1958

Healing of an Experimental Incision in the Human Attached Gingiva

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HEALING OF AN EXPERIMENTAL INCISION IN THE HUMAN ATTACHED GINGIVA

by

Henry Robert Mittelman, D.D.S.

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

June 1958
Our greatest glory is not in never failing,  
but in rising every time we fail.  

Confucius

A man in earnest finds means, or  
if he cannot find, creates.  

William Ellery Channing
LIFE

Henry Robert Mittelman was born on July 5, 1913, in Chicago, Illinois.

His preparatory education began at the Garfield Elementary School, and graduated in June, 1928, and from Crane Technical High School in 1931. He completed two years at Crane College, after which he was admitted to Chicago College of Dental Surgery in 1934. He graduated with a Doctor of Dental Surgery degree in 1938.

In December, 1938 he started to practice at 4737 N. Broadway in Chicago. In 1940 he was married and now has two daughters. He volunteered for dental service in the Navy during World War II, and served for more than two years. He resumed practice once again in 1946.

In 1955, Dr. Mittelman returned to his Alma Mater for post-graduate work under the personal supervision of Dr. Harry Sicher. Striving for more knowledge and experience, he was admitted into the Post-Graduate school in Oral Surgery for thirteen months, after which he passed the State Board examination for licensure to qualify him to practice as a specialist in Oral Surgery on January 10, 1958.

While in the Post Graduate program, he also began his studies in the Graduate School with the thought of furthering his education with a Master of Science degree.
His interest is for furthering the sciences and in this interest, the acquisition of knowledge, shall be continued.
ACKNOWLEDGMENT

The author is indebted to Doctor Harry Sicher, Doctor Frank Wentz and Doctor Patrick Toto for their excellent advice, guidance and encouragement in the preparation of this thesis. I, also, wish to thank Doctor Balint Orban for help in processing the specimen slides and Mr. John Blickenstaff for being so helpful in the processing of the microphotographs. Thanks is extended to the seven subjects who so graciously volunteered and submitted to be part of this experiment and Mrs. Mittelman who persevered through these trying hours and typed all the necessary copies during compilation of this thesis.
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CHAPTER I
INTRODUCTION

Little is known concerning the regeneration and repair of the oral mucous membrane, therefore, this investigation was designed to determine the manner of the healing following an incision wound in the human gingiva. The generally accepted view about the connective tissue and epithelial changes during the healing processes, may require a careful re-examination.

Findings in this study suggest new information relative to the basic biology of oral epithelium and connective tissue as it is compared and contrasted with the same tissues in the skin. Histologic investigation indicates a difference in the degree of the inflammatory reaction and rate of healing, if oral mucosa wounds are compared with skin wounds. Further investigation and knowledge of the healing phases may aid in the therapeutic management of diseases of the oral mucous membrane.
CHAPTER II
REVIEW OF THE LITERATURE

Studies of the wound healing in the oral cavity are mainly concerned with the healing of extraction of wounds (Claflin's experiments in dogs 1936) and the healing after gingivectomy (Orban and Archer 1945.) The introduction of the air abrasive technique stimulated an investigation of epithelial healing after the application of the abrasive to the gingiva (Kollar, Wentz, Orban 1955.) The interesting result of the latter experiment was the rapidity of epithelial healing that was complete in eighteen hours and the lack of inflammatory reaction.

No investigations were made on the dynamics and histology in the healing of simple incisions in health areas of the oral mucosa, though many studies were done on the healing of incisions on the skin (Hartwell 1929, '30, '55, Gilman 1955, et al.) In the skin sterility is probable, while asepsis is next to impossible in the mouth. Thus, the cause of injury and the site can vary the response of healing. (Lam, 1950) Heifetz (1952) advised that the best protection against ingress of microbial or foreign matter was the sealed clot.

Therefore, the present study was undertaken in human patients who volunteered for this experimental procedure.
Repair, as a rule, is considered to be the out-come of an inflammatory reaction to an injury. The described experimental findings seem to show that there are exceptions to this rule under certain special conditions. In other words, one has to conceive the possibility that repair can follow immediately after injury. This conclusion tends to draw a sharper line between the two commonly correlated processes of inflammation and repair.

Confirmation of the observation that wound closure occurs not so much by mitotic processes, but by migration of cells from the borders of the wound, is afforded by the interesting finding that epithelial repair of the surface break was complete at about eighteen hours after injury, before any increased mitotic activity was apparent. The latter was most prominent twenty-four hours after injury and after the wound surface was well epithelialized.

The present proposed experiment will be more delicate than the previous. Healing after a single incision will be studied. More information may thus be obtained by this more careful technique.

Other clinical evaluations are without corresponding histological evidence and thus are of limited value. The sparsity of histologic investigation on humans is understandable, because of the reluctance of most subjects to consent to experimental procedures. However, the ease with which one is able to biopsy the human attached gingiva overcomes this barrier and is the basis of the present experiment.
CHAPTER III

MATERIAL AND METHOD

In undertaking this experiment it was decided to avoid the complication in wound healing due to old age, growth changes in the young, and the hormonal variations that may occur in women. Selected were six men whose ages ranged from twenty three years to forty six years of age. There were two representative subjects of each decade. They were all well nourished, physically normal, healthy individuals.

The attached gingiva of the oral mucosa was the site selected for this study. The attached gingiva is a structure which is subjected and adapted to a mechanical function of stress, principally that of mastication. It is a thick immoveable structure firmly bound to the underlying periosteum and bone. The immoveability of this structure and the absence of any elastic fibers would prevent the formation of a gap after incision. Therefore, suturing or any other means of coaptation would be unnecessary. The easy accessibility of the attached gingiva located in the maxilla and mandible; from the first molars anteriorly to the mid line, was the area selected for making incision wounds and, subsequently, for taking biopsies. Clinically, the gingiva was normal (Plate I, Figure 1) in all subjects.

Histologically the gingiva (Plate I, Figure 2) is characterized by having
stratified squamous epithelium, which is Keratinized, and connective tissue which is devoid of any elastic fibers or glands. The epithelium is thin with long projecting ridges that penetrate into the connective tissue, enveloping the papillary pegs; binding and interlocking the two tissues together. This supporting tissue consists of dense wavy collagen fiber bundles, with an indefinite lamina propria, without any submucosa.

Nine vertical incisions were made approximately 5 mm in length and 1 1/2 in depth into the attached gingiva reaching into the interdental papilla, but avoiding the free margin. The timing of biopsies was derived from a pilot experiment in which healing of the incision was observed clinically, until no trace of the incision remained. In intervals from 0-72 hours, biopsies were taken that included the incisions and followed all good technique for biopsies. In order to eliminate any possibility of probable variance due to time; Zero hours was established as 8:30 P.M.

A short distance from the proposed incision site was anesthetized with 2% Ravocaine. The biopsy was secured with a #15 Bard Parker scapel and a pair of Orban periodontal knives. All biopsies were roughly triangular in shape, measuring 5 mm at the base and 7-10 mm in length.

All tissues were fixed in fresh Formal-Zenker solution (1-9) for exactly four hours and then washed for two hours in running water. Dehydrated in alcohol
and subsequently embedded in parafin and then sectioned at six microns. Every
tenth section was mounted on microscopic slides then stained (Hematoxylin and
Eosin) for histologic study. In two subjects Mallory's connective tissue stain was
used to specifically define collagen fibers. A light microscope was used to observe
the intimate details of these specimens.

Clinical records by color photographs of the incisions were taken before
each biopsy. An Alpa Camera #5 having a Kern-Switar F 1.8 lens, and using a
close-up stroboscopic circle light attachment was used. The film used was
Eastman's K 135 Kodachrome (daylight) color safety film.
CHAPTER IV

FINDINGS

Part A - Clinical Findings

To determine the approximate duration of healing, the wound was inspected and photographed at regular intervals. All subjects had a normal pink colored gingiva, with a characteristic texture indicated by the stippling seen in the normally attached gingiva. (Plate I Figure 1) The principal changes observed after surgery were the changes in color and texture. A serous exudate covered the edematous area of the incision. Clinical healing was considered complete after 72 hours. At this time no sign of the incision was visually perceptible.

After the incisions were made into the attached gingiva, the usual bleeding was observed. (Plate II Figure 3) This hemorrhage was slight and clotted almost immediately (Plate II Figure 3 - 3 to 6 minutes). A gaping of the tissue was not apparent. No other changes were noticeable under observation with the naked eye at this time.

The greatest changes occurred in the first 24 hours, the products of which were pallor and edema progressively with a loss of texture (stippling) between the 6th and 15th hours in 5 cases. In contrast, two cases revealed only such slight changes that it was deemed to be a minimal color change. The color of the pallor ranged from pale grey-pink to pink-grey and to grey. At the time of blanching, some exudate covered the wounds. (Plate III Figure 5)
Progressively, the tissue returned to their normal appearance after the twentieth hour. (Plate III Figure 5) By the twenty eighth hour, neither (Plate V Figure 9) greyness nor pallor was apparent and the length of the incision appeared shorter. (Plate V Figure 9) The tissues were again normal with good color and texture, (Plate VI Figure 11) remaining so thereafter until healing was complete. (Plate IX Figure 17) At the forty second hour (Plate VII Figure 13, the line of incision had become almost unnoticeable and was only observed by close scrutiny. (Plate VII Figure 13) By the forty eighth hour, it was no longer visible in the three cases and only very slightly visible (Plate VIII Figure 15) in the remaining four. (Plate VIII Figure 15) Seventy two hours after the incision, the only observable sign of the incision was a small depression (Plate IX Figure 17) in the gingiva in the area. Healing was considered to be complete at this time. (Plate IX Figure 17)
Part B - Microscopic Findings

The tissue response begins almost immediately after injury. (Plate II Figure 3) After an initial hemorrhage, a blood clot is formed that seems to be loose (Plate X Figure 19) and, therefore, was lost (Plate II Figure 4) in all the specimens during the first hours after incision. Later specimens show the clot is more firmly anchored to the edges of the wound, sealing the gap made by the incision. Also, at this time, the inflammatory reaction begins in the connective tissue. (Plate XII Figure 21) However, this inflammation is soon resolved. (Plate XIII Figure 22)

Soon thereafter, the epithelium starts to bridge the gap on the surface, (Plate III Figure 6) while in the depth, the perivascular connective tissue undergoes (Plate VIII Figure 16) proliferation with simultaneous formation of capillary buds. (Plate VIII Figure 16) This is also the time when there is a proliferation of the epithelium and, also, the first signs of production of collagen.

In the last phase of healing the epithelium produces keratin, (Plate VII Figure 14) the subepithelial gap narrows and the proliferation of the connective tissue elements continue. This sequence of events lends itself to a division of the process of healing into three phases, each one of which can be further subdivided into several stages. It is clear that there is a continuity of the entire process and that furthermore, many of these stages overlap.
CLASSIFICATION OF HEALING:

A. Initial Phase
   1. Bleeding and clotting
   2. Anchoring of the clot; inflammation
   3. Resolution of inflammation

B. Productive Phase
   1. Epithelial bridging
   2. Capillary budding and perivascular proliferation
   3. Epithelial proliferation
   4. Production of collagen

C. Maturation Phase
   1. Keratinization of the epithelium
   2. Narrowing of the cap by progressive proliferation
      and partial maturation of the connective tissue

PHASE I - Initial phase

1. Bleeding and clotting

   The first stage of this phase is characterized by bleeding and clotting (Plate XI Figure 20.) All individuals had one biopsy taken at 0 Hour to (Plate II Figure 4) establish the structures of the tissues and was used to serve as a control. This stage was relatively inactive.

0 Hour

   In all specimens observed, the epithelium, as well as the connective tissue, was normal and health (Plate II Figure 4) and showed a narrow gap (25-50 Microns.) The divided epithelium was of normal thickness, ridges were of normal length and either long thin or long medium pointed, or well rounded at their base. The basement membrane and basal cell layer, as well as the prickle cell layers, were well defined and
showed a typical arrangement. The granular layer was present in all keratinized specimens and was two cell layers in thickness.

In all but one specimen, a thin layer of keratin covered the surface. In this one exception the keratin was replaced by parakeratosis and the granular cell layers were absent.

A few mitotic figures could be found in all specimens in the basal layer or the deep portion of the prickle cell layers in the deepest part of the epithelial ridges.

In the gap in all sections there were frayed or ragged epithelial cells bordering the cut edges. (Plate II Figure 4)

The connective tissue was dense, consisting of heavy bundles of wavy collagen fibers. Interspersed between these collagen bundles some resting fibrocytes were seen. The papillary connective tissue was at right angles to the surface of the specimen, while the collagen of the reticular layer was parallel to the surface. Capillaries were cut and especially noticeable in the deeper reticular layer; also, some nerve fibers were cut. The wound gap formed a narrow V with its apex deep in the specimen presenting cut collagen fibers with folded and splayed edges. In the connective tissue, plasma cells, lymphocytes, monocytes and occasional polymorphonuclear and eosinophilic leucocytes were observed.

In all specimens the clot had not firmly sealed the wound, (Plate X Figure 19)
so that, it was lost during the histologic preparation. The only remains of this insecure clot were some red blood cells, stray epithelial cells, few bacterial placques and some keratin strands deep in the V of the gap.

2. Anchoring of Clot - Inflammation

Continuing from the sixth hour progressively to the eighteenth hour the formation of a well formed clot sealed and anchored the wound edges. This anchorage was noted in all subject specimens as a well defined clot. The clot protruded up through the gap, extended above the level of the keratinized surface and formed a mound overlying the wound. (Plate XI Figure 20)

The epithelial cells began to elongate slowly so that by the twelfth hour they had begun to fold into a gap. This elongation and infolding appeared as a rolled border consisting of three or four cell layers. Mitotic figures at the wound were absent.

The fibrin clot anchored the wound together, (Plate XI Figure 20) and at the same time the presence of a few inflammatory cells were noticed. Progressively thereafter, until the eighteenth hour there was a great increase in the number of inflammatory cells. (Plate XII Figure 21) Inflammation continued thereafter, but in lesser degree. The cells consisted of polymorphonuclear leucocytes, lymphocytes, macrophages (histiocytes) and undifferentiated mesenchymal cells.

3. Resolution of Inflammation

This was characterized by the progressive diminution of the inflammation from
the twentieth to the twenty fourth hour. (Plate XIV Figure 23) This decrease was characterized by a smaller number of inflammatory cells, and a diminution of degenerating red blood cells and hemosiderin. This resolution continued actively until the forty eighth hour (Plate VIII Figure 16) when a small amount of inflammation was seen. At the same time there was a continued activity of the epithelial cells.

At the twenty first hour the epithelial cells continued to migrate from the opposing edges in elongated finger-like projections, which almost contacted each other. (Plate III Figure 3) In one specimen the epithelial projections appeared to be in contact with each other, covering the wound gap, but this was uncertain. (Plate XV Figure 25)

In the connective tissue a budding capillary was seen. (Plate XIV Figure 23) In the perivascular spaces approximating the edges, great activity was noted, in that a large number of macrophages and undifferentiated mesenchymal cells migrated into the surrounding collagen and seemed to be moving toward the clot. (Plate XIV Figure 23) Endothelial cells from the cut capillaries at the clot edge were seen projecting into the wound, either degenerating or beginning to form new buds. Inflammation was subsiding in the more superficial reticular layer, but was acute in the deeper layer. Fibroblasts, noted for the first time, had migrated from the splayed collagen fibers into the clot.
PHASE B - Productive Phase

1. **Epithelial Bridging**

   At this stage of healing the epithelium narrowed the gap, so that by the twenty fourth hour it had fused in some specimens. (Plate III Figure 6) This stage signified the beginning of the productive phase of repair.

   The epithelium has joined by physical contact in either of two ways: the first way is by contact and fusion after the infolding (Plate III Figure 6) epithelium had migrated into the gap. This migration was first noted by the elongation of the epithelial cells. Some of the surface cells were either swollen or appeared vacuolated, and indicated degenerative changes. The peripheral cells from both edges folded into the gap until they contacted the clot; thereafter, the overlying cells from these extensions met by physical contact, fused and covered the wound surface. The second way was by the further migration of the epithelial cells through the clot; (Plate III & XVI Figure 6 & Figure 26) this was accomplished by finger-like projections from the epithelium, which appeared at the clot edge. These projections moved over the solid or semi-solid base of fibrin, which served a scaffold for the cells through the clot. Thus, 1-2 cell layers joined after migration through the clot and fused, covering the wound.

   This bridging of epithelium is apparent in the **twenty-fourth hour** (Plate XVII Figure 27) even in the presence of an unorganized clot and acute inflammation in the
connective tissue. Concurrently, the inflammatory state persisted, but continued to subside. In the connective tissue by special staining (Mallory connective tissue stain) in one subject, the young collagen fibers bundles were first noted within (36 hours) the clot area. (Plate XVIII Figure 28) Many fibroblasts were observed along the edges of the splayed collagen fibers. They had large oval nuclei with granular cytoplasm somewhat rounder or stellate in shape and projected into the clot. The nuclei in many cases were folded or curved. They continued to be liberated from the spaces between the layers of the cut collagen bundles and migrated into the clot. (Plate XX Figure 30)

2. Capillary Budding and Perivascular Proliferation

In this process of healing it is acknowledged that there is an orderly sequence in the progress of the healing. The many overlapping stages seem to present confusion but, actually, they indicate multiple processes being actively at work. The orderly sequence of activity of one action merges with the sequence of the previous action, yet both may act concurrently. This healing was aided in the twenty first hour by a cellular proliferation in the approximating connective tissue. Perivascular activity increased with formation of a large number of histiocytes, undifferentiated mesenchymal cells, eosinophilic and polymorphonuclear leucocytes and an occasional plasma cell, as previously noted. (Plate XVI Figure 26) These cells migrated into the clot through the substrate of splayed collagen and fibrin. In two subject specimens there were capillaries budding at the splayed collagen edge, and what appeared to be projecting reticulocytes from the pre-existing cut capillaries. (Plate XVI Figure 26) Special
staining revealed the production of young collagen fiber bundles increasing in quantity in these areas. Thereafter, this cellular production increased continually in its activity, until the seventy second hour, when there was a relative obliteration of the wound. (Plate IX Figure 18) Mitosis, as yet, was not seen either in epithelium or in the connective tissue.

3. **Epithelial Proliferation**

Progressive changes were seen after the twenty eighth hour with the epithelium thickening, as noted by the increase in cell layers. Most specimens had fully fused epithelium, although in two subjects, two of the serial sections revealed a remnant of the gap still present. Concurrently in the connective tissue, the perivascular stage continued and showed a diminution of inflammation with an increase of fibroblasts, histiocytes, undifferentiated mesenchymal cells and budding capillaries. At the thirty second hour (Plate VI Figure 12) the epithelium in all subjects was fused, however, the basal cell layer was, as yet, immature. On occasion, a mitotic figure was noticed in some specimens. The remaining clot had begun to appear narrower; this is probably due to the epithelial bridging, a contraction of the clot after anchorage and to the cellular production in the perivascular stage. No mitotic figures were seen in the connective tissue. In one subject, a large capillary space - dilation - was noted in the deep reticular layers. These reticular layers were heavily populated with
fibroblast, undifferentiated mesenchymal cells, budding capillaries and macrophages. Special stain showed continued connective tissue production from fibroblastic activity in the perivascular spaces and continued collagen production with a narrowing of the wound. After thirty two hours, the epithelium increased about eight cell layers in thickness without any break in the continuity in any of the subject specimens. By forty two hours, in four subjects, a small amount of keratin was noted (Plate VII Figure 14) and in two subjects, parakeratosis of the superficial layers remained. In those cases of beginning keratinization, the granular layer was present, but difficult to determine in all cases. The proliferation and production of fibroblasts and undifferentiated mesenchymal cells continued and a small number of inflammatory cells remained. The gap continued to narrow. Young fibroblasts were noted in the center of the remaining clot, where new collagen fibers were still being produced. At this time, mitotic activity of the epithelium was noted in the deeper layers with the beginning of maturation of the new basal layer.

**PHASE C - Maturation**

1. Stage of Keratinization of Epithelium

During this phase, beginning at the forty second hour, the epithelium was found to be keratinized in some subjects. Mitotic figures were noted as the epithelium continued its maturation until at seventy two hours, it was apparently healed. In returning to its normal resting stage, it has become keratinized where the granular layers and
the prickle cell layers are normal in depth and appearance and thickness and possess a well differentiated basal layer and basement membrane. The connective tissue from the forty second hour, continues its activity with the production of collagen from the existing and new fibroblasts, with the further differentiation of undifferentiated mesenchymal cells into fibroblasts. The budding capillaries are quite numerous (Plate IX Figure 18) and seem to have invaded the clot almost throughout the wound area.

2. Narrowing the Gap by the Progressive Proliferation and Partial Maturation of the Connective Tissue

At the forty eighth hour and until the seventy second hour there was a continual deposition of collagen which progressively narrowed the gap. (Plate IX Figure 18) This narrowing was not entirely completed in some areas of the specimens. The narrowing clot mass was reduced so that islands of clots were seen where new collagen fibers had joined the edges together, especially in the more superficial fiber bundles of the reticular layer. This gap was almost entirely eliminated by the seventy second hour, (Plate XIX Figure 29) but it is obvious that a subsequent period is necessary to complete the organization.

Mitosis was not evident in the epithelium but its healing (Plate IX Figure 18) was completed by the seventy second hour. Mitosis, in the connective tissue, was never apparent, except for one mitotic figure seen in one specimen at the forty eighth hour. Thus, while the healing was completed in the epithelium, the connective tissue continued with the generation and production of collagen, even at seventy two hours.
Therefore, an additional period for complete healing of the connective tissue was required.
CHAPTER V
DISCUSSION

Healing of a wound is a complex biologic phenomenon confirming to the laws of growth with alternating active and inactive factors. There is an interaction of local and systemic factors. The detailed mechanisms of healing, though often investigated, still need more clarification, especially differences in healing in different localities and different structures, deserve attention. Thus, the initiation of healing is quick and begins immediately, with a minimum of the adverse processes under ordinary circumstances.

The attached gingiva was utilized for our experiment for it was, easily accessible and was a firm, immovable tissue, bound to the underlying periosteum and bone. Furthermore, its lack of elastic fibers also prevented it from gaping.

A. CLINICAL ASPECTS

(1.) Rate of Healing

The interesting finding in this study was the rapidity in healing of the incision. After the incision was made, no gaping of the wound was evident. Clinically, hemorrhage and a small clot appeared to fill the defect. Edema and a minimal reddening of the gingiva occurred about the sixth hour and disappeared at twenty-one hours. All clinical signs of the wound disappeared by the forty-eighth hour. It is significant that all of the subjects showed healing at the same rate. This finding was anticipated as the selected subjects were young, healthy and well nourished men.
(2.) Influence of the Oral Environment upon the Healing of the Wound

The oral cavity lined by mucous membrane presents a warm and moist environment. Micro-organismal residents, desquamated cells and variable numbers of polymorphonuclear leucocytes are constant findings.

The saliva physically washes the mucosal surfaces and the teeth. Also, it serves to buffer any changes in pH of the oral cavity. Very important is the presence of the bacteriocidal property of saliva which inhibit excessive overgrowth of microbial residents. Nevertheless, it is evident that even in spite of this, an incision wound in the oral mucosa may be subject to invasion and possible subsequent infection by oral micro-organisms. Infection, produced by micro-organisms, will delay healing.

(3.) Influence of the Location of the Wound upon Healing

One interesting observation was that the incisions of the anterior gingiva appeared clinically to heal at a slightly more rapid rate than those in the posterior gingiva. Possibly the relation of the anterior gingival area to the air entering the mouth may have some influence on healing. (Lam, 1950) Owen (1946) pointed out that skin wounds left open to the air healed at a more rapid rate than wounds covered in a moist environment. However, this differs from the oral environment in that the salivary moisture is physiologically beneficial, while moisture in the skin wounds dressings only serve to incubate micro-organisms. This difference explains how microbial control in the wound environment may minimize infection, which in turn allows uninhibited healing, while in the skin it may promote infection and delay healing.
Influence of the Structure of the Attached Gingiva upon Healing

The healing response in the gingiva differs from that in the lining mucosa. The latter possesses elastic fibers, minor salivary glands, loose fibrous connective tissue and fat, and is freely moveable. Thus, an incision into this structure would present much gaping. This type of gaping wound would then expose the underlying tissue to a greater number of micro-organisms and, would require a greater tissue repair. Therefore, these tissues would require suturing for coaptation to overcome this excessive gaping. This greater connective tissue production results in a fibrosis which is clinically evident as a scar.

In Contra-distinction the attached gingiva lacks elastic fibers and glands in the lamina propria and it is firmly bound to the periosteum by dense fibrous connective tissue. This prevents gaping of the wound which, therefore, does not require surgical coaptation. Swelling may also be minimal. The absence of gaping reduces the amount of wound surface exposed to invasion by micro-organisms. It is also apparent that there is a minimum of space defect to be repaired. These factors, no doubt, prevent the formation of excessive fibrosis and scarring. Clinically, no scar formation was observed when healing was completed. These findings substantiate those of Orban (1948, 1952), Muhleman (1955) and Stahl, Soberman and Miller (1952). Also, (Wentz, Orban and Kollar 1953.)
B. MICROSCOPIC ASPECTS

(1.) Correlation of Clinical and Microscopic Findings

Microscopically, it appeared that the surface clot at first served the useful purpose in sealing the wound from the further invasion of foreign matter (Heifetz 1952.). Healing continued thereafter by the early epithelial closure of the wound. This closure was correlated to, and corresponded with, the forty-eighth hour of healing observed clinically, when the signs of the incision had been obliterated. However, the histologic picture of healing was at times at variance to the clinical picture. While clinically all signs of the wound had disappeared by forty-eight hours, however, microscopically healing was by no means complete. Though resolution of the inflammation began (twenty-first hour) and epithelialization was completed (forty-eighth hour), the deep anchoring clot in the connective tissue was still present at this time. In this clot fibroblastic activity was apparent, but production of collagen was still minimal. Even at seventy-two hours, the connective tissue had not fully healed. This was indicated by the differential staining (Mallory) for the presence of mature collagen fibers. This stain revealed fine unbundled fibers, interrupted by focal islands of degenerating clot. (Plate IX Figure 18)

(2.) Difference in Rate of Healing of the Epithelium and the Connective Tissue

The progressive sequence of healing wherein the epithelium healed in a short period of time and actually closed the wound, despite the unorganization and inflammation in the active underlying connective tissue. The migration epithelial cells occurred from the prickle cell layers in two ways; (1.) over the clot or (2.) through
the clot. When this migration occurred, the normally polygonal cells assumed a more or less elongated spindle shape. It is also significant that this early epithelial closure protects the underlying tissues by sealing the wound. This, therefore, allows the more complex healing of connective tissue with maturation of collagen to proceed without interference of intervening bacterial irritation from the outside environment. This may be compared to a dental infection at the root apex, which is aggravated constantly by the inaccessible bacteria present in the root canal. If the canal is sealed, resolution of inflammation and then the healing of the surrounding tissues may occur uninhibited.

(3.) EPITHELIAL HEALING

(a.) Clot Characteristics During Healing

The incisions into the gingiva evoked a response of hemorrhage and a clot to seal the wound from further injurious microbial and environmental elements; this agrees with the work of Heifetz (1952.) In the early hours after injury, the clot was infirm, as noted in all specimens wherein the wound was free of the clot (three to six hours.) This absence occurred during the specimen washing after the laboratory fixing, staining and slide preparation. It was considered insecure and fragile, thus requiring more time for the firmness and adherence to the approximating tissues. This had occurred by the twelfth hour, when it was firmly anchored and seen in all specimens. During this era of development, a concurrent inflammatory state was
evident. It was the reaction to the injury whereby the tissues may abort the degenerative changes which could cause the eventual death and necrosis of the tissue (Menckin 1950.)

Throughout the healing progress, the clot was an aid to the migration of cells, acting as a scaffold and pathway. This clot seemingly aided, also, in narrowing the wound gap and its contraction may have aided in the maintenance of a tight seal.

(b.) Time of Epithelium Healing

The action of the epithelium in the healing was evidenced by a migratory and amoeboid-like flow into, over and through the clot. This migratory action was almost immediate (twelfth hour.) Thereafter, the early elongation and eventual junctioning of the epithelium created a more or less permanent covering for the wound. This was characteristic and it occurred despite a lack of complete organization of the underlying tissues.

This rapid epithelial sealing may indicate a purposeful body response for survival and repair. The regeneration protects the underlying structures from the vicissitudes of the oral bacterial environment.

Interestingly enough, the epithelium healing revealed some variations. One of these worthy of notice is the fact that the healing was not uniform throughout the length of the incision. This is explainable only by the assumption that certain areas of the epithelium were more closely coated than others and, thus, healed more rapidly. This interdigitation is eventually obliterated by the continued epithelial regeneration.
so that, by the thirty-second to the thirty-sixth hour, complete fusion throughout the incision length is completed.

Full epithelialization was completed and matured by the seventy-second hour re-establishing a normal superficial surface microscopically revealing no scar formation—only a slight depression, that after a time, was also beyond recognition. In a follow up of some of the subjects, the re-establishment of the original clinical picture was entirely completed without any obvious defect.

(4.) Mitosis

The productivity and thickening of the cell layers of the epithelial cells is more physical than proliferative, due to the fact that mitosis was not apparent until later in the stage of healing. It is also obvious that there is not any accelerated mitotic activity in the adjacent cells to cause pressure and create the infolding cells into the gap. The infolding of epithelium is evident, but not from the mitosis of the neighboring epithelium, which would cause the infolding as the conclusions of Localio et al (1943) leads one to believe. Mitotic figures were noted only occasionally. This Mitosis occurred in the epithelium only after the cell layers had increased, which was about the time of keratinization (forty-two hours.) Apparently it is a fact that, mitosis is not the means for the motion or the production of cells during this activity in the epithelium. At no time were mitotic figures very obvious in number,
although some mitosis was seen at the end of the productive phase. Thus, mitosis is late in this healing process and compares to the mitosis seen in the healing in skin (Gilman, '55 and Hartwell '55.) Therefore, after the initial bridging (twenty-four hours) the production of cells increase locally without any mitotic figures, agrees with the findings in other studies and could lead one to believe that there is an atypical cell division (Loeb) for this multiplication. The latter phenomenon was not observed in this study. The gap was small, (twenty-five to fifty microns) and the thickness of migrating epithelium was so thin that only migration was all that was necessary. Therefore, neither mitosis nor amitosis was necessary for the closing of such a small gap. Thus, this presumptive evidence is more presentable than mitosis or amitosis. This is in contradiction to Loeb, who implied that there might be amitotic division.

5. CONNECTIVE TISSUE

(a.) Character of Clot

In the connective tissue the clot was not as insecure in the early hours as the clot of the epithelium, for some of it had remained attached to the edges of the collagen fibers. This indicated a beginning anchorage to the splayed fibers. After complete anchorage to the connective tissue fibers, the clot seemingly caused a contraction of the wound gap; thus, narrowing the incision wound. The clotting was early and more or less insecure up to the sixth hour, thereafter, it continually
became firmer and anchored the clot well. Throughout the balance of the experiment, the clot remained firmly anchored and aided the healing by functioning not only as a seal, but also as a scaffold for the migratory pathway of inflammatory, macrophagic and fibroblastic cells and the budding capillaries. The macrophagic activity of the histiocytes occurred during the healing process. It was noted that the hemosiderin pigment was no longer apparent after thirty-two hours.

(b.) Inflammatory Process

Inflammatory cells began to emigrate and invade the clot from the surrounding vascular spaces at the twelfth hour.

This lapse of time may indicate the local reaction of the mucosa to the injury by dilution of denatured protein of injured cells and phagocytosis by tissue histiocytes. Also, the ingress of micro-organisms, which may have occurred during the first six hours, increased the quantity of irritants which was greater than the local cellular and vascular components could handle. Such imbalance of irritant no doubt brought about the inflammatory reaction. Initially, this gingiva and its inelasticity brings forth only two responses clinically, milk redness and swelling. Pain was never noted and temperature was not taken at any time. This stage subsided in the initial phase, although the intimate cellular elements persisted until much later in healing. The inflammation was climaxed by the eighteenth to the twenty-first hour, when resolution began and continued throughout the balance
of the experiment, until about at the forty-eighth hour few inflammatory cells remained.

In essence, the inflammation was only moderately acute. This was corroborated by the clinical picture.

C. PRODUCTIVITY

(1.) Fibroblast and Collagen Production

At the beginning resolution of inflammation, there is noted an increased production of undifferentiated mesenchymal cells in the perivascular spaces. Thereafter, the production of such cells continued and (undifferentiated mesenchymal cells) contributed toward the regeneration and repair of the connective tissue. These new cells act as a parent cell, which differentiate into fibroblasts. These cells either migrate into the clot and produce collagen or, as observed, remained perivascularly and formed new collagen. This was interpreted as pushing the wound edge narrower and eliminating the gap. These cells appeared at about the twentieth hour and progressively increased so that by the forty-eighth hour they appeared in greater numbers. At the completion of the experiment, they appeared to be reduced in numbers, probably an indication of reduced stimulation from the injury. This is questioned, but may be explainable in that the greater repair is not as urgent. The fibrocytes, located between the curved, splayed collagen fiber bundles, are liberated into the gap. Such cells seem
expanded and appeared as a fibroblast (modulation.) These cells may contribute to collagen production; however, no such evidence was seen. On the other hand, such cells may undergo degeneration. In the differential stain (Mallory), there was collagen production in the middle of the clot at thirty-six hours, which is relatively late. However, this source of collagen was meager. The repair of connective tissue is noted mostly in the perivascular spaces by the increased liberation of undifferentiated mesenchymal cells and fibroblastic activity, which began after the epithelial bridging. The gap narrowed by the production and contraction of the new collagen fibers. A difference in lower animals are the fibroblastic and endothelial proliferation, which was seen after twelve hours (Pullinger & Florey) in the mouses ear, but no such observation was seen until after twenty-four hours in the human gingiva.

(2.) **Capillary Budding Production**

The new regenerated tissues required nourishment and this is achieved via the blood stream through capillary budding. Budding capillaries are extensions of the pre-existing cut capillaries in the connective tissue and increased in number as the perivascular cellular activity increased. Capillary activity was noted early (twenty-four hours) in the process of healing (twenty-one to twenty-four hours) with extensions into the clot. The new, as well as the old tissue, require
nourishment; therefore, this early proliferation of capillary budding is necessary. As the process of healing continues, the capillaries become more numerous by extensions into the surrounding area for this increased nutrition. In spite of these increased activities with the productivity of collagen and budding vascularity, the connective tissue at the conclusion of the experimental study (seventy-two hours) was only partially organized and would thus require an additional time to be fully matured, with eventual elimination of any clot areas.

(3.) Difference in Epithelial and Connective Tissue Healing

Through these observations, there is a special kind of healing in the attached gingiva of the oral mucous membrane. First, the epithelium healed rapidly to cover and protect the wound from further injury by foreign or infectious matter. This facilitated the organization of collagen, in which the underlying connective tissue is much slower in regeneration and repair.

First the epithelium regenerates by a direct extension of the pre-existing cells and proliferation is late in the process to fully bring about maturation. In contra-distinction, the collagen production is more of an indirect procedure, in that there must be a transformation of the fibrocytes and undifferentiated, mesenchymal cells to fibroblasts before there can be collagen production for connective tissue repair. Also, the vascularity of these tissues must be established for
nutrition. The epithelial repair occurs rapidly. This is indicated clinically as a healed wound. Furthermore, skin repair occurs without the formation of new cells. The connective tissue wound is only filled with a fibrin clot at this time. The repair is dependent upon the production of new fibroblasts and capillaries in the perivascular spaces. Such production of tissue migrates along the scaffold of the fibrin clot. The clot then is replaced and is reduced in mass. Subsequently, the wound gap reduces in volume as new collagen fibers are produced and undergo contraction.

(4.) Nutrition

The nutritional state of the subject has an important effect upon the rate of healing. The regeneration of wounds is bound closely to the stress associated with wounds. A particular protein is required to offer essential amino acids needed for production of cells and intercellular substance. It has been recommended by Williamson (1957) that an increase in daily requirements of proteins, before and after surgery, be instituted. The influence of vitamins likewise is required for intermediary metabolism and synthesis of protein necessary in healing. The effect of avitaminosis C is known to interfere with wound healing by the lack of maturation of collagen. The specific effect of cell maturation by the action of B 12 contributes to epithelial regeneration. It is known that the stress of injury increases the requirements of all nutriments.
CHAPTER VI
SUMMARY AND CONCLUSIONS

The purpose of this experiment was to examine the healing of an incision wound into the oral mucous membrane. The site selected was the attached gingiva of six humans. In the study, the clinical healing was correlated to the microscopic changes with particular attention given to the reaction of the epithelium and the connective tissue.

The following were the major observations:

(1.) The injury associated with the incision disrupted the continuity of the epithelium and connective tissue. Then hemorrhage and later clot formation filled the defect. A superficial clot sealed the epithelial break, while a deep clot anchored the connective tissue and acted as a scaffolding for the regenerating cells.

(2.) Microscopically, the epithelium covered the wound at the twenty-fourth hour after injury and corresponded to the healing noted clinically. The epithelium migrated into through or over the clot that served as its base.

(3.) Inflammation was moderate. A cellular infiltration appeared at nine hours. At eighteen hours the number of cells had reached optimum number; but at forty-eight hours, only a few inflammatory cells remained.

(4.) Connective tissue healing began at the thirty-sixth hour after injury, when collagen fibrils were observed in the anchoring clot. At seventy-two hours, healing was still not complete. Islands of clot separated some of the fiber bundles.
(5.) Some epithelial mitosis occurred at forty-two hours. Keratinization appears at about the same time. In the connective tissue, mitosis was seldom observed.
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Figure 1  Clinical photograph: The normal gingiva showing the normal pink color and the stippled texture.

Figure 2  Microphotograph x150: The normal attached gingiva; observe the keratinized stratified squamous epithelium and the underlying dense fibrous connective tissue.
Figure 3. Clinical photograph: Specimen immediately after incision into the interdental papilla of the normal attached gingiva. The incision is 5 mm long. Observe the hemorrhage and the clot between the lips of the wound.

Figure 4. Microphotograph x150: CONTROL SPECIMEN: Observe the epithelial gap produced by the incision at Zero hour. The connective tissue is also divided into the depth, showing splayed collagen fiber bundles.
PLATE II

Figure 3

Figure 4
Figure 5  Clinical photograph: Twenty hours after incision; there is edema and pallor with loss or stippling (texture.)

Figure 6  Microphotograph x150: Observe the physical contacted epithelial cells - seemingly fused. (Retraction of cells is due to fixing preparation) In the connective tissue inflammation and beginning resolution. Beginning perivascular activity is noted.
Figure 7  Clinical photograph: Twenty-four hours after surgery. The incision line is becoming obscure, especially at the superior and inferior ends.

Figure 8  Microphotograph x150: Twenty-four hours after surgery. Observed the infolded epithelium, resting on the underlying connective tissue and clot. (Tear is artifact) The connective tissue clot is well anchored.
Figure 9  
Clinical photograph: Twenty-eight hours after surgery. The incision has shortened. Color is almost normal.

Figure 10  
Microphotograph x150: Twenty-eight hours after surgery. Observe the infolding epithelium with the mushroom clot between these ends. Resolution is occurring, but the epithelial clot remains.
Clinical photograph: At the thirty-sixth hour after surgery, the tissue appears normal pink without edema and stipplings as may be observed. The incision is healing with the lips inverted. The wound is indistinct and is shorter.

Microphotograph x150: Thirty-six hours. Observe the fused and thickened epithelium. Basement membrane is irregular. Capillary buds are invading the clot.
Figure 13  Clinical photograph: At the forty-second hour after surgery, only a slight depression identifies the site of the incision.

Figure 14  Microphotograph x150: Forty-two hours after surgery. The epithelium is thickened and some keratin is on the surface. The basal layer of the epithelium is indistinct.
Figure 13

Figure 14
**Figure 15**

Clinical photograph: Forty-eight hours after incision shows a normal pink gingival color. Healing is almost complete.

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**Figure 16**

Microphotograph x150: Forty-eight hours after surgery. Observe the matured epithelium, slightly keratinized. Very few inflammatory cells are seen in the clot. The gap is narrower in the upper reticular layers. Active capillary budding is incurring in the clot.
Figure 17  
Clinical photograph: At seventy-two hours showing the normal color and stippling with a slight depression. Very close scrutiny is required to determine the site of the previous injury.

Figure 18  
Microphotograph x150: Seventy-two hours after surgery. The epithelium is healed -- only a slight depression is present. The surface is keratinized with two to three granular layers beneath. The basal layer is distinct. In the lower reticular layers some clot islands are seen.
Figure 19  Microphotograph x150: Sixth hour after surgery. Clot has been lost in the defect in the epithelium. A few inflammatory cells are present.
Figure 20  
Microphotograph x150: Twelfth hour after surgery. Observe the protruded mushroom clot in the gap.
Figure 21  Microphotograph x150: Eighteenth hour after surgery. Inflammatory cells have invaded the clot — histiocytes are apparent in the clot and connective tissue.
PLATE XII

Figure 21
Figure 22  Microphotograph x150: Twenty-four hours after surgery. The sealing clot in the epithelium and anchoring clot in the connective tissue is prominent.
Figure 22
Figure 23  Microphotograph x150: Twenty hours after surgery. Observe the resolution in the more superficial reticular layer, while the deeper reticular strata is still moderately filled with inflammatory cells. The epithelium is in close contact (the artifact tear is due to shrinkage.)
Figure 24

Microphotograph x500: Thirty-two hours.
Budding capillaries are migrating into the clot.

Figure 25

Microphotograph x150: Thirty-two hours. Observe the epithelium that has fused (tear is artifact), also, the migrating projections into the clot.
Figure 26  Microphotograph x150: Twenty-one hours. Migration of the epithelium through the clot is apparent. A few budding capillaries are observed.
Figure 26
Figure 27  
Microphotograph x150: Twenty-four hours. Observe the projecting finger-like epithelial 1 to 2 cells thick.
Figure 28 Microphotograph (Mallory stain) x500. Observe the collagen fibrils in the clot. Budding capillaries are also prominent.
Figure 29  Microphotograph (Mallory stain) x500. Seventy-two hours after surgery. Observe the island of clot. There is active fibroblastic activity in the approximating connective tissue.
Figure 29
Figure 30  

Microphotograph: (Mallory stain x500) Forty-two hours after surgery. Observe the young collagen fibrils extending from the collagen edges into the clot and projecting-budding capillaries. Some fibrils of collagen seemingly cross through the gap.
Figure 30
Figure 32  Diagrammatic drawing of the incision area in attached gingiva showing the incision and the biopsy site.

Figure 33  Chart of the healing: indicated in phases and depicting the process activity.

0 = None  + + + = Moderate quantity  
+ = Very little quantity  + + + + = Large quantity  
++ = Little quantity  ‡ = Very little or none
# PHASES

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Figure 31
PLATE XXII

ACTIVITY SUMMARY

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Figure 32

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