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Histogenesis of Repair of the Dental Supporting Tissues After Mucogingival Resection

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HISTOGENESIS OF REPAIR OF THE DENTAL SUPPORTING TISSUES 
AFTER MUCOGINGIVAL RESECTION 

by 
Malbern N. Wilderman 

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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LIFE

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CHAPTER I

INTRODUCTION

In recent years, a variety of surgical techniques have evolved for periodontal therapy where the attached gingiva extending from the tooth to mucogingival junction is inadequate or lacking. Clinical evidence has been introduced supporting the hypothesis that a complete regeneration of new and extended gingival occurs after surgical therapy.

The purpose of this histological investigation is to clarify the mechanism of regeneration of supporting tissues of the tooth after creating a surgical wound simulating the clinician's surgical therapy. The procedure exposed the dentogingival junction and vestibular bone by removing the gingiva and wound area periosteum.
CHAPTER II

REVIEW OF THE LITERATURE

I. Introduction

The mucous membrane surrounding the teeth, the gingiva, is subjected to forces of friction and pressure during mastication. The character of the tissue shows that it is adapted to meet these stresses.

This tissue is characterized by the thickness and keratinization of the epithelium, the thickness, density and firmness of collagenous fibers and finally its immovable attachment to the teeth and alveolar bone. The gingiva is sharply limited on the outer surface of the tooth jaws by a scalloped line, mucogingival junction, which separates it from the alveolar mucosa.

The structure of the alveolar mucosa, contrasts to that of the gingiva. It is characterized by a relatively thin non-keratinized epithelium and by the thickness of the lamina propria and submucosa, which is loosely and moveably attached to the deep structures to allow for the movement of the lips, cheek, and tongue.

The recognition of structural differences between the gingiva and alveolar mucosa and their adaptation to functional demands are keys to the understanding of the adverse effects if the gin-
giva is lost due to pathology. If the gingiva is lost, the remaining alveolar mucosa is not structurally adapted to resist the normal functional stresses, even after successful treatment of the initial causative pathological process. The field of mucogingival surgery was developed to remedy this structural deformity by attempting to re-create a new gingival tissue that will be functionally more acceptable.

II. Clinical Studies

Mucogingival problems have been classified by Orban (1958). This classification lists the clinical indications for mucogingival surgery and suggests the term gingival replacement operation to indicate the procedure. Other synonymous terms suggested are gingival extension operation, push-back, pouch operation, local extension of the vestibular trough, and repositioning of the attached gingiva (Gottsegen, 1954; Nabers, 1954; Goldman, 1956; Ariauo, 1957).

Friedman (1957), in a careful clinical study observed the healing of the mucogingival resection, and then concerning the origin of the gingiva in healing he quotes:

It has been contended that the new gingiva is formed from granulation tissue which comes from bone, and that a functional adaptation may occur to produce gingiva. It is important to know the origin of the granulation tissue that produces the new gingiva. Since we are dealing here with attempts at extending and creating new attached gingiva where it did not exist, the surgery is
done without prognostic conceptions unless we know if we will get new gingiva and how it is achieved. Therapeutically it becomes important to have this knowledge, and having it permits a wide range of surgical planning and a reasonable prediction of the results of the procedures.

The granulation tissue has three probable sources in the wound area. It may come from the cut edge of the alveolar mucosa, the bone, or the cut edge of the gingiva. From clinical observations of operations such as the pushback, where large areas of bone were exposed, the author noted that the tissue granulated from the interproximal gingival papillae and circled the cervical areas of the teeth. In the early stages of healing, then, there would be a thin rim of granulation tissue (or newly formed gingiva) around the cervical area of the teeth, an island of exposed bone, and the cut edge of the alveolar mucosa. As the healing progressed, the narrow band of gingiva would granulate apically until gradually it covered the bone and joined with the slowly granulating edge of the alveolar mucosa. The rapidity with which the gingiva granulates, in contrast to the alveolar mucosa and bone, accounts for the new gingiva and the increased zone of attached gingiva noted.

III. Histological Studies

Further studies at the histological level were attempted to answer the questions: First, what is the nature of the "new" gingiva? Second, what is the origin, rate of healing and mechanism of healing of this new tissue?

Human histological studies conducted determined the differentiation of replacement tissue (Grant, 1956; Ivancie, 1957). After repositioning the gingiva apically, it was evident that alveolar mucosa with elastic fibers was replaced with collagenous
connective tissue within eight months. Epithelial ridge formation, keratinization and orientation of mature collagen fiber bundles may be a result of functional adaptation. The new tissue is described as a less-differentiation and functional immature gingiva.

No clear cut studies relative to the origin, rate of healing and mechanism of healing of wounds after mucogingival surgery, have appeared in the literature. However, reference to previous studies of wound healing of mucous membrane, though less complex in nature may shed some light on this problem.

Abraded gingiva papilla (Kollar, Wentz, Orban, 1955) with aluminum oxide by an abrasive technique indicated a low vascular response with minimal inflammatory cellular reactions. Healing began at twelve hours with epithelial cell migrating from the borders of the wound and within eighteen hours completely covered the wound. Epithelial mitosis was evident at twenty-four hours.

A stab wound, one centimeter long in the gingiva (Mittelman, 1958), one and one-half millimeters deep, penetrated the epithelium and extended into the connective tissue. The inflammatory wound was covered in twenty-four hours. Since inflammation continued to be present at seventy-two hours in the papillary connective tissue, the conclusion was that epithelial regeneration is more rapid than connective tissue regeneration.

When the gingiva above the alveolar crest is removed (Orban and Archer, 1945), regeneration occurred by proliferation of
granulation tissue from the excised lamina propria within four to nine days. At fourteen days the epithelium covered the wound.

IV. Studies of Healing of the Dentogingival Junction

The repair of the dentogingival junction after surgical resection, the relation of the tissues, the amount of deformity is of special importance. Since the structural relations of the healing oral mucous membrane to the tooth may be different than the relation between the healing mucous membrane and bone.

Martino, 1958; Linghorne and O'Connell, 1950-55; Buebe, 1934, 1947; Swenson, 1947; and Linghorne, 1957 were able to demonstrate a new attachment of the mucous membrane to the tooth. However, the repair occurred with a deformity.

Martino (1958), created 10 mm. surgical pockets in ten young adult dogs with a flap producing operation and removing 6 x 7 mm. of crestal bone. A medicated pack between the flap and tooth was removed after three weeks. A new connective tissue of 1.25 to 2.50 mm. formed and attached to the tooth early, but organized slowly.

An epithelial attachment was evident by the twenty-third day of 1.50 to 2.50 mm. in length. Recession of the gingiva decreased the depth of pocket by one-third. Small amount of new bone was evident at the alveolar crest.

The purpose of this study is now clarified. The questions
after survey of the literature indicate that the mechanism of healing after mucogingival surgery needs clarification. The questions of timing, where, how and how much repair of the tissue actually occurs will be our problem. The correlation of these findings to the more careful clinical studies will be our secondary objective.
CHAPTER III

Materials and Methods

Ten young dogs with erupted permanent teeth were used in this experiment.

The surgery was performed in the right maxilla by making a vertical vestibular incision mesial to the first premolar and another distal to the third premolar. Two similar incisions were executed in the right mandible, one mesial to the second premolar and the other distal to the fourth premolar (Plate I, Fig. 1). All incisions penetrated the fibrous periosteum. A knife was used to form a flap between the incisions. The flap consisted of one-half of the papilla, the vestibular marginal and attached gingiva with the underlying periosteum. Clinically, the flap had for its base the mucogingival junction.

The cemento-enamel junction of the exposed teeth were carefully scraped and carefully polished with sulci wheel to remove all soft tissue attachments. The exposed bone was thoroughly scraped to remove any adhering periosteal remnants that remained. Areas were checked and rechecked visually for any possible tissue fragments. The soft tissue flap was excised at the mucogingival base with scissors. The cut wound base was sutured to the periosteum of the remaining alveolar mucosa with silk suture. All
All sutures were removed after seven days. Clinical measurements revealed an exposure of 5 by 32.5 mm. of alveolar bone. The amounts were repeated in every animal.

The surgery was performed under general anesthetic using one-half cc. of nembutal sodium per pound animal weight.

The only medication was an immediate post-operative intramuscular injection of six hundred thousand units of penicillin. A soft diet of pulverized dog meal mixed with water was fed throughout the experiment.

Color photographs were taken pre-operative, post-operative, and at time of sacrifice.

The animals were sacrificed by overdose of nembutal injected directly into the heart at the following intervals:

0 hrs.
2 days
4 days
6 days
10 days
14 days
21 days
28 days
93 days
185 days
The maxillary second premolar and the mandibular third premolar areas were considered central wound areas for histological examination.

Controls were taken from the areas of the unoperated opposite left jaw.

Histological examination of the operated areas was secured by the following method:

- **Fixation** - 10 percent neutral formalin solution
- **Decalcification** - large quantities of 5 percent aqueous nitric acid solution observed every two days until completely decalcified.

Specimen washed in running water for twenty-four hours, then neutralized in 10 percent formalin to which an excess of calcium or magnesium carbonate has been added; again washed in running water for 24 to 48 hours.

- **Dehydration** - 75 percent alcohol (24 hours)
  - 95 percent alcohol (24 hours)
  - 100 percent alcohol (24 hours)
- **Ether alcohol** \((\frac{1}{2} \text{ and } \frac{3}{2})\) (24 hours)

- **Embedded** - celloidin (thin - 1 week)
  - medium - 1 week
  - thick - 1 week

- **Sectioned** - serially - oral vestibular

- **Stained** - hematoxylin and eosin

  Mallory method
CHAPTER IV

A. MACROSCOPIC FINDINGS

After the gingival periosteal flap, measuring approximately 5 x 32.5 mm., was elevated and reflected from the premolar teeth and vestibular plate of alveolar bone, it was severed from its base. The remaining wound edge was sutured to the periosseum of the alveolar mucosa (Plate I, Fig. 1). While a clot formed at the edges of the wound, the denuded bone appeared in the central area free from hemorrhage. Two to four days later the soft tissue edges of the wound were reddened, rolled and elevated and a hemorrhagic exudate seeped from beneath. At this time the bone had a gray appearance.

The sixth to tenth day post-operative period was marked by the appearance of a bright, red, granular new tissue growth which covered the wound surface. A velvity tissue growing over the wound surface covered the entire defect from margin to margin with only a few small islands of bone visible.

Fourteen to twenty-one days after the surgery, the rolled elevated wound lips appeared to have extended further into the central area. A collar of tissue elevated higher than the surrounding area was apparent around each tooth. The entire wound was covered by smooth, glistening epithelium. The wound area no
longer appeared bright red, but had assumed a dark red coloration. At twenty-eight days the wound was completely healed. The surface now appeared smooth. The rolled irregular edges of the wound had disappeared and only a trace of edge is now visible. The color was rose pink. This light pink color, together with lack of pigmentation and stippling contrasting with the surrounded unoperative tissue served to outline the healed wound. The gingiva appeared as a loose collar around the teeth.

At ninety-three days the tissue at the site of the surgical flap was not rolled but firm and appeared well attached to the teeth. This tissue was not pigmented or stippled.

One hundred and eighty five days after surgery, a slight pigmentation of the new tissue had become apparent near the teeth. Stippling was not observed. The new tissue of the wound could still be distinguished from adjacent tissue.

B. MICROSCOPIC FINDINGS

1. Osteoclastic Phase (2-10 days)
   a. Clot Formation at Wound Edges

   Two days after surgery, a blood clot, with a superficial layer of degenerated cells, covered the wound edges (Plate V, Fig. 5). No clot was observed upon the exposed bone in the central area.

   At the superior wound edge, in the area corresponding to the
cut interdental papilla (Plate II, Fig. 2) and in the severed dentogingival junction (Plate IV, Fig. 4) the clot was well defined and in contact with the teeth above the exposed alveolar bone. At the lateral and inferior wound edges, the clot covered the space between the irregular edges of the wound and underlying bone (Plate II, Fig. 2; Plate IV, Fig. 4).

b. Inflammation

At four to six days, three layers in the clot could be distinguished; a superficial necrotic layer consisting of bacteria and cellular debris; a middle layer consisting of large numbers of polymorphonuclear leucocytes; a deep layer which was invaded by capillaries from below. Many fibroblasts with large nuclei could be distinguished.

The lamina propria beneath the clot at the interdental area, the incised periodontal tissue (Plate V, Fig. 5) and the connective tissue at the line of suture showed the presence of inflammatory cells. Inflammation was evident also in bone marrow spaces beneath the exposed bone, in those that were exposed by surgery (Plate III, Fig. 3) and in some of the haversian canals (Plate V, Fig. 5) in the compact vestibular plate. This inflammation extended further into the bone tissue from the wound by the fourth day.

c. Pattern of Osteoclastic Resorption

Two days after surgery the lucunae in the superficial layers
of the exposed vestibular plate were empty. The depth of the acellular necrotic bone averages .1 mm. In all the specimens, the remaining bone tissue appeared viable (Plate VI, Fig. 6).

An undermining pattern of resorption was observed as early as two days after surgery, while the greatest activity was apparent between four and six days. The activity diminished considerably between six and ten days, and little was observed at fourteen days. The osteoclasts differentiated in all areas of viable connective tissue close to exposed bone. These areas were observed in the connective tissue of the bone marrow beneath the necrotic zone, the connective tissue at the wound edges and in the connective tissue of the periodontal ligament.

The pattern of resorption varied with the detailed relation of anatomy of the part. Two areas, the area of the interdental septa and the radicular area (or the area of socket) showed significant differences.

Interdental Area:

The interdental septum consisted of an inner spongy bone and covered by an oral and vestibular thick compact bone (Plate II, Fig. 2) and bounded mesial and distal by a thinner alveolar bone of the teeth.

At two and four days, osteoclasts appeared in the marrow spaces of the interdental areas beneath the vestibular plate and in the Haversian canals of the vestibular plate (Plate III, Fig. 3;
Plate XXVII, Fig. 28B). Osteoclasts also were evident periosteal at the cut interdental tissue.

The greatest osteoclastic activity occurred between four and six days. Osteoclastic resorption resulted into an enlargement of the marrow spaces and Haversian canals (Plate IX, Fig. 9; Plate XI, Fig. 11; Plate XXVII, Fig. 29C). Periosteal resorption occurred at the crest (Plate X, Fig. 10) and sutured base (Plate XII, Fig. 12). The surface of resorption sloped from the lingual crest to labial edge of the sutured base (Plate VII, Fig. 7; Plate XXVII, Fig. 29C). The result was the disappearance of the exposed vestibular and crestal bone. The exposed surface of the remaining spicules were covered by a bacterial plaque, while the deep surface showed osteoclastic resorption (Plate VIII, Fig. 8).

The loss of vestibular and crestal plate exposed the spongy bone trabeculae. At this site, bone marrow spaces were filled with young connective tissue (Plate XI, Fig. 11) consisting of many capillaries, young fibroblasts, fibers and undifferentiated mesenchymal cells. In addition, many polymorphonuclear leukocytes, indicative of acute inflammation were present in the young connective tissue.

Through the six to ten day period, as osteoclastic activity declined, the deeper bone marrow spaces displayed osteoblastic activity (Plate XI, Fig. 11).

In the radicular area, the lingual alveolar bone proper is
fused with the oral plate to about three-fourths the distance from alveolar crest to fundus of the tooth and the labial alveolar bone proper is fused with the vestibular plate the same three-fourths of the bone distance (Plate IV, Fig. 4). In the same areas the bundle or lamellated bone of the alveolar bone proper was distinguished from the oral and vestibular plate. The bone plates contained many Haversian canals and only a few marrow spaces. Near the fundus of the tooth, spongy bone intervened as the diverging oral and vestibular plates separated from the alveolar bone proper.

In the early survival periods of two and four days, frequently osteoclasts had differentiated in the severed periodontal tissues. Within six days the periodontal ligament tissue became the site of the undermining osteoclastic resorption below the entire length of the exposed vestibular plate of bone (Plate XIII, Fig. 13). This resulted in, first, a widening of the periodontal space and, finally, in a progressive loss of the alveolar crest until the entire exposed bone was lost within ten days (Plate XIV, Fig. 14; Plate XXVIII, Fig. 33C). At fourteen days the resorption process in all areas had ended.

While in the interdental and radicular areas, the removal of the necrotic bone starts by undermining resorption, the bone in the area immediately at the wound edge experienced frontal resorption despite the absence of bone necrosis (Plate X, Fig. 10;
Plate XII, Fig. 12; Plate XV, Fig. 15). Osteoclasts were present in the connective tissue overlying this viable bone.

d. Connective Tissue Proliferation

Early, at two and four days after injury, a proliferation of young connective tissue was observed beneath the clot at the wound edges and cut periodontal tissue.

As undermining resorption occurred and the alveolar bone disappeared, a replacement of bone then occurred with the appearance of young connective tissue from the now exposed bone marrow. This young connective tissue extended from wound edge to wound edge as inverting, unbroken cover. This could be observed in both the interdental (Plate VII, Fig. 7; Plate XXVII, Fig. 29C) and radicular area (Plate XIV, Fig. 14; Plate XXVIII, Fig. 33B).

2. Osteoblastic Phase

a. Pattern of New Bone Formation (10-28 days)

The first sign of osteoblastic activity occurred already in the late stages of osteoclastic resorption. This was at ten days in the bone marrow, some distance to the adjacent exposed bone. Osteoblast and osteoid tissue outlined the trabeculae in these deep bone marrow spaces (Plate XI, Fig. 11).

In the same period, peripheral to the active frontal resorption site at the wound edge, osteophytes began to form. At times, osteophytic bone formation extended into areas of resorption.
(Plate XII, Fig. 12). The osteophytes presented a pattern of trabeculae formation that was just perpendicular to the original bone surface and later fused at external ends. The osteophytic trabeculae were coarse fibrillar bone covered by osteoid tissue and osteoblasts.

The 21-28 day period marked the time of the greatest bone formation. This occurred at the site of resorbed bone in the connective tissue covering the wound, both in the interdental (Plate XVI, Fig. 16) and radicular areas (Plate XVIII, Fig. 18). The covering tissue was either young connective tissue or within 14 days new collagenous connective tissue that differentiated from the young connective tissue. Both the interdental (Plate XVII, Fig. 17) and radicular areas (Plate XIX, Fig. 19) presented trabeculae formation of coarse fibrillar bone outlined with osteoid tissue and osteoblasts.

Simultaneously with this maximum bone formation in the radicular area, there was periodontal apposition of alveolar bone proper (Plate XIX, Fig. 19).

b. Epithelial Proliferation from Wound Edges

Soon after surgery, the epithelium at the incised interdental papilla and sutured base edge showed a long, thin extension in the direction of the exposed wound. Large vacuolated cells were evident in the prickle cell layer, and occurred later in the basal cell layer of epithelium. Normal epithelium consisted of ridges
and was fourteen to sixteen cells thick above the papilla. After surgery, in the proximity of the wound edges, the early covering of epithelium was without ridges and reduced to a six cell layer or about one-half the normal thickness of epithelium. Only a few mitotic figures were noted in the epithelium at anytime.

The thin extended epithelium at two days covered the incised connective tissue and stopped at the clot. In some sections it partially covered the clot, and sometimes it penetrated beneath the superficial and the fibrin layer of the clot.

The interdental specimen from six and ten days exhibited a continued proliferation of epithelium from the cut papilla and cut sutured edge. Some areas were covered with epithelium, but in general, the proliferating epithelium from the papilla had not contacted that from the base. The basal cell layer was well differentiated, and a disorganized, less-differentiated prickle cell layer was evident with large desquamated epithelial cells on the surface. Polymorphonuclear leukocytes were migrating through the epithelium to accumulate on the surface.

At fourteen days, at the interdental site, the epithelium was narrow with definite irregular ridges and acanthotic near the cut papillae, while near the cut sutured edge, the epithelium was narrow and lacking ridges. The basal cell layer and prickle cell layer were distinguished and some degenerated cells appeared on the surface. Similarly, the radicular area displayed a new mar-
original epithelium that was acanthotic and had irregular ridges. The remaining new epithelium of the radicular area adjacent to the cut sutured base, was narrow and ridgeless. The radicular area presented epithelium facing the tooth that was less differentiated and now attached to the root of the tooth.

At the twenty-first day the epithelium covered the connective tissue in the interdental and radicular areas. The basal and prickle cell layers were well differentiated. A few degenerated cells were present on the surface covering.

3. Phase of Functional Repair of the Dento-periodontal Unit (28 - 185 days)

a. Alveolar Bone

The vestibular bone at twenty-eight days displayed immature, coarse fibrillar trabeculae at the sight of previous resorption (Plate XVI, Fig. 16; Plate XVII, Fig. 17; Plate XVIII, Fig. 18; Plate XIX, Fig. 19). Within ninety-three days the immature trabeculae of immature bone was replaced largely by an intermediate type of bone forming a compact bony plate. The remaining compact plate was an intermediate type of bone (Plate XXII, Fig. 22) described by Wienmann and Sicher (1955), consisting of irregular arranged fibrils and lacking lamallation compared to the adjacent mature bone that exhibits alternation of fiber direction and displays lamellation. Slight periosteal apposition occurred on the new vestibular plate. Apical to the apposition the periosteal
bone surface presented Howship's lucanae indicating active resorption (Plate XXIV, Fig. 24). At one hundred and eighty-five days after surgery, apposition and resorption was evident to a slight degree.

The most apparent finding was the complete regeneration of the interdental alveolar septa (Plate XXI, Fig. 21; Plate XXVII, Fig. 27; Plate XXVII, Fig. 31E). The examination of the radicular area showed an entirely different picture. The vestibular alveolar bone crest at this site was at a lower level than the oral alveolar bone crest (Plate XXIII, Fig. 23; Plate XXVIII, Fig. 34C). As was stated in material and methods, all animals had an equal vertical strip of bone exposed that averaged four to five millimeters. All exposed bone in the radicular area was completely resorbed within six to ten days (Plate XIV, Fig. 14; Plate XXVIII, Fig. 33B). Measurements revealed that only 2.5 millimeters of bone regenerated.

It was apparent that within 93 days apposition of the alveolar bone proper now completely restored the width of the periodontal space (Plate XXIII, Fig. 23; Plate XXV, Fig. 25). In the area subjacent to the regenerated bone, the periodontal ligament appeared normal with the arrangement of cells and fibers perpendicular to the tooth root surface and new bone (Plate XXV, Fig. 25). Although the hematoxylin and eosin stain seemed to show regular fibers within 93 days, the specimens stained according to Mallory,
exhibited much earlier this regular arrangement. Mallory stain method indicated that Sharpey's fibers and a definitive intermediate plexus was evident in the periodontal space by the twenty-first day. At twenty-eight days, the Sharpey's fibers were similar to the normal specimens being more dense than the twenty-one day specimen.

A layer of cementoid was present in the cementum at the wound site at ninety-three days (Plate XXIV, Fig. 24).

b. Mucous Membrane

aa. Connective Tissue

In this period of functional adaptation, the residual chronic inflammation in the papillary layer of connective tissue diminished considerably from ninety-five to one hundred and eighty-five days after surgery.

During this period the collagen fibers are masked together in the reticular connective tissue layer that was free of inflammation. Only in the last specimens do they appear in the papillary layer.

At one hundred and eighty-three days, the collagen fiber bundles displayed a distinct orientation running between the ridges and at right angles to the surface mucosa. A chronic inflammatory process continued to be present in the papillary connective tissue.

bb. Epithelium
Previously, only the basal cell layer of the epithelium was well differentiated but by the twenty-first and the twenty-eighth day dogs presented in addition to a basal cell layer, evidence of distinct prickle cell layer with desquamated cells on the surface (Plate XX, Fig. 20). Acanthosis of marginal and interdental papillary epithelium was evident with some ridge formation (Plate XVI, Fig. 16; Plate XVIII, Fig. 18). However, the epithelium that proliferated from the base was fourteen cells thick and appeared as a thick even band (Plate XVIII, Fig. 18).

An increased ridge formation was present in both the ninety-three and one hundred and eighty-five days (Plate XXV, Fig. 25). The ridges were comparable to those found in the gingiva of normal specimens. Pigment in the new epithelium and underlying connective tissue was evident at one hundred and eighty-five days (Plate XXVI, Fig. 26). Stippling was never observed.

A granular layer of cells was never distinguishable in the dogs; however, a dense superficial prickle cell layer was present with elongated cells whose long axis was parallel to the epithelial surface. Keratin was never observed in the marginal and attached gingiva. Parakeratosis did occur in the normal specimen, and was evident in the operated areas by the ninety-third day (Plate XXVI, Fig. 26).

c. Dentogingival junction

   aa. Fibrous Attachment
The quantity of interdental connective tissue completely regenerated and replaces the tissue which was lost by resection of the mucogingival flap (Plate XXI, Fig. 21; Plate XXVII, Fig. 27; Plate XXVII, Fig. 31E).

The new connective tissue, coronal to the repaired crest and forming the fibrous attachment to the root of the tooth, measured 1.98 mm., double the .98 mm., measurement of the control (Plate XXV, Fig. 25; Plate XXVIII, Fig. 34C).

bb. Epithelial Attachment

In the operative site the epithelium attaches apical to the cemento-enamel junction. The normal epithelial attachment ended on the cemento-enamel junction and on the enamel. However, a few of the operated specimens exhibited a new attachment at this site. Occasionally the epithelial attachment had proliferated along the root of the tooth (Plate XXIII, Fig. 23). The epithelium attached to enamel (Plate XXV, Fig. 25), to dentin (Plate XXIII, Fig. 23), and to cementum (Plate XVIII, Fig. 18), but never on cementoid. The attachment was observed above as well as below and often in the notches on the tooth that were made by scraping the tooth to remove all tissue attachments.

The new epithelium facing the tooth was a narrow eight to ten cell layer and contained a one or two cuboidal basal cell layer and the remaining cells were elongated and parallel with the enamel surface of the tooth. A less differentiated new epithelium
attached to the tooth at fourteen days. At twenty-one days the epithelium facing the tooth contained a definite basal cell layer and a normal elongated prickle cell layer at one hundred and eighty-five days in the absence of dental deposits. Where deposits were present the superficial surface of the prickle cell layer showed enlarged degenerated cells. Epithelial strands proliferated into the underlying connective tissue in the ninety-three (Plate XXIII, Fig. 23) and one hundred and eighty-five day (Plate XXV, Fig. 25) animals. In the last stage, the new epithelium facing the tooth resembled the crevicular epithelium of the normal specimen in that it consisted of a definite basal and prickle cell layer (Plate XXVI, Fig. 26). The difference was that the normal showed no long proliferating ridges of epithelium.

Dental deposits on the teeth may be an objectionable variable.

The epithelium in the radicular other than that facing the tooth, exhibited the same characteristics as the epithelium on the interdental areas.
CHAPTER V
DISCUSSION

Initial Connective Tissue Response

The repair of the oral mucosa and the dentogingival junction after reflection and resection of a mucogingival flap was complicated by necrosis of the alveolar bone.

Regeneration of all the tissues, including bone, occurred in all the interdental areas. In contrast, the radicular areas exhibited only a functional repair of the dentogingival junction. At this site, not all the resorbed bone was replaced.

The most significant finding is the abundant formation of young connective tissue (granulation tissue) from the periodontal ligament, as well as other connective tissue exposed during surgery. The cells of the tissue and the tissue itself undergo varied differentiation. Osteoclasts and osteoblasts serve for destruction and formation of new bone. Lymphocytes and macrophages were derived from the proliferating connective tissue cells in addition to the inflammatory blood elements. The young connective tissue itself can differentiate into dense connective tissue, lamina propria of the gingiva, or into connective tissue of the periodontal ligament. This young connective tissue was immovably attached to the repaired bone and cementum and formed the basis
for the reestablishment of the new dentogingival junction.

The site of origin of the granulation or undifferentiated connective tissue was the sum of all cut edges of the mucosa and the tissue of marrow spaces of the adjacent bone. The cut edges were the interdental papillae, the cervical edge of the periodontal ligament and finally to a minor degree the sutured wound edges.

Pattern of Osteoclastic Resorption

Some osteoclastic activity was observed soon after the surgical operation, and though slight during the second day, it was most active between the fifth and tenth day, and then gradually diminished and ended within fourteen days.

The resorption of the exposed necrotic bone could be achieved only by undermining resorption. The osteoclasts responsible for this resorption originated in the adjacent underlying bone marrow spaces of the wound. Resorption also proceeded from the periodontal surface in the radicular areas where the vestibular plate was fused with the alveolar bone proper. This fused plate was thin and here the marrow spaces were absent or scarce.

The removal of the exposed necrotic surface bone and, of course, the viable osseous tissue beneath was accomplished within fourteen days by osteoclasts. It was interesting to note that immediately peripheral to the wound edges, frontal resorption of
the vestibular plate started six days after sacrifice. The bone in these areas does not show any clear signs of degeneration and contained osteocytes in their lucunae.

At the end of the resorption phase, the compact vestibular plate of the alveolar process in the interdental area, had been lost in the entire area of exposure. The results of the resorption was a "ramping" configuration of interdental bone from the lingual crest to the excised wound edge, thus exposing the underlying spongy bone. In contrast to the interdental areas, in the radicular areas, the process of periodontal resorption caused a total loss of the exposed vestibular bone. As a result, the crest of the alveolar bone was considerably lowered.

The lost bone was replaced by the proliferating, undifferentiated, young connective tissue. However, the tissue was formed in excess and started to bulge into the wound area beyond the resorbed bone.

Pattern of Repair

The first sign of repair was the appearance of osteoblasts in the proliferating, young connective tissue. As a result of this, bone apposition started early, occurring during the six to ten day period on the inner surface of the exposed trabeculae. At the same time, osteoclastic activity was occurring on the outer surface. Due to the lack of marrow spaces, this was not evident in
the radicular area.

Reconstruction of the resorbed vestibular plate began by formation of trabeculae somewhat later, but before twenty-one days and it continues beyond twenty-eight days in the new connective tissue of the wound. All new trabeculae consisted of coarse fibrillar bone.

It was of great interest that after resorption, where a broad bone scaffolding remained (the interdental area), the formation of new bone leads to a fairly complete restoration of resorbed bone which was anatomically acceptable. In contrast, the radicular area, where the scaffolding was restricted to a narrow area or where the resorption stops on a compact plate, bone apposition was slow and never completely reconstructed the resorbed bone. Here only one-half of the bone was rebuilt, therefore, this wall of the socket in the radicular area remained low.

In later stages, more of the immature bone was removed and was replaced by a more mature type that, however, one hundred and eighty-five days still did not show all the detailed specialization of fully mature lamellated or Haversian bone.

Cementum Formation

There was no resorption of cementum in the wound area. During regeneration of this wound area, a layer of cementoid was formed on the root of the tooth within ninety-three days.
Maturation of Connective Tissue

Between ten and fourteen days, there was an increase of collagenous fibers and the number of cells decreased. In the last stages, a mature, dense connective tissue was present in the area of newly formed bone. This new connective tissue cover of newly formed bone was firmly attached to the bone and the tooth. The formation of a connective tissue led to the establishment of a new periodontal ligament in the radicular area. Also, the connective tissue fibers became imbedded in the cementum above the alveolar crest. In all of these described areas, the connective tissue developed into a thick, and uniform layer that showed all the characteristics of gingiva.

It was interesting that the newly formed "attached" gingiva was first attached to the bone and later gained attachment to the newly formed cementum. From this, the fibrous component of a new dentogingival junction was established. Significantly, the length of the regenerated fibrous attachment, the gingiva, above the alveolar crest was double the original gingiva at the radicular area. This increase in gingiva compensated for the failure of complete regeneration of the radicular alveolar bone.

Epithelial Migration

Epithelial migration from the wound edges started immediately after surgery and continued so that by the twenty-first day, the
wound was completely covered. Finally, the epithelium exhibits characteristics similar to that of normal gingiva.

The epithelial attachment to the tooth varied depending on the amount of bone formation or connective tissue regeneration or both. If the connective tissue attachment was not enough or the regeneration of bone was limited, or both, the epithelial attachment was present on the root apical to cemento-enamel junction.

**Histological Differentiation**

Histologically, this experiment indicates that the regenerated soft tissue was gingiva and not alveolar mucosa. Two influencing factors, seemingly, determined the regeneration of gingiva. One was genetic and the other was the adaptation of structure to function. Tissues will develop long before function occurs, and this is applicable to the gingiva, which forms around the necks of the teeth and attaches to the bone and teeth during wound healing. This does not mean that functional influences do not play any role, but that both the genetic and functional influences are necessary. Tissue formation is first, without which there would be no function. Functional influences play a dominant role as a pre-requisite for differentiation of the connective tissue in the cervical area. Maintenance of a tissue depends on function.

One cannot justify that this tissue differentiates to gingiva
because a portion of the proliferating young connective tissue is derived from the connective tissue of the cut gingival papilla. In this experiment, a large portion of this proliferating connective tissue arises from the bone marrow and the periodontal tissues.

The experimental findings indicate that proliferating young connective tissue occurred mainly from the fixed connective tissue sites, namely, the cut papilla, bone marrow spaces and the periodontal ligament tissue. A lesser amount of proliferation of young connective tissue occurred at the mobile excised sutured edge. The immovable fixation of the proliferating young connective tissue seems to be essential.
CHAPTER VI
SUMMARY AND CONCLUSIONS

This investigation represented a histological evaluation of repair of the oral mucosa and the dentogingival junction after resection of a mucogingival flap with exposure of the vestibular bone.

Ten adult mongrel dogs with full permanent dentition served as subject material. The area of surgery was in the maxillary and mandibular premolar areas. The animals were sacrificed at 0 hrs., 2, 4, 6, 10, 14, 21, 28, 93, and 185 days.

The repair of the oral mucosa and dentogingival junction was complicated by the necrosis of alveolar bone.

Within twenty-one days an epithelium covering of the wound was visible. However, not until the later stages, one hundred and eighty-five days was the wound functionally repaired.

Regeneration of all the tissues including bone, occurred in all the interdental areas. In contrast, the radicular areas exhibited only a functional repair of the dentogingival junction. At this site, not all the resorbed bone was replaced.

The dentogingival junction was altered as the result of a deficiency in repair of the vestibular bone. The fibrous connective tissue attachment increased to compensate completely or partially.
for this lack of bone regeneration. Frequently this increase of connective tissue was not enough to allow the epithelium to attach at the cemento-enamel junction, thus, resulting in a more apical attachment on the root surface of the tooth.

Microscopic findings indicate that there was complete regeneration of the oral mucosa in the interdental areas. However, the presence of a tooth and its specialized supporting structures altered the repair of the dentogingival junction. The dentogingival junction exhibited a functional repair but with an anatomic deformity.
CHAPTER VII

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PLATE I

Figure 1:

Clinical photograph of exposed bone in premolar area with surgical flap excised and the alveolar mucosa wound edge sutured to the periosteum.
CHAPTER VIII
Appendix

A. Photomicrographs

PLATE I

Figure 1
PLATE II

Figure 2:

Photomicrograph of 2 day specimen - maxillary right, interdental area. (X12)

Note:

a) Compact oral and vestibular plate and underlying spongy bone

b) Clot of wound edges - the cut papilla and sutured wound edge

c) Exposed bone between the clots
PLATE III

Figure 3:
Photomicrograph of 2 day specimen - maxillary right second premolar. High magnification of Plate II, Fig. 2. (X50)

Note:

a) Clot wound edge

b) Fibrous type of marrow - young connective tissue

c) Osteoclast in marrow spaces

d) Inflammatory cells and dilated blood vessels in marrow spaces
PLATE III

Figure 3
PLATE IV

Figure 4:

Photomicrograph of 2 day specimen - right mandibular third premolar (X12)

Note:

a) Compact vestibular plate with few marrow spaces
b) Clot at severed dentogingival junction
c) Clot at interior wound edge (excised wound edge)
d) Inflammatory processes beneath clot
Figure 4
PLATE V

Figure 5:

Photomicrograph of 2 day specimen - right mandibular radicular area. High power magnification of Plate IV, Fig. 4. (X50)

Note:

a) Clot at cut periodontal ligament
b) Proliferation of young connective tissue
c) Inflammation below clot
d) Osteoclasts in Haversian systems
e) Inflammation in Haversian systems
Figure 6:

Photomicrograph of two day specimen (X350)

Note:

a) Bone necrosis - empty lucunae

b) Presence of viable bone beneath necrotic layer of bone

c) Remnant of Periosteum
Figure 6
PLATE VII

Figure 7:

Photomicrograph of 6 day specimen - right mandibular interdental area. (X12)

Note:

a) Resorbed crestal and vestibular bone plate and exposure of marrow spaces
b) Young connective tissue covering the wound
c) Epithelial migration at wound edges
Figure 7
Figure 8:

Photomicrograph of 6 day specimen - right mandibular interdental area. High magnification of Plate VII, Fig. 7.

Note:

a) Spicules covered with bacterial plaque
b) Osteoclasts on under surface of spicules
c) Osteoclasts on superficial surface of exposed trabeculae
d) Osteoblastic activity occurring on under surface of same trabeculae
e) Fibrous marrow space
f) Proliferating young connective tissue with presence of inflammatory cells
PLATE IX

Figure 9:
Photomicrograph of 10 day specimen - maxillary right interdental area. (X12)

Note:

a) Resorbed compact bone and exposure of marrow spaces

b) Enlarged crestal marrow spaces

c) Osteophytic bone adjacent to sutured wound edge

d) Wound covered by young connective tissue
PLATE IX

Figure 9
PLATE X

Figure 10:

Photomicrograph of 10 day specimen - maxillary interdental area. High magnification of crest area of Plate IX, Fig. 9. (X50)

Note:

a) Frontal or periosteal resorption on viable bone at crest
Figure 11:

Photomicrograph in the vestibular area at 10 days - right maxillary interdental area at wound surface. Higher magnification of Plate IX, Fig. 9. (X50)

Note:

a) No resorption on remaining exposed vestibular bony plate

b) Resorption decreasing and apposition on trabeculae on marrow space side

c) Frontal resorption on viable bone above exposed trabeculae

d) Osteoid and osteoblasts outlining trabeculae in deep marrow spaces
PLATE XI

Figure 11
PLATE XII

Figure 12:

Photomicrograph of 10 day right maxillary specimen - interdental area adjacent to sutured wound edge.

High magnification of Plate IX, Fig. 9. (X50)

Note:

a) Osteophytic bone trabeculae below frontal resorption

b) Osteophytic bone trabeculae in frontal resorbed area

c) Frontal or periosteal bone resorption at sutured base
Figure 12
PLATE XIII

Figure 13:

Photomicrograph of 6 day specimen, right maxillary radicular area. (X50)

Note:

a) Osteoclastic activity below entire vestibular plate

b) Inflamed periodontal tissue and widened periodontal space

c) Bacterial plaque on bone surface

d) Connective tissue proliferation contained within radicular bony plate
PLATE XIII

Figure 13
PLATE XIV

Figure 14:

Photomicrograph of 10 day specimen - mandibular third premolar. (X12)

Note:

a) Complete resorption of vestibular alveolar bone plate
b) Widened periodontal space
c) Notch that was scraped in tooth
d) Spicule of bone
PLATE XV

Figure 15:

Photomicrograph of 10 day specimen - mandibular third premolar. Higher magnification of Plate XIV, Fig. 14. (X50)

Note:

a) Resorption occurring in marrow spaces and on surface of vestibular bone plate below sutured wound edge
b) Resorption of the crest
c) Spicule with plaque on external surface and resorption on internal surface
d) Inflammatory reaction in proliferating young connective tissue in the former periodontal space
e) Inflammatory reaction of bone marrow and at sutured wound edge
f) Apposition of bone in periodontal space
PLATE XVI

Figure 16:

Photomicrograph of 28 day specimen - left mandibular interdental area. (X12)

Note:

a) Osteophytic bone trabeculae in resorbed wound area

b) Collagenous connective tissue

c) Epithelium is acanthotic and contains ridges in the area of the cut papilla

d) Adjacent to alveolar mucosa a thick even band of epithelium
PLATE XVII

Figure 17:

Photomicrograph of 28 day specimen - mandibular interdental area. High magnification of Plate XVI, Fig. 16 (X50)

Note:

a) Coarse immature trabeculae outlined with osteoid tissue and osteoblasts

b) Long, thick band of epithelium
c) Epithelium with ridges
d) Collagenous connective tissue
Figure 17
PLATE XVIII

Figure 18:

Photomicrograph of 28 day specimen - maxillary third premolar (X12)

Note:

a) Osteophytic bone trabeculae at crest

b) New collagenous connective tissue attachment to tooth

c) Inflammatory reaction - subepithelial

d) Epithelium attached to tooth

e) Marginal epithelium contains broad ridges

f) Band of epithelium and lacking ridges near alveolar mucosa
PLATE XIX

Figure 19:

Photomicrograph of 28 day specimen - right mandibular third premolar area. High magnification of Plate XVIII, Figure 18. (X50)

Note:

a) Osteophytic bone trabeculae at crest

b) Periodontal apposition of coarse fibrillar bone
Figure 19
PLATE XX

Figure 20:

Photomicrograph of 28 day specimen - mandibular third premolar area (X50). High power magnification of Plate XVIII, Fig. 18.

Note:

a) Epithelial attachment in scraped notch
b) Epithelial ridges and acanthosis in marginal area, absence of epithelial ridges near alveolar mucosa
c) Desquamated cells on epithelial surface
d) Inflammation in papillary connective tissue
e) New connective tissue attachment to cementum of root
Figure 20
PLATE XXI

Figure 21:

Photomicrograph of 93 day specimen - right mandibular interdental area (X12)

Note:

a) Restoration of vestibular bony plate

b) Apposition of bone at the crest
Figure 21
PLATE XXII

Figure 22:

Photomicrograph of 93 day specimen - right mandibular interdental area. High power magnification from Plate XXI, Fig. 21. (X50)

Note:

a) Normal bone with Haversian system

b) Intermediate type of bone near the crest
PLATE XXIII

Figure 23:

Photomicrograph of 93 day specimen - right mandibular third premolar. (X12)

Note:

a) Deficient height of radicular vestibular plate
b) New connective tissue attachment to root of tooth
c) Epithelial attachment at lower level
d) Subepithelial inflammation
e) Periodontal space almost normal width
PLATE XXIV

Figure 24:

Photomicrograph of 93 day specimen - right mandibular third premolar area. High magnification of Plate XXIII, Fig. 23. (X50)

Note:

a) Apposition occurring on crest
b) Intermediate type of bone - irregular coarse fibrillar bone
c) Osteoclastic activity on periosteal bone surface below irregular bone formation
d) Cementoid on root adjacent to new periodontal ligament and new bone
e) Regular arrangement of periodontal fibers
Figure 24
Figure 25:

Photomicrograph of 185 day specimen - maxillary second premolar radicular area. (X12)

Note:

a) Deficient bony crest regeneration
b) Long, new connective tissue attachment on root
c) Inflammation due to calculus
d) Epithelium on the cemento-enamel junction and on the enamel
e) Proliferation of strands from new crevicular epithelium
f) Marginal epithelium ridges almost normal
Figure 26:

Photomicrograph of 185 day specimen - maxillary second premolar area. High magnification.

Note:

a) Ridge formation of epithelium

b) Parakeratosis on epithelium

c) Pigment in epithelium and connective tissue
PLATE XXVII

Diagrammatic illustration of operative site at interdental area.

Figure 27 (A) Normal

I. Direction of incisions

Figure 28 (B) 2 - 4 days
→ Direction of resorption
C. Blood clot

Figure 29 (C) 6 - 10 days
→ Direction of resorption
Z. Trabeculae formation - coarse fibrillar bone

Figure 30 (D) 28 days
R. Trabeculae bone formation at wound

Figure 31 (E) 185 day - Return to normal
Diagrammatic illustrations of operative site at radicular area.

Figure 32 - Normal
Figure 33 - 6-10 days
Figure 34 - 185 days

I - Incisions
A - 4 mm. bone exposure and resorption
B - 2 mm. new bone formation
C - 2 mm. loss of bone
D₁ - Original connective tissue attachment
D₂ - New connective tissue attachment
E₁ - Original epithelial attachment on cemento-enamel junction
E₂ - Epithelial attachment apical to cemento-enamel junction
X - Epithelium at lower level
G - Young connective tissue
H - Tooth
Fig. 32A  Fig. 33B  Fig. 34C
<table>
<thead>
<tr>
<th>Author</th>
<th>Nature of Surgery</th>
<th>No. of Humans and Time</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orban 1945</td>
<td>Resection of gingiva above alveolar crest</td>
<td>1 Human 14 days</td>
<td>Two day specimens displayed a blood clot. Mitotic activity was evident in the connective tissue at 4 days. The epithelium advances from both sides of the wound at nine days and within fourteen days completely covered wound.</td>
</tr>
<tr>
<td>Kollar Wentz Orban 1955</td>
<td>Abraded gingiva with aluminum oxide powder</td>
<td>6 Humans 48 hrs.</td>
<td>Epithelial migration from borders of wound began at six hours and covered the surface within eighteen hours. Healing was complete at forty-eight hours. The only sign of inflammation was a vascular response.</td>
</tr>
<tr>
<td>Grant 1957</td>
<td>Removal of gingiva with a muco-periosteal flap that extended into alveolar mucosa</td>
<td>14 Humans 60 days</td>
<td>New gingiva in eight subjects displayed absence of elastic fibers, while five exhibited a few elastic fibers and one had enough to be considered alveolar mucosa. The new epithelium was either normal or similar to gingival epithelium.</td>
</tr>
<tr>
<td>Ivancie 1958</td>
<td>Removal of gingiva with a muco-periosteal flap that extended into alveolar mucosa</td>
<td>20 Humans 8 Months</td>
<td>New epithelium at one month appears like gingiva. Complete regeneration and functional adaptation of gingiva within eight months.</td>
</tr>
</tbody>
</table>
TABLE I (continued)

REVIEW OF LITERATURE

<table>
<thead>
<tr>
<th>Author Year</th>
<th>Nature of Surgery</th>
<th>No. of Humans and Time</th>
<th>Results</th>
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<tbody>
<tr>
<td>Mittelman 1958</td>
<td>Stab wound in gingiva</td>
<td>7 Humans 72 hrs.</td>
<td>Epithelium covered the inflamed wound within twenty-one hours. Inflammation continued to be present in the papillary connective tissue in the seventy-two hour specimen. Epithelial regeneration is more rapid than regeneration of connective tissue.</td>
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## TABLE II
REVIEW OF LITERATURE

<table>
<thead>
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<th>No. of Animals and Time</th>
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</thead>
<tbody>
<tr>
<td>Buebe and Silvers 1934</td>
<td>Heterogenous powder in labial bone defect</td>
<td>8 Dogs 9 Weeks</td>
<td>Little evidence of bone formation in control with no bone powder, while in the experimental wound the original plate doubled.</td>
</tr>
<tr>
<td>Skiller and Lundquist 1935</td>
<td>Surgically created 8 mm. pocket</td>
<td>Dogs 4 Months</td>
<td>The pocket healing occurred with a new 1.5 to 2.0 mm. connective tissue attachment, an elongated epithelial attachment and remaining pocket by recession.</td>
</tr>
<tr>
<td>Buebe 1947</td>
<td>Flap operation with removal of 6 mm. of bone</td>
<td>7 Dogs 2 Years</td>
<td>Bone formation and cementoid formed within 7 days. New periodontal membrane was evident by 2 years.</td>
</tr>
<tr>
<td>Swenson 1947</td>
<td>Creation of a soft tissue pocket 8x4 mm. in dogs 150 days</td>
<td>100 pockets</td>
<td>Pocket lined with epithelium. Main finding was two to three mm. of gingival recession.</td>
</tr>
<tr>
<td>Linghorne and O'Connell 1950</td>
<td>Gingival flap creating a pocket and 9 mm. defect of bone</td>
<td>4 Dogs 1 Year</td>
<td>Resorption of cementum and later deposition of new cementum. New attachment of connective tissue and apical proliferation of oral epithelium, with one to three mm. of recession. Slight regeneration of alveolar crest was evident.</td>
</tr>
</tbody>
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TABLE II (continued)

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<tr>
<td>Linghorne and O'Connell 1951</td>
<td>Autogenous bone chips in 7x6 alveolar bone defect</td>
<td>3 Dogs 130 Days</td>
<td>Within seventeen days osteoclasts disappeared and osteoblasts appeared. Bone grafts stimulated bone formation. Bone apposition occurred on bone cementum and dentin particles.</td>
</tr>
<tr>
<td>Ramjford 1951</td>
<td>Soft tissue products were produced by copper bands</td>
<td>4 Monkeys 137 Days</td>
<td>Attachment was possible on root of tooth preceded by resorption of root. Degree of inflammation determines whether new attachment will be epithelial or collagenous in nature. Alveolar bone formed at crest.</td>
</tr>
<tr>
<td>Linghorne and O'Connell 1955</td>
<td>Produced 10 mm. pocket and bone defect</td>
<td>5 Dogs 3 Months</td>
<td>Regeneration of epithelium and connective tissue with a simultaneous new formation of cementum and periodontal ligament. Average bone regeneration was 6 mm.</td>
</tr>
<tr>
<td>Linghorne 1957</td>
<td>Autogenous graft placed in bony defect after the gingival flap operation</td>
<td>7 Dogs 117 Days</td>
<td>Pocket displayed a 2-3 mm. regeneration and recession. Resorption of mesial and distal walls of bony defect. Grafts induced 4-7 mm. of new bone formation. Epithelization was complete in 35 days.</td>
</tr>
</tbody>
</table>
### TABLE II (continued)

**REVIEW OF LITERATURE**

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</tr>
</thead>
<tbody>
<tr>
<td>Marfino 1958</td>
<td>Surgical creation of 10 mm. pocket. 7x6 removal of alveolar bone.</td>
<td>10 Dogs 183 Days</td>
<td>New connective tissue attachment of 2.0 - 2.5 mm. and elongation of epithelium 1.50 - 2.50 mm. contributed one-third to the elimination of pocket. Remaining one-third of pocket was eliminated by gingival recession. The connective tissue formation occurred early but organization late. New cementum in all cases. Dentogingival junction is functionally acceptable, but without total regeneration of original morphology.</td>
</tr>
</tbody>
</table>
APPROVAL SHEET

The thesis submitted by Dr. Malbern N. Wilderman has been read and approved by three members of the Departments of Anatomy and Oral Anatomy.

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.

[Signature]

Date: [May 26, 1954]