastic resorption on the vestibular alveolar plate.

This osteoblastic activity increased in the fourteen day specimens

along the vestibular alveolar plate area and was noted at maximum in the twenty-one day specimens. Osteoid tissue apposition was noted in the twenty-one day specimens on the vestibular and crestal alveolar surfaces.

The sixty day specimens showed a complete restoration of the alveolar crest to preoperative height but with a reduction in thickness of vestibular alveolar bone in a bucco-lingual dimension. All relationships of the alveolar crest to the dento-gingival junction were restored to preoperative status.

The osteoclastic resorption noted following the second surgical injury was of a lesser magnitude than that noted following the initial surgical injury. Both surgical procedures were performed in an identical manner. This lessened loss of bone following the second procedure must then be the result of increased localized resistance. This increased localized resistance is attributed to two factors, both resultant from the initial surgical injury: the dense fibroperiosteum noted on the vestibular alveolar surface following the initial surgery and the resorption resistant osteoid layer apposed on the vestibular alveolar surface noted at the time of second surgery.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to study histologically the effect of surgical re-entry upon the healing sequence of a split thickness mucosal flap.

Five adult mongrel dogs with fully erupted permanent teeth were used as experimental subjects. Surgery was performed in the region of the maxillary and mandibular premolar teeth. The animals were sacrificed at specified intervals following re-entry surgery.

The following were the major observations:

1. Microscopically, the epithelium in the incision area of the second split thickness mucosal flap covered the clot in forty-eight hours.
2. Connective tissue in the incision area following preparation of the second split thickness mucosal flap did not show any decrease in healing time nor any increased healing potential.
3. Following initial surgery, the vestibular alveolar bone underwent osteoclastic resorption and subsequent osteoblastic repair with apposition of an osteoid layer on the vestibular alveolar surface prior to re-entry surgery.
4. Following initial surgery, there was increased fibroblastic activity in the periosteal connective tissue layer adjacent to the vestibular alveolar bone. This increased fibroblastic activity resulted in the development of a dense fibroperiosteum.
5. Loss of alveolar bone due to osteoclastic resorption was noted following both initial and re-entry surgery. That loss noted following the surgical

re-entry was in a lesser magnitude than the osteoclastic resorption following initial surgical entry.

From the noted observations it is concluded that:

1. Epithelial regeneration following a second surgical injury is more rapid than that following an initial surgical injury.
2. Connective tissue repair is neither accelerated nor enhanced by initiation of a second surgical injury.
3. Localized resistance to osteoclastic resorption is greater to a second surgical injury than to initial surgical injury.
4. The noted localized resistance to the osteoclastic resorption of a second surgical injury results from the first surgical procedure which induces the apposition of a resorption resistant osteoid layer and the development of a protective dense fibroperiosteal connective tissue layer.

CHAPTER VII

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CHAPTER VIII

APPENDIX

- 1) Clinical photographs
- 2) Photomicrographs
- 3) Diagrammatic illustrations

PLATE I

Figure 1

Clinical photograph of a preoperative premolar area.

Figure 2

Photomicrograph of the control specimen. (X43)

Note:

- 1) Normal continuity of epithelium, lamina propria, and periosteal connective tissue.
- 2) Contour and height of vestibular alveolar bone.



Figure 1

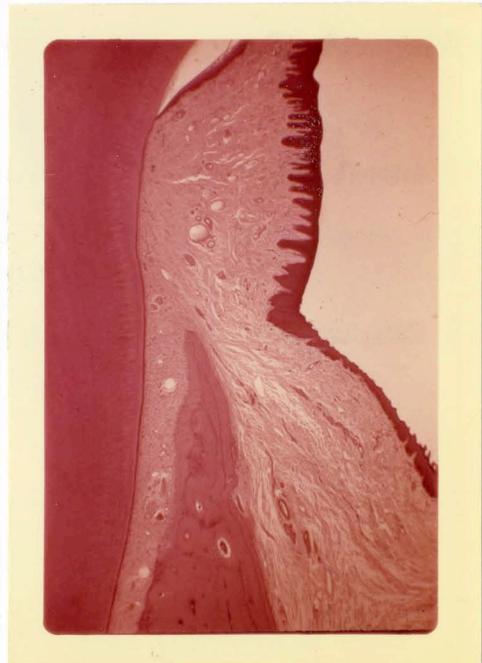


Figure 2

PLATE II

Figure 3

Clinical photograph of the split thickness mucosal flap in the premolar area demonstrating the periosteal connective tissue remaining on the vestibular alveolar bone surface.

Figure 4

Clinical photograph of the premolar area of operation with split thickness mucosal flap sutured in position.



Figure 3



Figure 4

PLATE III

Figure 5

Photomicrograph of zero-hour specimen. (X₁0)

Notes:

- 1) Early fusion of split thickness mucosal flap and remaining attached connective tissue, mediated by the clot.
- 2) Undermining resorption of vestibular alveolar crest.
- 3) Remaining connective tissue still attached to the tooth and periosteum.

Figure 6

Photomicrograph of zero hour specimen. (X₁00)

Notes:

- 1) Undermining resorption of vestibular alveolar crest.
- 2) Differentiation of osteoblasts adjacent to resorbed alveolar bone surface.
- 3) Apposition of osteoid tissue on periosteal and periodontal surfaces of alveolar crest.



Figure 5

Figure 6

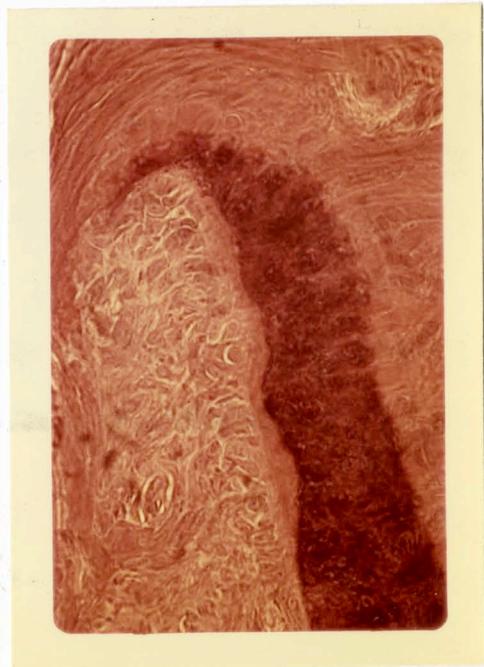


PLATE IV

Figure 7

Clinical photograph of operated area two days after re-entry surgery.

Figure 8

Photomicrograph of two day post-operative specimen. (X140)

Note:

- 1) Epithelium bridging superficial incision area.
- 2) Fibrinous clot occupying incision area.

Figure 9

Photomicrograph of two day post-operative specimen. (X160)

Note:

- 1) Infiltration of polymorphonuclear leukocytes.
- 2) Bridging of incision by epithelium.
- 3) Intracellular and intercellular edema of superficial epithelial cells.



Figure 7



Figure 8



Figure 9

PLATE V

Figure 10

Photomicrograph of two day post-operative specimen. (X100)

Note:

- 1) Undermining resorption of vestibular alveolar crest.
- 2) Osteoblastic activity adjacent to resorbed vestibular alveolar bone surface.

Figure 11

Photomicrograph of two day post-operative specimen. (X100)

Note:

- 1) Osteoblastic activity adjacent to vestibular alveolar bone surface.
- 2) Osteoclastic activity.
- 3) Cellular activity of cambium layer of periosteal connective tissue.
- 4) Proliferating undifferentiated mesenchymal cells from marrow space.



Figure 10

Figure 11

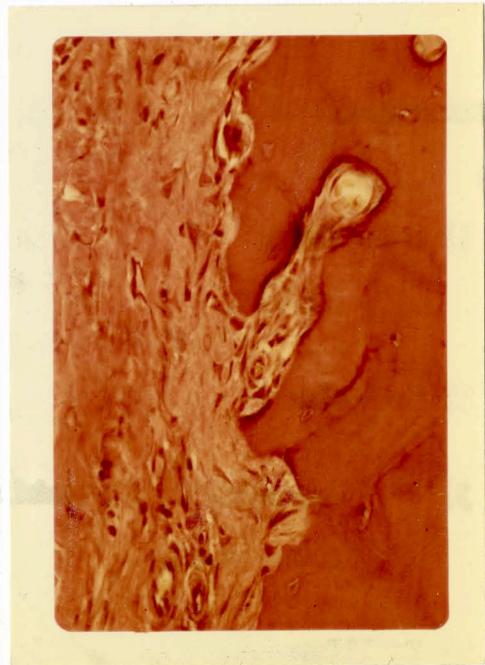


PLATE VI

Figure 12

Clinical photograph of operative site six days after re-entry surgery.

Figure 13

Photomicrograph of six day post-operative specimen. (X40)

Note:

- 1) Scalloped resorption pattern of vestibular alveolar plate.
- 2) Incomplete healing of connective tissue of incision area.

Figure 14

Photomicrograph of six day post-operative specimen. (X400)

Notes:

- 1) Osteoclastic action.
- 2) Remnant of previous osteoid layer between Howship's lacunae.
- 3) Cellular activity of cambium layer of periosteal connective tissue.



Figure 12



Figure 13

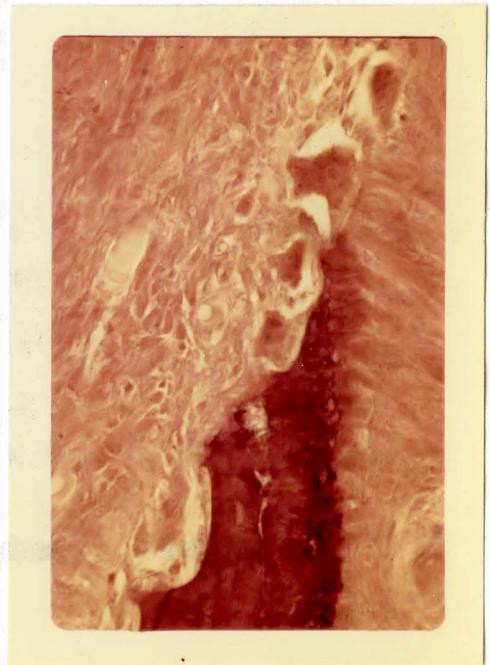


Figure 14

PLATE VII

Figure 15

Clinical photograph of operated area fourteen days after re-entry surgery.

Figure 16

Photomicrograph of fourteen day post-operative specimen. (X10)

Note:

- 1) Epithelial scarring in incision area.



Figure 15



Figure 16

PLATE VIII

Figure 17

Photomicrograph of fourteen day post-operative specimen. (X100)

Note:

- 1) Vascularity of periodontal ligament space.
- 2) Osteoclastic resorption of vestibular alveolar bone surface.
- 3) Dense fibroperiosteal connective tissue.

Figure 18

Photomicrograph of fourteen day post-operative specimen. (X100)

Note:

- 1) Osteoblastic activity in marrow space.
- 2) Proliferating undifferentiated mesenchymal cells adjacent to capillaries.



Figure 17

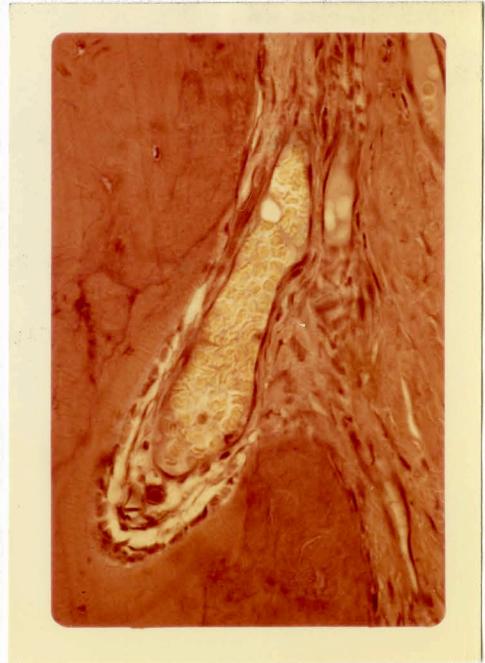


Figure 18

PLATE IX

Figure 19

Clinical photograph of operated area twenty-one days following re-entry surgery.

Figure 20

Photomicrograph of twenty-one day specimen. (X25)

Note:

- 1) Resorption of vestibular alveolar crest area.
- 2) Epithelial repair complete.
- 3) Incision area discernible due to increased cellularity.



Figure 19



Figure 20

PLATE X

Figure 21

Photomicrograph of twenty-one day post-operative specimen. (X100)

Note:

- 1) Osteoblastic activity adjacent to vestibular alveolar bone surface.

Figure 22

Photomicrograph of twenty-one day post-operative specimen. (X100)

Note:

- 1) Osteoblastic layer adjacent to vestibular alveolar bone surface.
- 2) Osteoid tissue appositional layer.
- 3) Reversal lines.

Figure 23

Photomicrograph of twenty-one day post-operative specimen. (X630)

Note:

- 1) Osteoblastic layer adjacent to vestibular alveolar bone surface.
- 2) Osteoid tissue appositional layer.
- 3) Proliferating undifferentiated mesenchymal cells adjacent to capillaries.



Figure 21

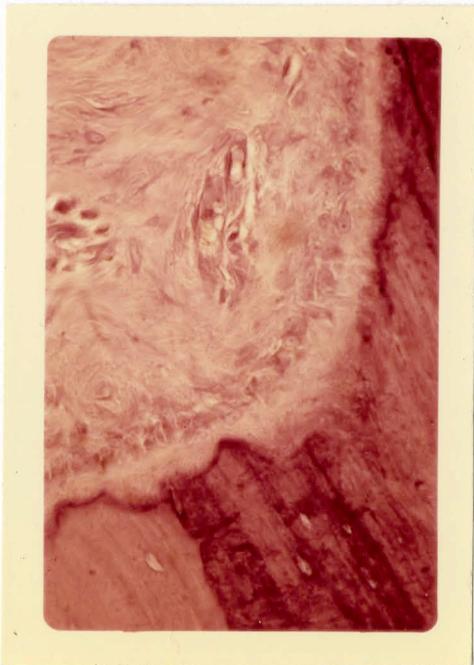


Figure 22



Figure 23

PLATE XI

Figure 24

Clinical photograph of operated areas sixty days following re-entry surgery.

Figure 25

Photomicrograph of sixty day post-operative specimen. (X10)

Notes:

- 1) Connective tissue restored to normal.
- 2) Vestibular alveolar crest restored to normal height.

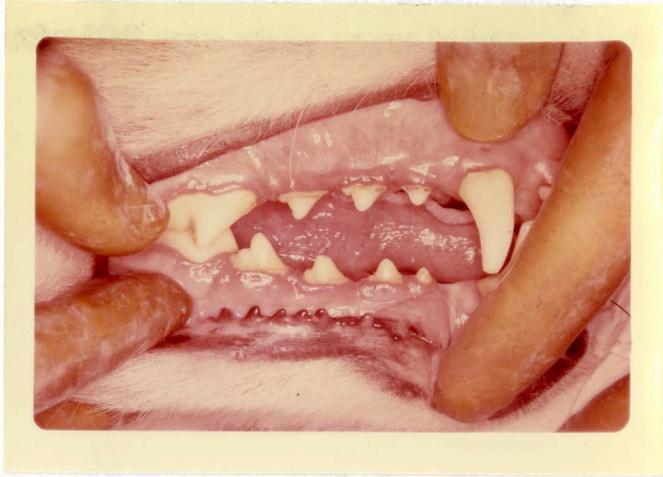


Figure 24



Figure 25

PLATE XIII

Diagrammatic Illustrations

Figure 26

Diagram of split thickness mucosal flap

- A - Enamel
- B - Epithelium
- C - Remaining tag of epithelial attachment
- D - Clot
- E - Remaining stump of connective tissue
- F - Alveolar bone
- G - Periosteal connective tissue covering bone
- H - Tooth

Figure 27

Diagram of three zones of split thickness mucosal flap.

DIAGRAMMATIC ILLUSTRATIONS

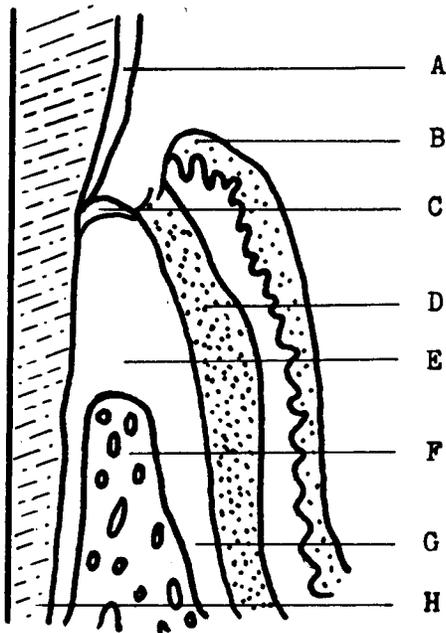


Figure 26

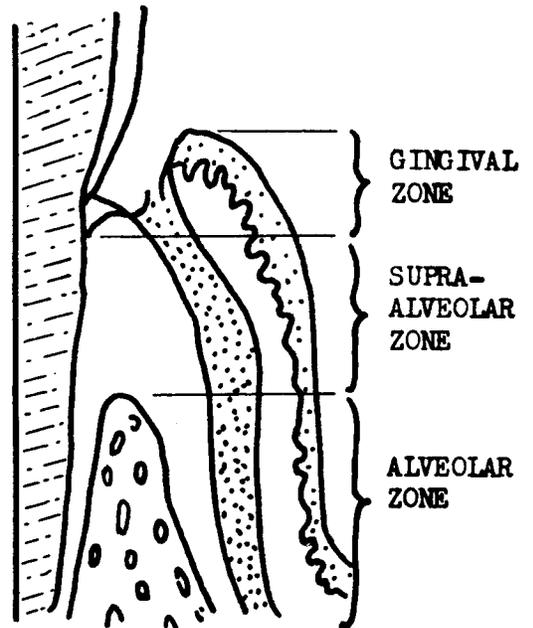


Figure 27

PLATE XIII

Figure 28

Graph comparing osteoblastic activity in two different experiments. Abscissa is in days while ordinate is expressed in arbitrary degrees of 1 through 5.

Legend

Solid line - split thickness mucosal flap re-entry surgery - (Giblin)

Dashed line - split thickness mucosal flap surgery - (Staffileno)

OSTEOBLASTIC ACTIVITY

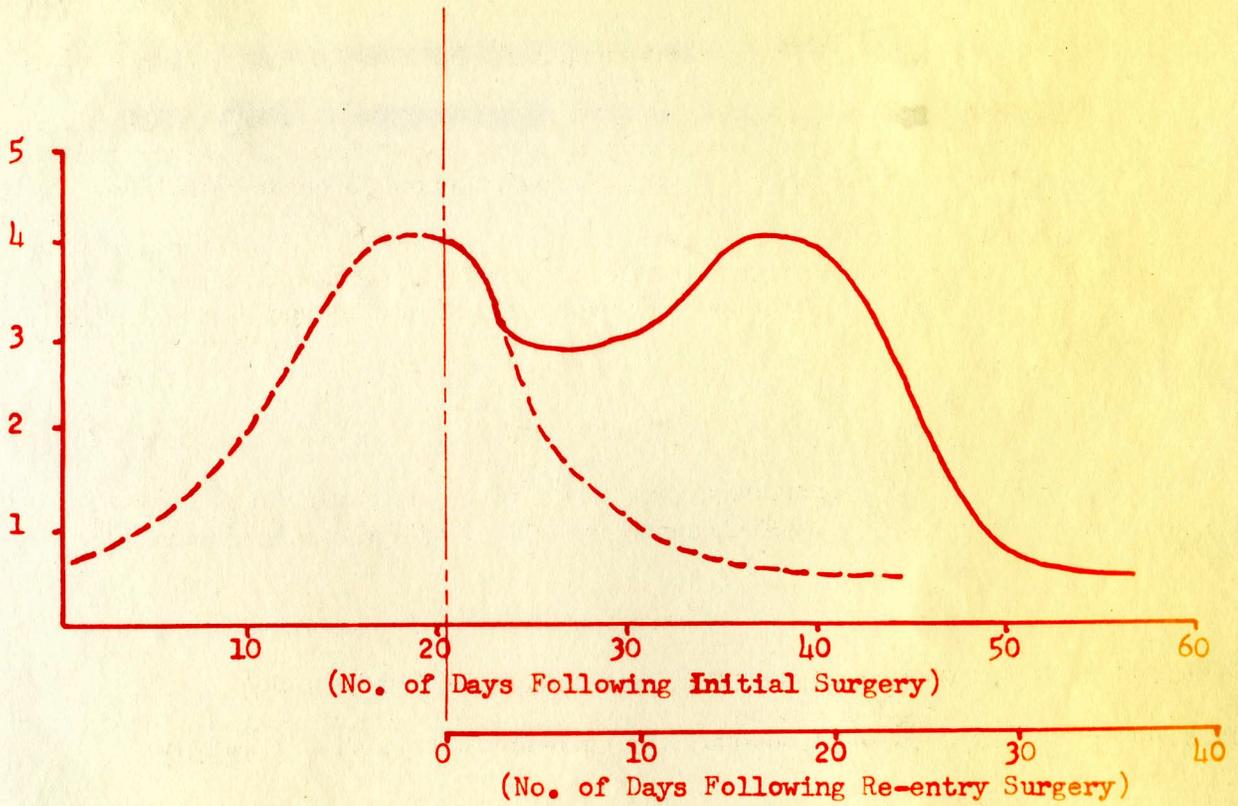


Figure 28

PLATE XIV

Figure 29

Graph contrasting osteoblastic activity in two different experiments. Abscissa is in days while ordinate is expressed in arbitrary degrees of 1 through 5.

Legend

Solid line - split thickness mucosal flap re-entry surgery - (Giblin)

Dashed line - split thickness mucosal flap surgery - (Staffileno)

OSTEOCLASTIC ACTIVITY

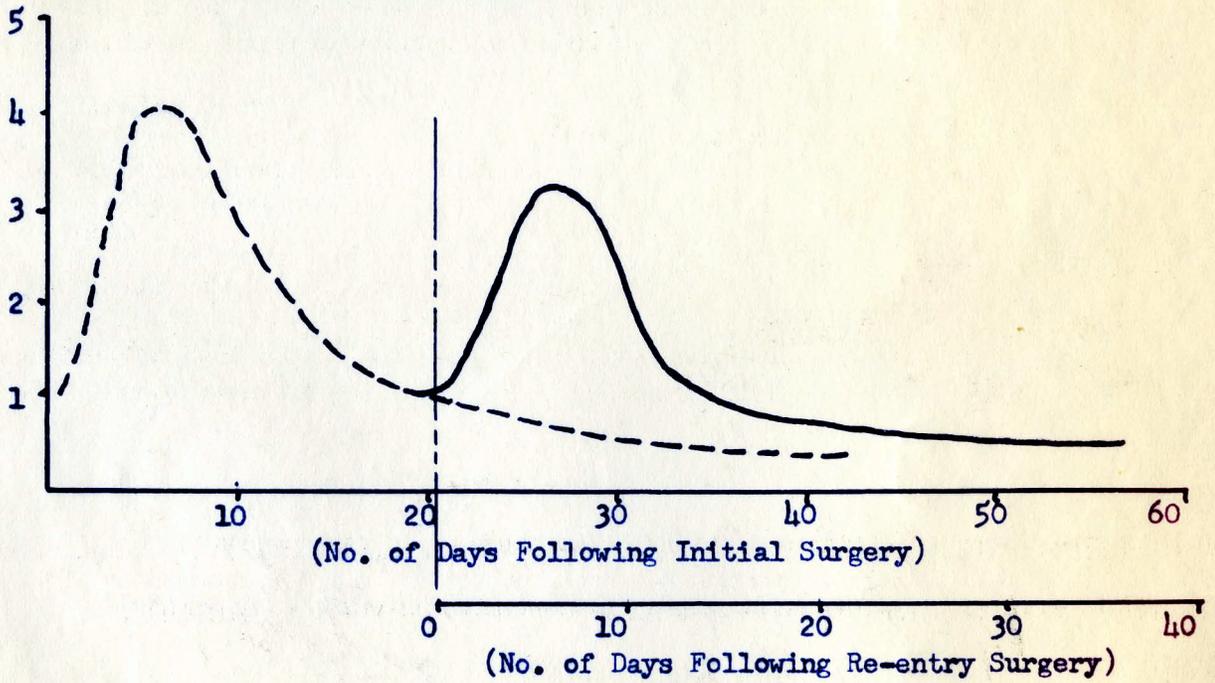


Figure 30

PLATE XVI

Figure 31

Graph contrasting osteoclastic activity in two different experiments. Abscissa is in days while ordinate is expressed in arbitrary degrees of 1 through 5.

Legend

Solid line - split thickness mucosal flap re-entry surgery - (Giblin)

Dashed line - split thickness mucosal flap surgery - (Staffileno)

OSTEOCLASTIC ACTIVITY

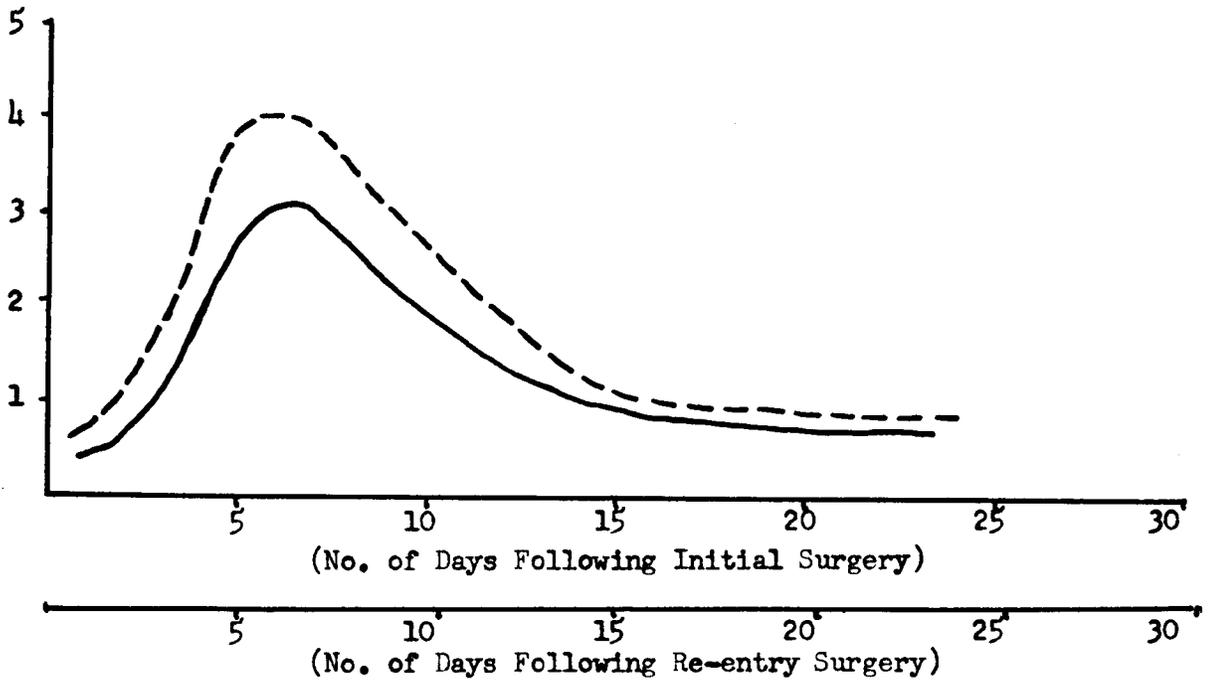


Figure 31

APPROVAL SHEET

The thesis submitted by Dr. James M. Giblin has been read and approved by three members of the Graduate School Faculty.

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

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

May 4, 1964
Date

Anthony W. Longino, BS, M.S., Ph.D.
Signature of Adviser