Comparative Healing of Electrosurgical and Scalpel Inflicted Incisional Wounds

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COMPARATIVE HEALING OF
ELECTROSURGICAL AND SCALPEL INFLECTED
INCISIONAL WOUNDS

by

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A Thesis Submitted to the Faculty of the Graduate School of
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the Requirements for the Degree of
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BIOGRAPHY

James Theodore Ozimek was born on February 4, 1944 in Chicago, Illinois.

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INTRODUCTION

The purpose of this study was to evaluate the extent to which the healing of an electrosurgical incisional wound differed from that of a scalpel blade incisional wound.

Histologic sections were prepared to detect alterations in healing of incisions made in the maxillary antemolar region of Sprague-Dawley rats.

Controversy exists because some previous research has been critical of electrosurgery on the basis of retarded rates of periodontal wound healing. The compared wounds in these previous studies were not produced with analogous modes of instrumentation. In this investigation a needle electrode rather than a loop electrode was used to create the incision in the oral mucosa to be compared with the scalpel blade incision.

This paper will introduce the reader to the observed differences seen in the healing of the tissues involved in the electrosurgical incision wounds when compared with tissues involved in scalpel incisions.
REVIEW OF LITERATURE

The use of electronic current for the treatment of tissue dates back to 1891. d'Arsonval (1893) reported that high frequency current would pass through the body without evoking pain or muscle contraction. Alternating currents of a frequency higher than 10,000 cycles per second caused formation of heat in a localized area of tissue at the tip of the electrode although the electrode itself remained cold.

Application of the principle of "electrothermic penetration" to tissue lesions was made by Doctor Joseph Riviere (Strong, 1968). Using an apparatus similar to that of Professor d'Arsonval, Riviere observed resolution of a previously unsuccessfully treated skin ulcer. Healing of the lesion was attributed to an accidental spark from an Ouiden or Tesla coil.

Battig (1968) stated that these spark-gap units produced pulses of dampened frequency current of sine wave form. The frequency employed was of little significance in regard to the end result sought by the surgeons of the time. The frequency range was from one to three megacycles per second with no adjustment for power output. The performance of these units was not altered by changes in local impedance from patient to patient.
Iredell and Turner (1919) employed a patient ground plate in their procedures. This allowed distribution of the surface charge on the patient, minimizing the risk of tissue burns at the site of the passive electrode.

The utilization of endothermy (localized production of heat by high frequency current from a spark gap generator) was considered valuable in removing cancerous lesions. Wyeth (1923) stated the major benefits were believed to include the sealing of lymph and blood vessels and the destruction of cancerous cells resulting in lessening the possibility of surgical induced metastasis.

Clark (1924) reported the application of electrodessication and electrocoagulation to remove oral malignant tumors. Histologic study revealed that cells in areas of electrodessication retain their outline but are shrunken, elongated, and appear divided up. Blood vessels in the immediate area were thrombosed. Areas of electrocoagulation displayed cells that were fused into a homogeneous mass with a hyalinized appearance.

Clark reported the clinical manifestations of more fibrosis in the healing tissues and sequestration or bone sloughing of bone in some cases following electrocoagulation. Oringer (1969) stated that the use of the indifferent plate creates a biterminal circuit with the end result of deeper penetration of the electrocoagulation effect.
The difference in results observed by Clark were produced by the use or non-use of Iredell and Turner's indifferent plate. Without the indifferent plate used in conjunction with the spark-gap generator of the time, electrodessication resulted. This was a shallow effect due to the lower level of heat which merely dried the tissue. Use of the indifferent plate with the spark-gap generator created a biterminal circuit resulting in higher current density at the surgeon's active electrode. The end result being a hotter and, thus, more deeply penetrating tissue effect.

Orban (1944) conducted a study of healing following electrocoagulation treatment of gingival tissues. This result was severe inflammation necrosis, sloughing of underlying bone and delayed healing.

The Orban study of 1944 was conducted utilizing the electrocoagulation modality. A more refined current had been available since DeForest's discovery of the vacuum tube oscillator. This three element vacuum tube generator had led to the development of an improved "electrothermic endothermy" technique. The improvement was a more controlled delivery of the amount of electrical power delivered. These new partially rectified electrosurgical units delivered an undampened sign wave electrical current.
McClean (1929) had recognized that variations in wave form produced by the unit affected tissues differently. His article, with comments by Bovie, discussed the electrosurgical theory. In essence, the paper stated that the recently introduced vacuum tube oscillator produced current with a wave form that resulted in a predominance of cutting over coagulation.

Electrosurgery and electrocoagulation were continued for some time for removal of tumors. A refinement of the technique known as acusection and later electrosection became more widely used. Wyeth (1925) stated that with the technique of the proper current levels generated in a vacuum tube and use of a needle electrode (termed an "endotherm knife"), tissue was cut by a minutely localized disintegration of cells at the tip of the electrode. The net result of the technique was cutting predominated over coagulation.

Oringer (1960) reiterated Wyeth's claim that cutting action predominated over coagulation with the single vacuum tube oscillator. Of prime importance was the fact that coagulation still accompanied the cutting action. Oringer thought that a cutting effect was produced by fully rectified current provided by power vacuum tubes in conjunction with either mercury vapor rectifying tubes and transistors or dry selenium rectifiers.

Ward (1926) had reported that histologic examination of tissue along the line of incision revealed an area 0.1 mm in thick-
ness of hyalinized tissue. Thus, a limited area of coagulation occurred, but healing was reported to be "satisfactory".

Mitchell and Lumb (1962) provided a more recent and technical explanation of the cause of tissue volitionalization. They stated that vacuum tube oscillators produced continuous frequency sine wave current with no dampening.

"Tissue reaction was not due to heat at the sharp edge of the needle electrode, but rather to the dissol­ution of the molecular structure. Cell morphology was altered by dissolution of molecular structure by an electric arc in the immediate path of the electrode. Radiofrequency current behaved much like electrostatic charges in that they were distributed on the surface of a body rather than uniformly throughout the body possessing the charge. The needle electrode was thought to concentrate high densities of current at points and provided intense local power responsible for the tissue effects."

Crowell (1931) and Kelley (1931) stated that from their observations the use of electrosection resulted in healing equal or superior to that of conventional knife surgery in the field of gynecological surgery.

Ellis (1931) measured the tensile strength of numerous healing incisions which were made with a knife or by electrosection on dog skin, muscle and stomach. He found no differences in the case of muscle tissue. However, the percentage of skin lesions which had primary union was 60% in the case of electrosection and 97% following knife surgery. When union did occur the electrosection lesions were weaker under tensile stress than knife lesions. Results in stomach tissue were similar to those of skin
It is evident that by 1931 there were conflicting reports being made as to the superiority of electrosurgery over the scalpel blade. It should be noted that claims were being answered with counter-claims based on observations of healing in entirely different types of tissue and a variety of electrosurgical currents.

The dynamics of wound healing in humans after gingivectomy were ascertained in a study by Orban and Archer (1945). Only reparative processes of connective tissue and epithelium were studied. Incision of tissue resulted in hemorrhage and clot formation that covered the wound after two days. This clot was seen to be demarcated by leukocytes into an outer necrotic zone and an inner protective zone. Leukocytes and the outer necrotic layer of the clot provided the inner layer with protection to allow it to organize, to undergo infiltration of capillaries and fibroblasts, and to form granulation tissue. By the ninth day the granulation tissue has provided a viable nutrient bed for epithelial cells and so enabled them almost completely to cover the wound.

The importance of an intact clot was substantiated by Toto and Annoni (1965). Their investigation into the source or undifferentiated cells in the regenerative blastem in Amphibians indicated that the fibrin clot and inflammatory exudate over the wound site acted as a temporary sealant and local defense mechanism.
The full extent of the histological response had not been determined in the Orban and Archer study of 1945 since osseous reaction in the healing of the gingivectomy wounds was not in the scope of the study.

Wider application of electrosurgery to dentistry occurred during the 1930's and 1940's. Ogus (1941) reported favorable clinical results utilizing electrosurgery for gingivoplasty.

Sagirian (1943) also reported successful clinical results applying electrosurgery to pocket removal.

Observations on the success of electrosurgery as applied to dentistry were not all in agreement. Phillips (1938) pointed out that faulty technique may lead to unnecessary loss of soft tissue or contact with bone to its destruction because of the low conductivity of hard tissues.

The extent to which soft tissue response to electrosurgery varies due to the type and location relative to calcified tissue has been observed. Hardwick (1953) provided histologic evidence that high resistance of bone resulted in coagulation of bone cells by a current which does not substantially coagulate soft tissue.

Evidence that soft tissue can be adversely affected while calcified, current resistant structure appeared uninjured has been offered. Using currents above normal in strength and duration, Beube (1953) and Agnew (1952) elicited severe pulpal dam-
Their evidence indicated that even at normal current strength excessive contacts to teeth induced vascular distension in one pulp and considerable inflammation in another.

Prior knowledge of individual tissue components at an electrosurgically induced wound site have been shown to exhibit a variety of responses. The extent to which all the tissue component responses influence the overall healing of the wound is unknown.

Harrison (1966) stated that tissue management using a partially rectified current was less desirable than with fully rectified current. He found significant difference in the self-limiting capabilities of partially and fully rectified units. Lateral dissipation of heat from the partially rectified set was evident histologically by cellular damage several layers thick and a thin coagulum along the incised margins.

Pope (1968) utilized a partially rectified unit and a loop electrode in a gingivectomy procedure on dogs. He observed retarded healing, osseous involvement, and lowered height of gingival contour. Previous studies on the importance of the fibrin clot and connective tissue damage seen in his slide led him to conclude that thermal trauma was the etiology of the delayed healing.

Oringer (1969) placed lateral heat dispersion in proper perspective to coagulation. He felt that the number of one second entries and the total number of electrosurgical instrumen-
tations was unimportant in deep pocket debridement. He stated that the factors of importance were: time interval of each interval, motion within the pocket during current flow, and an adequate lapse of time between entry to allow heat dissipation. Also stated was the fact that the coagulation effect producing bone necrosis was a function of a type of current other than fully rectified. He felt the blending of cutting and coagulating current produced the hazard. Inadequate unit output of a fully rectified instrument can result in coagulation concurrent with cutting. Oringer stated the cause inadequate power to create an energy density high enough to produce an arc resulting in total disintegration and volitalization of cells.

The extent to which thermal trauma could have altered the subjacent connective tissue would certainly be a factor influencing the rate of epithelialization and ultimately healing. Hartwell (1955) reported that one of the main factors in controlling the rate of advance of epithelial cells was the viability of the connective tissue base these cells had to migrate over.

Catchpole (1957) considered the ground substance important in wound healing. He thought that was a two-phase system of equilibrium with a water rich portion and a colloid rich portion. Ground substance was hypothesized to be a culture medium for cellular growth, activity, and reorganization. Catchpole stated
that the ground substance might be considered a sensitive transporter between blood stream and cell.

A study of the function of the epithelial layer in periodontal repair by Klingberg and Butcher (1963) revealed that removal of the epithelial layer evokes production of fibroblastic karyolysis and "periosteal atrophy". The fact that removal of epithelium with a scalpel blade in even a relatively slight wound constituted a rather severe periodontal injury was made evident by bone loss. The extent to which healing was delayed increased with the amount of overlying connective tissue removed with the epithelium.

Evidence that the epithelial layer can be electrosurgically instrumented without clinical manifestation of any periosteal alteration was shown by Armstrong (1966). Armstrong used this perfected technique to deepen the gingival sulcus slightly before taking impressions. Measurements of several treated teeth indicated that the levels of the free margin of the gingiva and the base of the sulcus were restored to normal within two weeks after surgery.

The factor of operator variance in a given procedure enters into initiating an electrosurgical wound. Klug (1966) used electrocoagulation for gingival retraction. Histologic examination revealed tissue destruction along the cut surface with some necrosis as well as vesicular changes and hydropic degeneration
in the underlying connective tissue. After healing, regeneration of gingival height was within 0.1 mm of the original which the author states is clinically acceptable. In spite of using a less desirable mode of tissue retraction, Klug attained acceptable clinical results while other operators were reporting necrosis and bone loss with a similar technique.

The effect to which thermal trauma alters the rate of healing was studied by Kelly (1968). He demonstrated re-epithelialization of electrosurgically treated gingiva in monkeys within one week. Histologic comparisons with comparable knife wounds demonstrated no retardation of healing by electrosection and in some cases a more rapid maturation of tissue.

Malone and Manning (1968) in a histologic and clinical study of ten patients found no evidence of delayed healing or retarded maturation. The gingivoplasty procedure was performed with an 893 electrode and a 255 partially rectified Cameron-Miller instrument.

The question of the appearance of cells in the line of incision when cut in ideal circumstances both by electrosection with a needle electrode and using a conventional knife was examined by Eisenmann, Malone and Kusek (1969). Human gingival papillae were surgically removed and immediately placed in glutaraldehyde fixative. Light and electron microscopic examination of cells in the line of incision revealed no differences in their appearance
whether cut with a knife or by electron. Cellular organelles and inclusions appeared to be completely unaffected by either method.

Healing followed with re-epithelialization and keratinization within one week of surgery was reported by Malone, Kusek and Eisenmann (1970). Clinical data on the gingivectomy wound healing was provided by the same subjects used in the preceding reference.

A study by Glickman and Imber involved shallow and deep resection of gingiva by knife and electrosurgical methods. The healing rate and level of the healed gingival margin were very similar following both methods in the case of the shallow resection. However, following deep resection by electrosurgery, bone necrosis and delayed healing ensued. After twelve weeks the wounds were completely healed, but in the case of those created by electrosurgery there was a statistically significant decreased gingival height and apparently more bone loss than in comparable wounds made with a knife. The study compared repeated incisions made with Kirkland #15 and #16 knives to repeated applications of a loop electrode.

The Malone and Manning (1968) study had shown that improper selection of electrode points or too slow progress though the treated tissue resulted in necrotic tissue. This characterization of tissue destruction was made on the basis of loss of
cellular definition and vacuolization.

A review of the literature leaves the reader with a variety of statements of comparative wound healing induced by using electrosurgical or blade instrumentation. None of these studies seems to allow a valid comparison of the two methods. To evaluate the comparative healing one study has yet to incorporate the following considerations.

1) Use of an instrument that produces the most refined current available.

2) Use of an electrode demonstrated to produce the least destruction to cells along the line of incision, i.e., a thin needle electrode.

3) Incision with a needle electrode, not a loop electrode, which is difficult to handle, clinically and experimentally.

4) Selection of a more easily standardized wound type, i.e., incisional.

5) Wounds in an area of a specific animal for which the base line healing dynamics have been established through independent study, i.e. auto-radiographic.
MATERIALS AND METHODS

A. Materials

Twenty-four male Sprague-Dawley rats approximately ninety days old were utilized. Male rats were chosen to minimize variations in fluctuations of the mitotic rate of epithelial tissue with stages in the oestruos cycle reportedly found in female rats by Bullough (1950) and Ebling (1954). As many of the rats as possible were requested to be litter-mates. This would minimize discrepancies in specimen age and pre-experimental stage conditioning.

All rats were maintained on Purina Rat Chow and water ad libitum in animal room at 75 degrees Fahrenheit. To facilitate handling, three animals at a time were given a light anesthetic of 60% ether and 40% alcohol. These animals were given intra-peritoneal injection of 3% nembutal solution. The amount of nembutal was determined by specimen body weight, at a dosage of 0.1 cc per 100 grams body weight. An equal volume of isotonic saline solution was injected with the nembutal through a 1 cc tuberculin syringe. Redmann (1969) utilized this anesthetic procedure with a minimal loss of specimens.

Each of the three animals in a group were anesthetized, housed and sacrificed together. Within the group the A animal was operated on first and the C animal last. Animal designation
was determined by one of three ear markings. Any change in operator dexterity within a given series of incisions could more easily be noted in the data.

Each rat was placed on the passive plate electrode to insure adequate contact for the biterminal circuit of the fully rectified Cameron-Miller 26-255R electrosurgical unit. A 26-854 needle electrode was used to create an incisional wound in the alveolar tissue of the right maxillary antemolar region (Figure 1).

Trott and Gorenstein (1963) had previously established the daily mitotic rates of rat oral and gingival epithelium. This data was reviewed to provide a normal base line of activity in the maxillary antemolar area. They found that the attached gingiva had the lowest mitotic rate, and was not influenced by function as were the cheek and palatal mucusa.

The healing dynamics of scalpel wounds in the same area and strain of rat had been recorded by Stahl et al (1968) in an autoradiographic study. While the periods of sacrifice extended over a longer span of time, many of the initial sacrifice times in this study were made to coincide to those established by Stahl.

The current strength selector was set at 3½ for all electrosurgically incised specimens. This setting exhibited neither tissue carbonization, indicating too strong a current, nor electrode drag which would indicate too weak a current. An
Incisional wound to the periosteum was attempted without excessive lag in the tissue minimizing the incidence of thermal damage to soft tissue.

To minimize operator variance in the current strength selection, a pilot study had been run on five male Sprague-Dawley rats. A scalpel incision from the first molar extending to the midline was made in the palatal tissue. Bard-Parker #15 scalpel was used. The contra-lateral site in each was incised with a 24-854 electrode at a setting of 4. The incisions were made to the periosteum. Examination under a dissecting microscope on day five found the incision sites at both sites undetectable. The conclusion from the pilot study was that the instrument power setting was within the proper range for this strain of laboratory animal. Sloughing and delayed healing were not evident.

A comparable incision wound was made in the left maxillary antemolar region with a Bard-Parker #15 scalpel blade. This incision was also made to the periosteum with a new sterile blade for each specimen. This precaution was taken to minimize the chance of connective tissue inflammation due to bacteria cross-contamination being attributed to surgical trauma. Sterilization of the electrode between specimens was unnecessary in accord with the findings of Schamburg and Malone (1969). Cell volitization due to the nature of the current produced during the electrosection immediately rendered the electrode free of viable pathogenic
Three animals were sacrificed in an ether chamber at each of the following stages of healing: immediately after incision, and at 1, 2, 3, 4, 5, 7, 10 day intervals. After decapitation the resected maxillae were placed in 10% formalin solution. This was to insure adequate fixation due to the affinity of formalin for the edipose tissue in the mass of the initially fixed specimen. The consistency of the tissue facilitated the necessary reduction of specimen size for the customary procedures of histologic sectioning.

Decalcification of the specimens was accomplished in formic acid-sodium citrate (Custer's) solution. Embedment was in paraffin and six micrometer thick serial sections were cut and every fifth section was stained with hematoxylin and eosin.

In view of the tissue response observed under the light microscope the study was extended to the electronmicroscopic level. Four male Sprague-Dawley rats were incised on the mandibular incisal eminance. The line of incision was made parallel to the long axis of the tooth using a 26-854 electrode with the power setting at 3½. The same electrosurgical unit was used in this portion of the study as for the light microscopic evaluation portion.

The change in the site of the incisional wound was prompted by the knowledge that the tissue turnover rate was more rapid in
this area Santangelo (1962). Any minute deep tissue responses were anticipated to more readily observable in this area due to the higher cell dynamics associated with rapid incisor eruption.

All specimens were sacrificed on the fifth day by arterial perfusion of a 4 per cent glutaraldehyde solution. The cacodylate-buffered glutaraldehyde maintained a pH range from 7-7.2. The area of incision was identified, ultimately post-fixed in 2 per cent osium tetroxide, and embedded in Araldite. Prepared ultrathin sections (1/20 micron) were stained with uranyl acetate and lead citrate. Examination of the incision sites was done on a Hitachi 7S electronmicroscope. Photographs of the sections were taken at a magnification of X 6,750 and 8,850 (Plates 1-5).

B. Methods

The method of evaluation was observational on the macroscopic, microscopic, and electron microscopic levels.

Macroscopic criteria were selected to delineate any differences in tissue response other than those normally expected in an incision wound with subsequent healing. See Tables A & B.

1. Prolonged hemorrhage after inflicting the wound.
2. Redness or blanching of the tissue immediately adjacent to the incision as an indication of the vascularity of the area. The state in which the connective tissue capillaries provided blood components for clot formation, inflammation, organization and maturation would be reflected.
3. Presence or absence of a visible wound during the
healing intervals. Determination to be made by the ability to locate the incision, union or non-union of the apposed incision edges.

Microscopic evaluation of the hematoxalyin and eosin stained sections was recorded for the initial, proliferative and beginning organizational stages. Photomicrographs were taken of histologic sections at 40, 100 and X250.

Observation of the wound surface, state of inflammation, epithelial bridging, connective tissue proliferation and osteoclasia were recorded (Tables C & D). The length of this study did not allow evaluation of the maturation stage.

Electron microscopic sections were evaluated on validity of tissue fixation and ultrastructure definition.

Cells along the line of incision were evaluated utilizing the desmosomes, tonofilaments, and glycogen granule integrity. The extent of traumatic disturbances was also monitored on the basis of collagen fibers and fibroblasts along the line of incision.
FINDINGS

I. Macroscopic evaluation

Observation of the wounds was done immediately after incision and sacrifice. All electrosurgically induced incisions were identical in manifesting blanching at the edges of the wound (Figure 2). This indicated some capillary constriction and lymphatic shut-down. The underlying connective tissue exposed by the incision appeared red, indicating the blanching was limited to the surface of the alveolar mucosa.

Approximately two out of every three electrosurgical incisions did hemorrhage. All scalpel blade incisions bled freely. This presented an impression that all scalpel incisions and two-thirds of the electrosurgical incisions were provided with adequate circulatory components for clot formation and wound protection.

Final macroscopic evaluation of inflammation and wound closure was recorded in Table A for electrosurgical incisions, and Table B for scalpel incisions. Scalpel incisions exhibited a lateral redness until day three as an indication if inflammation present in the lamina propria.

Table A illustrates the fact that redness was present due to inflammation of the lamina propria until day four in two out of three specimens and until day five in one specimen. Specimen A
sacrificed on day seven was the only electrosurgical incision to exhibit prolonged inflammation surrounding the wound. This was noted and histologic evidence for this observation can be seen in Figures 11A and 11B.

By day three scalpel incisions were beginning to be undetectable. Electrosurgically induced incisions were still visible on day three. Only one of these incisions on the fourth day was undetectable.

It should be noted that the fifth and seventh day A-specimens of electrosurgical incisions were clinically disconcernable. A-specimens were always the first to be incised, and presence of a delayed closure may have indicated operator variation in incisions when compared to specimens B and C.

II. Microscopic findings

A. Immediately after sacrifice

Histologic findings observed in sections made of the site immediately after incision revealed epithelial, lamina propria and calcified tissue alterations.

Epithelial cells adjacent to electrosurgically induced wounds were observed to display vacuolization and pyknotic nuclei. Evidence of loss of cell definition was seen (Figure 3B).

Basal cells were seen to be disrupted from intimate contact with the overlying epithelium lateral to the incision. An undermining of the connective tissue support of epithelium to the
definition near the incision, and pyknotic nuclei in basal cells more remote from the incision. Normal rete peg configuration of the connective tissue was observed to be absent. The connective tissue subjacent to the epithelial exhibited loss of cellular and fibrillar definition, and increase uptake of stain. This indicated a degenerative state in the lamina propria lateral to the incision.

Deeper portions of connective tissue were not as affected as evidenced by the ability to maintain blood clot formation. Figure 5B illustrates blood clot with outer necrotic and inner fibrinous layers protecting the deeper connective tissue cells.

Observation of scalpel inflicted incisions demonstrated the presence of a protective clot. Figure 6B illustrates a lack of epithelial migration even though there was no evidence of vacuolization in the epithelial layer. These sections presented a normal picture of the basal cell layer and connective tissue organization. Ground substance and fibrillar nature of the lamina propria does not demonstrate the degradation visible in electrosurgical incisions of this same healing period.

C. Wound healing after the second and third day

Inability to locate electrosurgical and scalpel wound sites in both day two and three specimens made a comparison of those stages of healing impossible.
lateral to the incision was observed.

Vacuolization and deeper staining of the lamina propria adjacent of electrosurgical incisions was observed. This indicated an alteration in the ground substance of the connective tissue. Lack of any dilated capillaries or cellular blood components indicated no hemorrhage into the wound. The soft tissue defect space was filled with a pale pink stain interpreted as coagulated fibrin protein material.

The more organic cementum surface of the root in Figure 3B appeared more drastically altered than the deeper dentinal layer.

Histologic observation of the scalpel incision immediately after the incision revealed a defect through the entire epithelial layer into the lamina propria. Epithelial cells displayed a normal configuration from the basal cell layer to the surface. Cellular atrophy and increased density of nuclear chromatin could be attributed to normal maturation.

Underlying connective tissue was observed to be in close apposition to the basal cell layer. Uniform staining of the lamina propria to the wound defect indicated no wide spread alteration of ground substance. (Figure 4B)

B. Histologic observation after day 1 (one) healing

Tissue incised with electrosurgery exhibited vacuolization within the epithelial layer. Basal cell layer disruption consisted of undermining of the epithelial layer, loss of cell
D. Fourth day of wound healing

Electrosurgically inflicted incisions demonstrated attempts at epithelial migration over the wound. Closure was not complete as illustrated in Figures 7B. Fusiform epithelial cells with large nuclei were seen layered at the edge of the incision site. The lamina propria exhibited evidence of acute inflammation through the presence of vacuolization in connective tissue cells, lack of fibrillar definition, infiltration of polymorphonuclear leukocytes, capillary enlargement and the presence of histiocytes. This indicated a less organized connective tissue bed than was necessary for epithelial migration over the wound.

Histologic sections of scalpel incisions after the fourth day of healing demonstrated a lamina propria with less extensive signs of inflammation, loss of fibrillar definition lateral to the incision site, and alteration of ground substance staining properties. The unraveled collagen fibers and freed radicals on the polymucosaccharide molecules provided sites for greater attraction of the stain. Figure 8B illustrated a less involved connective tissue response. What appeared to be complete epithelial migration can be attributed to the fact that this section was taken near the end of the incision.

E. Fifth day of wound healing

Evidence of epithelial coverage was evident in electrosurgical wounds by day five. These wounds did not involve injury
to calcified tissue. In spite of evidence of acute inflammation within the lamina propria, epithelialization was accomplished (Figure 9B).

Scalpel incisions after a comparable time also exhibited epithelialization. They differed from the electrosurgically induced incision in the quality and quantity of epithelial proliferation. The lamina propria was less extensively involved lateral to the wound site and presented a more advance picture of organization. Figure 10B illustrates the greater mitotic activity of fibroblasts, cellular orientation indicating cellular mobilization, through loose connective tissue, and a ground substance that stained with less sol state characteristics.

F. Seventh day of healing

Electrosurgical incisions in the proximity to calcified tissue, i.e. bone, showed a departure from the healing pattern developed so far in this study. Epithelial cells exhibited recession from the wound edges. Vacuolization was observed above the basal cell layer. Cells in the outer layer of exposed lamina propria exhibited a lack of definition, pyknotic nuclei is conjunction with lack of surrounding fibrillar definition. Disruption of the periosteum and osteoclastic activity was visible (Figure 11B).

The comparable scalpel incisions involving calcified tissue, i.e. bone, were epithelialized. Immature epithelial with a
thinner stratum sinosum can be seen in Figure 12B. Subjacent lamina propria was less proliferative and organized than the scalpel incision wounds after five days of healing. Periosteal involvement and osteoclastic activity was evident, but not to the extent seen in electrosurgical incisions to bone. Osteoclastic response was less than that seen in electrosurgical injury and was covered by a protective epithelial closure by day seven.

III. Electron Photomicrographic Evaluation

To focus on the calcified tissue response underlying the delayed healing seen in Figure 11B a series of electron photomicrographs were taken. The area of incision was the mandibular incisal eminence illustrated by the H & E stained slide in Plate 1.

The labial surface of alveolar bone was seen to be covered with the deepest portion of degenerated lamina propria and periosteum. Extracellular vacuoles, hydropic degeneration within the cytoplasm, and pyknotic nuclei, and disrupted intracellular bridging was evident (Plate 2).

Areas of the labial plate of bone exhibited osteoclastic activity. Plate 3 illustrates osteoclast activity in response to the overlying acute inflammation of the lamina propria.

Another source of osteoclastic activity was observed in Plate 4. The degradation of an osteocyte within the mature bone of the labial plate was observed. Evidence of non-viable mature
bone was evident beneath the electrosurgical incision.

The extent of the destruction of the mature bone was observed to continue to the periodontal ligament space opposite the incision (Plate 5).
DISCUSSION

Both types of incisional wounds were kept relatively equal in length and depth. The A specimens subjected to electrosurgical incision exhibited more frequent lack of bleeding after the insult than did specimens B and C for each healing period. This lack of hemorrhage at the time of electrosurgical incision was correlated to vesicular formation and hydropic degeneration of the epithelium and lamina propria.

Histologic sections of electrosurgical incisions that exhibited hemorrhage immediately after insult also demonstrated evidence of coagulation necrosis. This appeared to be more extensive in those incisions involving calcified tissue. From this study it can be assumed that minimal blanching and hemorrhage immediately after incision is of no value in gauging the amount of epithelial and connective tissue degeneration is involved.

While some electrosurgical incisions were found to retain the ability of protective blood clot formation and maintenance, those sections exhibiting extensive inflammatory response in the lamina propria demonstrated inadequate clot formation. This study would appear to agree with the results of Orban and Archer (1945) and Toto and Annonit (1965) on the importance of a pro-
tective blood clot. From the evidence visible, the lamina propria of electrosurgical incisions without protection exhibited a more intense inflammatory response. This response extended lateral to the incision much more than in protected incisions. This could be interpreted as a result of the missing protective zone preventing capillary and fibroblast proliferation.

The interpretation could also mean the lamina propria, in those examples of inadequate blood clot protection, was unable to supply needed serus and cellular components to the wound. Observation of loss of cellular definition, vesicularization, hydropic degeneration, pyknotic nuclei, and altered ground substance staining could be presented to support this view. Instances of marked connective tissue alteration occurred in conjunction with lack of adequate blood clot formation. Hartwell (1955) had also stated the importance of a viable connective tissue bed.

The observable delay in electrosurgical incision epithelialization can also be attributed to severe alteration of the lamina propria. Evident was the altered staining of the ground substance lateral to the incision. If we can accept consideration that the ground substance is the transporter of nutrients between blood stream and cells, we can understand why delayed proliferation of epithelium was present (Catchpole, 1957). Of particular interest was the lack of basal cell continuity to the lamina
Epithelialization of scalpel incisions appeared to be within the normal range even when calcified tissue was involved. Wound healing in regard to epithelial proliferation agree with the findings of Stahl, et al. (1968) and Trott and Gorenstein (1963). The healing times required in their autoradiographic and colchicine studies provided the norm of comparison for the scalpel wound in this study. Scalpel incisions but not electrosurgical incisions epithelized within the proper time period. The mitotic index and cell renewal rates in the electrosurgical incisions were reduced; as evidenced by the slower rate of proliferation.

An increase in connective tissue response was observed when electrosurgical incisions involved calcified tissue. Both degeneration and acute inflammation were more extensive. Two possible explanations are available.

It is known that mature bone offers high resistance to electric current (Akerman, 1962). Increased resistance of a fully rectified high frequency current results in degradation yielding a blended current (Oringer, 1969). This mixing of cutting and coagulating current because of bone resistance results in protein denaturization. The high resistance of bone with the resultant power reduction produce coagulation rather than volitalization of the cells. This coagulation was observed in the epithelium and lamina propria of electrosurgical incisions.
calcified tissue.

The second explanation of the more extensive acute inflammatory response in electrosurgical incisions could be the existence of a large amount of degraded tissue. The more degraded tissue in the area to be removed the more extensive the acute inflammation. The electron photomicrographs provided evidence of non-viable bone as a source of increased inflammatory reaction in deep electrosurgical incisions.

Lipp (1967) sited the interrelationship of blood serum proteins and the mineralization of bone ground substance. It is plausible that if bone resistance is high enough to alter ground substance transport ability in the lamina propria than the organic portion of mature bone could be altered.

The mucopolysaccharides in the ground substance separating the osteocyte from the mineral matrix of bone is part of the lacunocanalicular system of metabolic traffic (Baud, 1968). It is reasonable to expect that alteration in ground substance within mature bone as well as in adjacent connective tissue can result in disruption of necessary nutrients to maintain the osteocyte. The osteocyte degeneration in Plate 4 could be attributed to the destruction of the supporting ground substance of the lacunocanalicular system.

The observation of the extensive amount of non-viable bone in the electron photomicrographs is offered as another explana-
tion of the increased inflammatory response in deep electrosurgical incisions.

The formulation of some clinical guidelines for the use of electrosurgery were implied in this study. Electrosurgery should be selected as a surgical mode in situations requiring soft tissue management in restorative dentistry. Incisions for envelope and split thickness flaps as well as the preparation of graft sites are contra-indicated.

The inflammatory and osseous response at the edge of a flap incision or gingival graft is critical to the success of the subsequent healing following the procedure. The increased inflammatory response evident when electrosurgery involves ossified tissue warrants a critical evaluation before its use in selected clinical cases.
SUMMARY AND CONCLUSIONS

This observational study was conducted to evaluate the extent to which healing of an electrosurgical incisional wound differed from that of a scalpel blade incisional wound. Epithelialization, connective tissue, and calcified tissue response were the areas of primary interest.

Twenty-four male Sprague-Dawley rats ninety days old were used for this study. Each animal received and incision in the maxillary antemolar area with a fully rectified electrosurgical unit utilizing a 26-854 needle electrode. A comparable incision was made in each animal with a Bard-Parker #15 scalpel blade on the contra lateral side. All rats were injected with a 3\% nembutol solution at a dosage of .1 c.c. per 100 grams of body weight to facilitate handling.

Sacrifice in an ether chamber immediately after incision and at days 1,2,3,4,5,7 and 10 was conducted. Three specimens were allotted for each healing time period. Animals were decapitated and fixed in 10\% formalin for the microscopic portion of the study. Decalcification in Custer's solution was followed by washing, paraffin embedment, section into six (6) micron sections, and hematoxylin and eosin staining.
Electron photomicrographic specimens were sacrificed on the fifth day by ether chamber depression and arterial perfusion with 4 per cent glutaraldehyde solution. Ultimate post-fixing with 2 per cent osmium tetroxide and embedment in Araldite followed. The ultrathin sections (1/20 micron) were examined at X 6,750 and X 8,850 after staining with uranyl acetate and lead citrate. An incision to the bone of the incisal eminence was the area of concern.

1. Electrosurgically induced incisions exhibited more extensive connective tissue inflammation than tissue incised with the scalpel.

2. A viable protective clot comparable to that formed over a scalpel incision was maintained over the electrosurgical incision even though calcified tissue was involved in the wound.

3. A one day delay in epithelialization was seen in electrosurgical incisions when compared to scalpel incisions.

4. Epithelial migration over electrosurgical incision wounds when it did occur, did so over lamina propria exhibiting more extensive responses than those seen in scalpel wounds.

5. Osteoclastic activity in deep electrosurgical incisional wounds was seen to be initiated by a cause other than inflammation of the overlaying lamina propria with periosteal disruption. Electron photomicrographs illustrated osteocyte degeneration and lack of mature bone viability in the area of incision severe enough to bring about osteoclastic activity.
6. Lack of predictable healing responses was seen to occur when electrosurgical incisions involved calcified tissue, i.e. bone or cementum.

AGNES, R.G. and KAISER, W.F.: Effects upon the dental pulp of the macacus rhesus of externally applied high frequency electrosurgical currents. IADR Abstract #105, 30th Meeting Colorado Springs, 1952.


• Ellis, J.D.: The rate of healing of electrosurgical wounds as expressed by tensile strength. JAMA, 96: 16-18, 1931.


ILLUSTRATIONS
Figure 1 - Kodachrome of a typical rat maxilla specimen after 24 hours of fixation. The antemolar area on each side is shown to be sectioned distal to the first molar, mesial to the incision site and along the palatal raphe.
Figure 2 - A typical macroscopic comparison of the incisional wound sites. The left antemolar wound was produced with the electrosurgical instrument and the right wound with a scalpel blade. Blanching is evident along the periphery and to a minimal depth in the electrosurgically inflicted wound. The vascularity of the connective tissue bed was still evident in the deeper portions of the wound.
Figure 3A - Histologic section showing initial electrosurgical incision through attached, crestal, and crevicular gingiva. Note incision depth is to the cemental layer of the root. (H & E X40)

Figure 3B - Higher magnification of same incision. Vacuolization and lack of cellular definition is evident in the epithelial layer at the periphery of the incision. Disruption to intimate contact of epithelium to the lamina propria at the basal cell layer is evident. Note the deeper staining of the connective tissue lateral to the line of incision. Note disruption of the cementoid layer on the root surface. (H & E X100)
Figure 4A - Histologic section of an initial scalpel blade incisional wound. Incision extended through the epithelium and into the lamina propria. Note that no calcified tissue is involved at the deepest portion of the incision. (H & E X40)

Figure 4B - Higher magnification of the same histologic section. Evident is basal cell layer integrity lateral to the line of incision, uniform staining of the ground substance in the lamina propria in close proximity to the incision edges. Hemorrhage into the wound is evident by the accumulation of blood cells and fibrin clot on the connective tissue surface. (H & E X100)
Figure 5A - Histologic section showing electrosurgical wound one day after incision. Note presence of clot, deeper staining of lamina propria at the surface near the wound. As evident in the initial electrosurgically induced incision, there is disruption of basal cell layer apposition to the connective tissue below. Also, note the notching effect on the cementoid surface of the root. (H & E X40)

Figure 5B - Identical incision wound at higher magnification. Vacuolization and pyknosis is evident in the epithelial layer. Loss of intimate contact of epithelial cells to underlying connective tissue lateral to the line of incision is visible. Intense leukocytic infiltration of lamina propria indicates initiation of acute inflammation. The blood clot present can be divided into outer necrotic and inner fibrinour layers with a limiting wall of leukocytes interposed. (H & E X100)
Figure 6A - Histologic section showing one day scalpel incision wound. Blood clot is observable at the site of injury. Underlying lamina propria is stained uniformly up to the edges of the wound. (H & E X40)

Figure 6B - A higher magnification of the one day old incision reveals continuity of the epithelial, basal, and connective tissue cells. Blood clot with its outer necrotic and middle leukocytic layers is visible but dislodged from the wound surface. (H & E X100)
Figure 7A - Histologic section of electrosurgical incision at the fourth day of healing. Note the lack of epithelial migration over the wound, the cleft in the lamina propria, and the darker stain at the periphery of the cleft extending laterally into the connective tissue. (H & E X40)

Figure 7B - Histologic section at higher magnification of the fourth day electrosurgical incision wound. Evidence of connective tissue disruption is seen by the presence of polymorphonuclear leukocytes and vacuolization in the lamina propria subjacent to the basal layer. Lack of epithelial migration even over a short distance can be seen when the lamina propria is degenerative rather than proliferative. (H & E X100)
Figure 8A - Histologic section of scalpel incision at the fourth day of healing. Lack of epithelial disruption indicates this section is near the end of an incision. An inflammatory response in the lamina propria can be noted. The presence of polymorphonuclear leukocytes in the area of incision is contrasted by the relatively low vascularity of the gingiva lamina propria. Note incision did not approach calcified tissue. (H & E X40)

Figure 8B - Histologic section of fourth day healing at a higher magnification illustrating the invasion of the injured area by polymorphonuclear leukocytes. High cell density and lack of vacuolization indicates nutrients for repair are readily available and adjacent connective tissue integrity has not been interrupted. (H & E X100)
Figure SC. - Histologic section of scalpel incision at fourth day of healing. Note the upper part of section retained an undisturbed cellular fibrular and ground substance configuration. The lower area is sharply defined from the normal connective tissue. Unravelling of collagen, presence of polymorphonuclear leukocytes and darker staining indicate an alteration in the sol-gel balance of the ground substance immediate to the area of repair. (H & E X250)
Figure 9A - Histologic section at the fifth day post-electrosurgical incision. Note the epithelial migration is still attempting closure. The connective tissue cleft is surrounded by a wide diffuse area of cellular infiltration. (H & E X40)

Figure 9B - Higher magnification reveals a capillary with margination of leukocytes, migration into the connective tissue, and vacuolization. This would indicate that there is an acute inflammation, at this late date, below epithelial closure. (H & E X100)
Figure 10A - Histologic section at fifth day of healing after scalpel incision. Note closure of wound surface by epithelial migration and limited connective tissue response within the lamina propria. (H & E X40)

Figure 10B - Higher magnification illustrates immature epithelial cells at the surface covering the incision noted by their large nuclei. Lack of a definite basal cell layer and normal rete peg configuration indicate the immaturity of the epithelia closure. (H & E X100)
Figure 11A - Histologic section of electrosurgically inflicted incision after seven days of healing. Note the lack of epithelial bridging, disruption of underlying connective tissue, and close proximity of alveolar bone. (H & E X40)

Figure 11B - Higher magnification of electrosurgical incision after seven days of healing. Note the lack of epithelial migration, vacuolization in the upper layer of lamina propria with disruption of the periosteum. Lack of connective tissue, capillary proliferation indicate a nutritive bed to support epithelial migration or protective connective tissue is not available. (H & E X100)
Figure 12A - Histologic section of the scalpel incisional wound at the seventh day. Wound surface is epithelialized. Stratum spinosum is thinner than in adjacent uninjured area. Note scalpel incision is in close proximity to alveolar bone. (H & E X40)

Figure 12B - Higher magnification of the identical incision on the seventh day. Epithelial cells over the wound contain larger nuclei at the surface. Stratum spinosum is not as thick as in the adjacent epithelial layer. Basal cell layer is intact across the wound and in apposition to connective tissue cells. Note the leukocytic infiltration of the connective tissue near the bone indicating inflammation. Osteoclastic activity is observable on the surface of the mature bone. Disruption of the periosteum on bone surface nearest the overlying wound is evident. (H & E X100)
TABLES
I. Macroscopic Findings

Visible evidence of clot formation indicating wound protection as the result of post-incisional hemorrhage is denoted by the letter 'C'. 'R' indicates the observation of redness at the incision periphery as an index on connective tissue inflammation. Inability to locate the site of incision after subsequent healing is recorded as N.O. (Not observable).

Table A-Electrosurgical Incisions

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Table B-Scalpel Incisions

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II. Microscopic Findings

The letters N.O. indicate that the incision site was not observable. An 'X' denotes the existence of a histologic situation in the given column for the healing period and specimen.

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<th>Inflammatory Response</th>
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Plate 1 - Illustrating the wound location on the mandibular incisal eminence. (H & E X100)
Plate 2 - Electron photomicrograph of the deep lamina propria adjacent to the labial surface of alveolar bone. Evidence of pyknotic nuclei, extracellular vacuoles, hydropic degeneration within the cytoplasm and pyknotic nuclei were seen. (X6,750)

Plate 3 - Visible osteoclasts along the labial surface of bone amid cellular debris of deep lamina propria. (X8,850)
Plate 4 - Overlapping area showing the same osteoclast in Plate 3 and its proximity to a degenerated osteocyte housed in mature bone. Lack of organelle definition and granular cytoplasm indicate a non-viable osteocyte. (x8,850)

Plate 5 - Vacuolization, hydropic degeneration, loss of intercellular bridging evident on the periodontal ligament space adjacent to the non-viable labial bone. (x6,750)
APPROVAL SHEET

The thesis submitted by James T. Ozimek has been read and approved by the members of the faculty of the Graduate School.

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.

Date \textit{5/19/72}

William F. Malone, D.D.S., M.S.

\[\text{Signature}\]