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A Sequential Histologic Healing Study Following Gingivectomy in Streptozotocin Induced Diabetic and Non-Diabetic Rats

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A SEQUENTIAL HISTOLOGIC HEALING STUDY FOLLOWING
GINGIVECTOMY IN STREPTOZOTOCIN INDUCED
DIABETIC AND NON-DIABETIC RATS

by

Steven Segall

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

May

1980
DEDICATION

I dedicate this thesis to my father and my mother, Walter and Gerda Segall and my brother Hank Segall, whose encouragement, understanding, patience, and great sacrifices made it possible for me to further my education.
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I wish to express my sincere gratitude to Dr. Joseph J. Keene Jr. for his personal interest, guidance, and direction during the preparation of this thesis.

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VITA

The author, Steven Walter Segall, was born on August 7, 1947, in Chicago, Illinois.

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INTRODUCTION

The literature reports that rates of known diabetes within the universal population have ranged from less than .1% to more than 4%. European population rates of about 1% are now typical. In 1969 the United States Public Health Service estimated that 5% of middle aged Americans had diagnosed diabetes and 2% had undiagnosed diabetes.\(^1\) Periodontal disease is most prevalent in this age group.

This significant percent of the population with diabetes mellitus makes the literature reports of delayed wound healing in the diabetic especially important to the periodontal therapist. The surgical management of diabetics with periodontal disease is a problem because the healing of wounds may be delayed. Wound healing studies by Goodson and Hunt, Rosenthal, Prakash, and Abbey support the theory that a delay in the wound healing process is a characteristic of the diabetic state.\(^2,^3,^4,^5\)

In a periodontal healing study of alloxan diabetic rats, Glickman concluded that healing was retarded following gingival surgery because of altered fibroblastic activity, collagen formation, development of osteoblasts, and new bone formation.\(^6\)

The purpose of this study is to create a surgical incision of the type used in periodontal therapy and to compare the periodontal post-operative histologic healing response in non-diabetic and streptozotocin induced diabetic rats. The gingivectomy procedure, a secondary intention healing wound, will be utilized as a model in that it best describes all
phases of wound healing. Non-diabetic and streptozotocin induced diabetic rats will be sacrificed at pre-determined time intervals and healing wound tissue specimens will be examined histologically to compare the process. Special emphasis will be placed upon inflammatory cell types during each time interval. It is hoped that this investigation will histologically demonstrate inflammatory cell turnover effected by the diabetic state.
HEALING IN DIABETES

Experimental studies document that diabetics heal slower post-surgically than non-diabetics. In 1977 Goodson and Hunt conducted a study on secondary intention wound healing using rats that had been made diabetic using streptozotocin. Their conclusion was that non-diabetic rats healed faster than the diabetic rats, implicating that insulin was needed for adequate wound healing. They speculated that insulin replacement in the diabetic rats was needed for adequate wound healing. They also speculated that insulin replacement in the diabetic rats was more vital in the early as opposed to the late phase of healing.2

Rosenthal, Lerner, DiBiase, and Enquist using alloxan diabetic rats studied the relation of strength to composition (ground substance, collagen, and water) in diabetic wounds and their results coincided with those of Goodson and Hunt.3 Rosenthal et al., found that the gain in tensile strength, as measured by the amount of cross-linkage in the collagen molecule, of sutured abdominal wounds was greatest in the well controlled diabetic, least in the poorly controlled diabetic, and intermediate in the normal rats. No explanation for the superior healing in well controlled diabetic rats was found. There was no correlation between wound strength and the wound content of collagen (hydroxyproline), ground substance (hexosamine), or water as measured by the procedures
of Neumann and Logan and Rondle and Morgan. They also found that impaired wound strength in the poorly controlled diabetic animals was not related to hypoalbuminemia.

In 1974 Prakash, Pandit, and Sharman studied wound healing in alloxan diabetic rats. The tensile strength, histology, histochemistry, and collagen content of the wounds were studied. Protamine zinc insulin was used to control diabetes. Wound healing was markedly retarded in uncontrolled diabetic animals. The scar formed had less tensile strength and, histologically, fibrous tissue was of poor quality and quantity. In contrast, Prakash et al., described wound healing in the controlled diabetic animals as normal. Normal rats which received neither alloxan nor insulin served as the control group and healed normally. 4

In 1972 Abbey, Cohen, and Shklar studied the effect of streptozotocin-induced diabetes on the healing of artificially produced tongue wounds in rats. Histologic studies in the experimental group revealed an increase in inflammation, a delay in fibroblastic proliferation, and an exaggerated response to foreign bodies in the later stages of wound healing. Abbey et al., concluded by saying that their work supports the theory that a delay in the wound healing process is one of the characteristics of the diabetic state. 5

In 1967 Glickman, Smulow, and Moreau stated that in alloxan diabetic rats, retarded healing following gingival surgery resulted from inhibited fibroblastic activity, collagen formation, development of
osteoblasts and new bone formation; however, it did not effect the epithelium or the gingival sulcus. They further pointed out that retarded wound healing showed its most marked effect in the long term uncontrolled diabetic animals. Furthermore, the measurable wound healing differences between diabetic and control animals is temporal as at 7 days it was difficult to discern the effects of diabetes upon healing while at 14 days the differences between the diabetic and non-diabetic rats became apparent.  

In 1977 Golub, Garant, and Ramamurthy studied the inflammatory changes in gingival collagen in the alloxan-diabetic rat. They induced chronic inflammation by repeatedly injecting ferritin-antiferritin (AgAb) complexes into the gingiva of normal, untreated, and insulin treated alloxan diabetic rats. Injection into the non-diabetic produced typical chronic inflammatory cell infiltrates, fibroblasts containing profiles of intracellular collagen (electron microscopy), disruption of extracellular collagen fibrils and a reduction in collagen concentration primarily affecting the insoluble fraction (Stegmann analysis) while the inflamed gingiva of the diabetic rats appeared similar histologically to that of the normal animals. The macrophages appeared to be more congested with phagocytosed material and the fibroblasts appeared smaller and less well developed. Additionally, unlike the normal rats, the collagen content of the diabetic gingiva was not reduced during inflammation. The loss of the inflammatory effect on gingival collagen content during experimental diabetes was explained by three possibilities:
that diabetic fibroblasts had a decreased ability to effect collagen remodeling during inflammation, (2) that the inflammatory cells responsible for gingival collagen degradation were less active in diabetes, and (3) that diabetic gingival collagen, because it was more highly crosslinked than normal, resisted the degradative enzymes produced in increased amounts during the inflammatory process.  

HEALING BY SECONDARY INTENTION

Gingivectomy results in the excision of a large portion of the gingival connective tissue core. In addition, the junctional and sulcular epithelia are lost, as well as the coronal portion of the oral epithelium. The resulting wound consists of open connective tissue limited on one side by the tooth and on the other by the cut edge of the oral epithelium.

Healing of the oral and sulcular epithelia of the gingiva at the ultrastructural level was studied in dogs by Innes in 1970. Gingivectomies were done in the canine area of mongrel dogs and the healing wounds studied at intervals of one to fourteen days. Innes reported that the source of the new epithelial cells in healing arose from the basal and deeper spinous layer of the epithelium at the wound edge, as a two to three cell layer thick sheet of cells. This was noted as early as the first day after surgery. The cells appeared to migrate towards the tooth over a fibrinous covering, where the leading cells were in intimate contact with the fibrin. Five to six cells behind the leading edge, an electron-lucent zone formed between the fibrin and the epithelial cells.
This was accompanied by the appearance of hemidesmosomes and patchy sections of lamina densa. It required approximately five days for the connective tissue bed to re-epithelialize completely. At approximately five days, the fibrin had become completely resorbed and replaced by a connective tissue bed. By seven days the new epithelial covering had thickened and had differentiated sufficiently to give rise to a stratum corneum; by eight days a new sulcular epithelium had formed.\(^8\)

Other investigators found that although epithelial cells occasionally cover certain wounds by migrating along the fibrinous exudate as described by Innes this is generally not the case in skin wounds. In skin wounds the advancing edge of the epithelium is more likely to dissect its way between the connective tissue bed and the overlying coagulum. The epithelial edge seems to advance by a tumbling action of the cells according to what Krawczyk wrote in 1971. As the cells of the epithelial edge become fixed to their substrate by means of hemidesmosomes and a new basal lamina; the overlying cells glide forward and toward the substrate where they in turn, establish a new attachment to the substrate. The process is repeated until the wound is re-epithelialized. Interestingly, the migration of epithelial cells through wounded tissue may be facilitated by the apparent ability of the cells to phagocytose cellular debris.\(^9,10\)

In 1945 Orban and Archer studied the dynamics of wound healing in humans after gingivectomy. Only the 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 demarkated by leukocytes located in an outer necrotic zone and an inner protective zone. This observation was interpreted by the explanation that leucocytes 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 had provided a viable nutrient bed for epithelial cells and so enabled them almost completely to cover the wound.¹¹

In 1963 Ramfjord and Costich, using ten human specimens from nine patients, reported on the histologic healing of a gingivectomy over a zero to fourty-four day postsurgery period. The patients' periodontal health varied from clinically normal gingiva to moderately advanced periodontitis. The surgical excision was accompanied by root planing and extended to the bottom of the clinically measured crevice. Within two days, the wound was covered by a clot and necrosis was seen at the wound margin. Beginning epithelization could be seen at the borders of the wound. At four days postsurgery, partial epithelization of the wound was noted with the epithelium growing under the inflammatory exudate and on the wound surface. Also, at four days, evidence of new bone formation was present at the alveolar crest. Six days after the surgery epithelization was completed and a new epithelial attachment had formed and attached to the cementum. No evidence of crestal resorption was noted at the sixth post-op day. Seven days after surgery maturation of the epithelium was evident as some rete peg formation was seen in the epithelium which completely covered the wound. Although mild inflammation
extended into the periodontal membrane, new bone formation was seen at the alveolar crest.\textsuperscript{12}

In 1972, Stahl, Slavkin, Yamada, and Levine reviewed histologic studies of healing in gingivectomy wounds. In humans the wounds epithelialized in 7 to 14 days while in rats it took 8 days. It took 14 to 28 days for connective tissue maturation in humans; 15 to 30 days in rats.\textsuperscript{13}

Engler, Ramfjord, and Hinniker (1966) studied sequential healing of simple gingivectomy wounds in three monkeys utilizing histologic and autoradiographic techniques. The initial response was necrosis at the wound margin and acute inflammation. Migration and increased synthesis of DNA in epithelial cells started between 12 and 24 hours after the surgery and reached peak activity at the border of the wound between 24 and 36 hours after the excision. The migrating epithelial cells wedged themselves between the leukocytic zone and the healing connective tissue and reached the tooth in 5 to 7 days. The outer surface of the gingiva was completely healed and keratinized in 2 weeks; but, it took between 3 and 5 weeks to complete the healing in the new gingival sulcus. The critical period for the complete healing of the gingivectomy wound was from two to five weeks after surgery.\textsuperscript{14}

In 1969 Novaes, Kon, Rubin, and Goldman carried out a study for the purpose of observing the wound healing process and the behavior of the blood vessels when gingivectomy was performed. Eight mongrel dogs received a standard gingivectomy on the labial side of the lower incisors. Animals were sacrificed at intervals from zero hour to eighty-five day postsurgical time period. Intra-arterial injection with
filtered carbon black suspension was performed prior to sacrifice of the animals in order to visualize micro-vascularization of the healing wound. They noted that the vasodilation and increase in vascularization identified by the presence of carbon black in the second and fourth day specimens decreased as the healing progressed, thereby, achieving a near-normal appearance at sixteen days. Also, at sixteen days the epithelium matured as manifested by rete peg formation in the keratinizing surface. The wound revealed a complete epithelial covering in the seventh day specimen. Damage to blood vessels was noted in the second to fourth day healing specimens. An incipient inflammatory reaction was observed fifty-five days after injury.\textsuperscript{15}

In 1965 Stahl studied the effects of gingival wound size on healing. Twenty-five rats received gingival wounds of 1 mm diameter at the right and 3 mm diameter at the left maxilla. Animals were sacrificed over a one month post-injury period. Results indicated that enlarged diameters of the wound led to increased inflammation and delayed epithelization at the larger wound sites than was seen at the smaller sites. The tissue responses at the larger wound sites were associated with marked accumulation of debris and other irritants. These findings suggest that the size of the gingivectomy wound, i.e. surgical exposure of underlying connective tissue also may affect repair timing.\textsuperscript{16}

HEALING BY PRIMARY INTENTION

In 1854 Jacob Bigelow described an uncomplicated incised wound as "self limiting" or as one "to which there is due a certain succession
of processes, to be completed in a certain time; and which time and processes may vary with the constitution and condition of the patient, and may tend to death, or to recovery, but are not known to be shortened, or greatly changed by medical treatment. 17

According to Howes, Sooy, and Harvey in 1929 "Recovery in the case of a wound accomplishes two main purposes: the reconstruction of the gross continuity of the tissue involved and the restoration of its function. When the latter is that of weight bearing or of the transmission of physical stress, it is accomplished in the greater part by the reestablishment of the continuity of the tissue and the solidification of the scar up to the point at which it is able to bear the stress imposed on it. This is brought about in the main by the multiplication of fibroblasts and their maturation into the adult cell of connective and osseous tissues." They concluded their investigation of wound healing in dogs by suggesting that the tensile strength of a healing wound is a function of the fibroblastic process, i.e. growth of fibroblasts. 18

In a subsequent study Howes, Harvey, and Hewitt concluded that the rate of fibroplasia is the same in the healing of cutaneous wounds in the rat, the guinea pig, the rabbit, the dog, and the cat, and the difference of species does not affect the rate. 19

In 1965 Levenson, Geever, Crowley, Oates, Berard, and Rosen published a study dealing with the healing of sutured rat skin wounds. At the fifth day wound margins were loosely held together. (Sutures were
not removed until the seventh day). The wound surface was covered with fibrin which overlay multiple layers of squamous epithelium and the deeper tissues consisted of cellular granulation tissue which contained numerous fibroblasts, endothelial cells, undifferentiated adventitial cells, histiocytes, and lymphocytes. Less numerous were eosinophils, polymorphonuclear leukocytes, and in the margins, mast cells. The stroma contained pink proteinaceous material, probably fibrin. Young fibroblasts were numerous. At fourteen days the cellular reaction was less than in the five day wounds and collagen deposition was abundant. The new epidermis was slightly thicker than normal. No elastic fibrils were present. Young fibroblasts and vascular channels were more numerous than in the uninvolved dermis. The twenty-one day wound was found to be only slightly more cellular than the nearby uninvolved dermis. Within the wound the collagen fibrils were arranged in loose interlacing fascicles. Young fibroblasts were still slightly more numerous than mature forms. The forty-two day wounds continued to be relatively acellular and collagen deposition was extensive within it. Although the fibers were more compact than seen in younger wounds, their fibrillar character was still more easily recognized than in the nearby intact collagen. The felt-work design of the normal dermal collagen was not attained, however, young fibroblasts appeared somewhat more numerous than mature forms but the ratio approached that of the uninvolved dermis. Vascularity was the same as the uninvolved dermis. The six month wounds revealed approximately the same collagen fiber size, compactness, and
number as seen in the forty-two day wounds. Mast cells and endothelial elements were also present. The 365 day wounds revealed increased fiber caliber and compactness as compared to the 42 day wounds. The cellular population was sparse, mainly mature fibrocytes and endothelial elements. Rare mast cells were visible. Levenson concluded that wound collagen increased for six to seven weeks after the wound.²

In 1961 Ross and Benditt did a quantitative histological study of cells and extracellular elements in the guinea pig. They found that shortly after wounding the skin, the defect is filled with tissue debris, erythrocytes, leukocytes, fibrin, and fluid. In the case of a linear skin incision, emigration of leukocytes from vessels occurs within the first six hours; their numbers increase to a maximum during the first day, remain at this level until 2 or 3 days and then decrease. This increase and decrease in granulocytes coincides with an equally prominent rise and fall in the amount of fibrin present in the wound. The granulocytes are followed into the wound by large mononuclear cells or macrophages which reach their maximum concentration within approximately 48 hours. Lymphocytes are found in large numbers at a somewhat later stage of wound repair reaching their maximum concentration at about the sixth day after wounding. Spindle-shaped cells, identified as fibroblasts, solely on the basis of their shape, can be seen within the wound in the first 48-72 hours. They are followed into the wound by capillaries that continue to proliferate until approximately the eighth day. After the seventh or eighth day, the numbers of fibroblasts and
blood vessels begin to decrease and appear to become constant by the fourteenth day. Collagen fibers may be detected by the light microscope within the intercellular spaces after approximately four days, and continue to increase in both number and size for several weeks when the formation of a scar has occurred. The length of each of these phases is dependent upon the size of the defect. Connective tissue formation proceeds more rapidly near the wound margin, whereas the central area, particularly in larger wounds, is generally the last to heal.  

In 1970 Ross, Everett, and Tyler studied the effect of wound healing in irradiated rats. They concluded that wound fibroblasts do not arise from hematogenous precursors (monocytes?) and, therefore, must arise from the adjacent connective tissue cells.  

In a 1968 study in rats, J.W. Madden and E.E. Peacock measured the rate of new collagen deposition by administering radioactive proline and determining the specific radioactivity of non-dialyzable hydroxyproline at the end of a 24 hour injection period. They found that the net rate of collagen synthesis and deposition in cutaneous wounds was maximal around the fourteenth day and remained significantly higher than normal skin controls through the seventieth day after wounding. The rate of new collagen deposition was correlated with gain in tensile strength through the first ten weeks of healing.

**STREPTOZOTOCIN INDUCTION OF DIABETES**

Streptozotocin in an antibiotic extracted from Streptomyces
acromogenes and prepared in highly purified form. Its molecular weight is 265 with the empirical formula $C_{8}H_{15}N_{3}O_{7}$.

In 1963 Rakieten reported that streptozotocin is diabetogenic, since its intravenous administration led to frank diabetes in dogs and rats. On the basis of their histologic studies, they attributed this diabetes to damage to the pancreatic $\beta$-cells.\(^{24}\)

Evans, while confirming the diabetogenic action, suggested that it might not result from permanent damage or necrosis of the $\beta$-cells, but rather from an inhibition of production and/or secretion of insulin.\(^{25}\) Similarly Arison, Ciacco, Glitzer, Cassaro, and Pruss concluded from their studies, including electron microscopy, that streptozotocin produces degranulation of $\beta$-cells without necrosis. They reported that the additional effects of streptozotocin are: (1) development of cataracts in four months after injection of 65 mg. per kilogram of the drug; (2) accumulation of glycogen in the proximal convoluted tubules of the kidney after 65 mg. per kilogram; (3) lesions in the exocrine cells of the pancreas after 100 mg. per kilogram; (4) persistence of small, possibly secretory granules in the golgi zone of $\beta$-cells in diabetic rats.\(^{26}\)

In 1967 Junod, Lambert, Orci, Pictet, Gonet, and Renold studied the diabetogenic action of streptozotocin. They concluded that streptozotocin was a highly effective cytotoxic agent for pancreatic $\beta$-cells. After intravenous injection of 65 mg. streptozotocin per kg., they reported damage to $\beta$-cells which was apparent as early as one hour after
injection. Frank necrosis associated with phagocytosis is best seen after 7 hours, when pancreatic insulin release and hypoglycemia were also noted. By 24 hours, pancreatic insulin content was reduced to 5% of normal or less. While the \( \beta \)-cell cytotoxic effects of streptozotocin resembled those of alloxan, their specificity was very much greater, as demonstrated by a wide margin between diabetogenic dose and general toxicity.\(^{27}\)

Ganda, Rossini, and Like in 1976 also reported that streptozotocin produced \( \beta \)-cell necrosis in the rat. The study suggested that the \( \beta \)-cytotoxic effect of streptozotocin was dependent on certain interactions with the cell surface or vicinity. Complete protection afforded by 3-O-methylglucose when administered immediately prior to streptozotocin made it unlikely that intracellular events were responsible for its cytotoxic effect. However, the lack of protection by glucose and the failure of mannoheptulose to alter the protective action of 3-O-metnyglucose against streptozotocin, unlike that against alloxan, strongly indicated the possibility that the two compounds interact with different sites on the cell surface.\(^{28}\)

In the same year Like and Rossini presented evidence that streptozotocin given intravenously or intraperitoneally to laboratory mice in the usual multiple subdiabetogenic doses, induced pronounced pancreatic insulitis, followed by eventual destruction of insulin secreting \( \beta \)-cells and diabetes mellitus, and enhanced replication of type C virus particles within pancreatic \( \beta \)-cells. The timing and appearance of the
inflammatory islet lesions suggested, but did not prove, that streptozotocin may initiate a cell mediated immune reaction directed against the C virus infected β-cells. The relevance, if any, of the increased number of type C virus particles to the inflammation and β-cell destruction was unknown.²⁹

In 1977 Rossini, Like, Chick, Appel, and Cahill similarly reported that multiple small injections of streptozotocin produced a delayed, progressive increase in plasma glucose in mice within 5-6 days. Examination of the pancreas also showed pronounced insulitis and the induction of type C viruses within β-cells. Multiple subdiabetogenic doses of streptozotocin in rats and multiple injections of another β-cell toxin, alloxan, in mice did not induce insulitis although hyperglycemia followed the injection of large quantities of both agents. In mice, the prior injection of 3-O-methyl-D-glucose (3-OMG) or nicotinamide attenuated the diabetic syndrome produced by streptozotocin; however, 3-OMG was more protective than nicotinamide. Rabbit antimouse lymphocyte serum, alone, provided partial protection but, when given together with either 3-OMG or nicotinamide, effectively prevented the streptozotocin-induced diabetic syndrome. Cessation of these preventive treatments was followed by the appearance of insulitis and diabetes. These findings suggested that multiple injections of streptozotocin induce, in susceptible animals, the triad of direct β-cell cytotoxicity, virus induction within β-cells, and cell mediated autoimmune reaction. These factors, acting separately or in concert, appear to induce a
destructive insulitis and severe diabetes in animals. 36

EFFECTS OF DIABETES MELLITUS ON LEUKOCYTE FUNCTION

Although it is well accepted that infection complicates the control of blood glucose and ketosis in patients with diabetes, there is little agreement that patients with diabetes have an increased susceptibility to bacterial infections. However, when infections do occur in diabetic patients, they tend to be protracted and severe. 31 Bybee and Rogers described a defect in phagocytic activity by leucocytes from diabetic patients during periods of acidosis. 32 Perillie, Nolan, and Finch found depressed accumulation of neutrophils at the site of skin abrasion in diabetic patients with acidosis. 33 In both of these studies neutrophil function returned to normal with correction of acidosis.

Studies by Brayton, Stokes, Schwartz, and Louria 34 and Mowat and Baum 35 showed depressed leukotactic activity in adult diabetics in good metabolic control.

In 1974 Begdade, Root, and Bulgar studied the impaired leukocyte function in patients with poorly controlled diabetes. Patients with poorly controlled but non-ketotic disease were studied before and after therapy. Before treatment, phagocytosis was significantly reduced and, consequently, the rate of killing the test organism (pneumoccus) was decreased. Following antidiabetes therapy phagocytosis improved significantly. These studies suggest: (1) PMN function may be impaired during periods of poor diabetes control, as has been shown previously in ketoacidosis, and (2) hyperglycemia or a closely related factor may contribute
to the defect.\textsuperscript{36}

In 1978, Bagadade, Stewart, and Walters reported that in addition to the previously reported abnormalities in migration and the ingestion and killing of bacteria, granulocyte adherence may also be impaired in poorly controlled diabetic patients. This functional abnormality correlated directly with fasting glucose and is reversed by insulin treatment. Bagdade et al., concluded that a defect of this type may compromise the normal inflammatory response in some diabetics and impair the capacity to resist infection.\textsuperscript{37}
MATERIALS AND METHODS

Nineteen Sprague-Dawley (Holtzman) male rats weighing from 333 to 393 grams were utilized in this study.

Experimental animals were divided into two groups (A and B). Group A consisted of nine rats that were chemically made diabetic and wounded. Group B consisted of nine non-diabetic rats which were wounded. One additional animal was housed as a complete control and not subjected to either the diabetic inducer or wounding.

Group A rats were injected intraperitoneal (I.P.) with 65mg./kg. streptozotocin* diluted in a 1 ml. citrate buffer solution. Each animal was injected within five minutes of the solution preparation. Care was taken to insure good asepsis at the time of injection.

Gloves were worn to protect the investigators from the streptozotocin which has been shown to produce pancreatic tumors in experimental animals.

Citrate buffer solution:
Preparation of the citrate buffer was as follows:

Sol.A--1.114 gm citric acid H$_2$O was diluted with 100 ml. NaCl 0.9%
Sol.B--1.382 gm Na citrate 2 H$_2$O was diluted with 100 ml. NaCl 0.9%

*Streptozotocin: Lot No. 60, 273-3, U9889 was obtained from Dr. W. Dulin, The Upjohn Co., Kalamazoo, Michigan.
Solution A was mixed with solution B until the pH of the citrate buffer solution was 4.5.

The diagnosis of diabetes mellitus was made when the animal evidenced the following characteristics:

A) Weight loss or absence of normal weight gain during growth period.

B) Polydypsia and Polyuria

C) Glucosuria (see table 2)

D) Hyperglycemia (see table 2)

Animals from Group A that did not become diabetic within seven to nine days were subsequently reinjected I.V. in order to induce the diabetic state.

Each of the diabetic, (Group A), and non-diabetic (Group B) rats were subjected to the gingivectomy periodontal surgical procedure. The gingival resection was performed palatal to the maxillary incisors.

One each of the diabetic (Group A) and non-diabetic (Group B) rats was sacrificed at each of the following post-gingivectomy time intervals: 3, 6, 24, 36, 48, 96 hours; 7, 14, and 28 days.

Immediately upon sacrifice the gingival tissue palatal to the maxillary incisors was dissected. All tissue was immediately fixed in 10% formalin and prepared for histologic sectioning.

Specimens were placed alcohol and xylenes, embedded in paraffin and sectioned with the microtome approximately 5 microns in thickness, floated on glass slides, dried and deparaffinized according to conventional histologic technique for staining with hematoxylin and eosin.
Sections of each tissue specimen were viewed with the light microscope under oil immersion using an eyepiece containing a 100 micron square grid. All neutrophils and monocytes within the grid were counted. The areas counted were with 200 microns of the wound margins. Three areas were counted on each slide; an average number of neutrophils and monocytes was computed for each slide.

Each tissue specimen was viewed with the light microscope under low power (40X) and under higher power (100X) in order to characterize the healing sequence histologically.
RESULTS

3 Hour Diabetic

Low Power- This section shows keratinized stratified squamous epithelium with a break in the continuity of the epithelium where the incision was made. Beneath the epithelium and in the incision area the lamina propria is infiltrated with inflammatory cells. Also present in this slide are mature connective tissue and muscle tissue.

High Power- Extravasation of RBC's and fibrin formation can be seen in the connective tissue. Collagenolytic and sarcoelytic activity are present. The inflammatory cells in the connective tissue consist of PMN's, monocytes, and mast cells.

3 Hour Non-diabetic

Low Power- This section shows keratinized stratified squamous epithelium with a break in the continuity of the epithelium where the incision was made. Beneath the epithelium and in the incision area the lamina propria is infiltrated with inflammatory cells. Also present in this slide are mature connective tissue and muscle tissue.

High Power- There is extravasation of RBC's in the connective tissue. The inflammatory infiltrate consists of a few PMN's and a few monocytes. Collagenolytic and sarcoelytic changes are apparent.
6 Hour Diabetic

Low Power- This section shows keratinized stratified squamous epithelium with a break in the continuity of the epithelium where the incision was made. The lamina propria beneath the incision area and epithelium is heavily infiltrated with inflammatory cells. Necrotic debris is visible. Also present are mature connective and muscle tissue.

High Power- Extravasation of RBC's and fibrin formation are apparent within the wound. The connective tissue infiltrate consists of PMN's, monocytes, and macrophages. Collagenolytic and sarcolytic changes can be seen.

6 Hour Non-diabetic

Low Power- This section shows keratinized stratified squamous epithelium with a break in the continuity of the epithelium where the incision was made. The lamina propria beneath the incision area and epithelium is heavily infiltrated with inflammatory cells. Necrotic debris is visible. Also present are mature connective and muscle tissue.

High Power- Extravasation of RBC's and fibrin formation are apparent within the wound. The connective tissue infiltrate consists of PMN's, monocytes, and macrophages. Collagenolytic and sarcolytic changes can be seen.

24 Hour Diabetic

Low Power- This section is characterized by loss of epithelium
at the wound site except for an epithelial remnant at the edge. The lamina propria beneath the incision area is infiltrated by inflammatory cells. The necrotic tissue on the wound surface is stained deeply basophilic. Mature connective tissue and muscle tissue are present in this slide.

High Power- This section demonstrates formation of a fibrinopurulent exudate at the wound surface. Vasodilatation is evident in the connective tissue. The inflammatory infiltrate consists of PMN's, monocytes and mast cells. There is margination of PMN's along blood vessels. Collagenolytic and sarcoytic activity are seen in this slide.

24 Hour Non-diabetic

Low Power- This section shows keratinized squamous epithelium (stratified) with a break in the continuity of the epithelium where the incision was made. The connective tissue and muscle tissue in and around the wound site are infiltrated with inflammatory cells. The wound site also shows a fibrinopurulent exudate. Mature connective tissue and muscle tissue are present in this slide.

High Power- There is evidence of epithelial migration in this section. The fibrinopurulent exudate contains PMN's, monocytes, and mast cells. There is no vasodilatation or engorgement of the capillaries. Collagenolytic and sarcoytic degeneration are seen. Fibroblast-like cells are visible in the connective
tissue, along with PMN's, monocytes, and mast cells.

36 Hour Diabetic

Low Power- This section shows keratinized squamous epithelium (stratified) with a break in the continuity of the epithelium where the incision was made. The connective tissue and muscle tissue in and around the wound site are infiltrated with inflammatory cells. The wound site also shows a fibrinopurulent exudate. Mature connective tissue and muscle tissue are present in this slide.

High Power- The section shows three distinct zones: 1) Necrotic, 2) Leukocytic, 3) Fibrinous. The fibrinopurulent exudate contains PMN's, monocytes, and mast cells. The connective tissue inflammatory infiltrate contains PMN's, monocytes, and mast cells. There is vasodilatation and extravasation of RBC's in the connective tissue. Undifferentiated mesenchymal cells can be seen around the blood vessels. Collagenolytic and sarcolytic degeneration are seen.

36 Hour Non-diabetic

Low Power- The sections are characterized by loss of epithelium at the wound site except for some epithelial remnants. The connective tissue and muscle tissue at the wound site are infiltrated by inflammatory cells. The wound site also shows a fibrinopurulent exudate. Mature connective tissue and muscle tissue are present in this slide. Dilated blood vessels can be seen in the connective tissue.
High Power- The section shows three distinct zones: 1) Necrotic, 2) Leukocytic, 3) Fibrinous. The fibrinopurulent exudate contains PMN's, monocytes, and mast cells. The connective tissue inflammatory infiltrate contains PMN's, monocytes, and mast cells. There is vasodilatation and extravasation of RBC's in the connective tissue. Undifferentiated mesenchymal cells can be seen around the blood vessels. Collagenolytic and sarcolytic degeneration are seen. Fibroblasts can be seen in the connective tissue. Undifferentiated mesenchymal cells surround many of the blood vessels in the connective tissue.

48 Hour Diabetic

Low Power- The sections are characterized by loss of epithelium at the wound site except for some epithelial remnants. The connective tissue and muscle tissue at the wound site are infiltrated by inflammatory cells. The wound site also shows a fibrinopurulent exudate. Mature connective tissue and muscle tissue are present in this slide. Dilated blood vessels can be seen in the connective tissue.

High Power- The section shows three distinct zones: 1) Necrotic, 2) Leukocytic, 3) Fibrinous. The fibrinopurulent exudate contains PMN's, monocytes, and mast cells. The connective tissue inflammatory infiltrate contains PMN's, monocytes, and mast cells. There is vasodilation and extravasation of RBC's in the connective tissue. Undifferentiated mesenchymal cells can
be seen around the blood vessels. Collagenolytic and sarcolytic degeneration are seen. The connective tissue contains streaming monocytic cells—probably fibroblasts. There is margination of PMN's around the blood vessels within the connective tissue. Macrophages are present within the connective tissue.

48 Hour Non-diabetic

Low Power—This section shows keratinized stratified squamous epithelium with a break in the continuity of the epithelium and connective tissue where the incision was made. Three distinct zones: 1) Necrotic, 2) Leukocytic, 3) Fibrinous can readily be seen. The necrotic layer stains basophilically. The connective tissue and muscle tissue are infiltrated with inflammatory cells. There is a fibrinopurulent exudate at the wound site. Mature connective tissue and muscle tissue are present in this slide.

High Power—The section shows three distinct zones: 1) Necrotic, 2) Leukocytic, 3) Fibrinous. The fibrinopurulent exudate contains PMN's, monocytes, and mast cells. The connective tissue inflammatory infiltrate contains PMN's, monocytes, and mast cells. There is vasodilatation and extravasation of RBC's in the connective tissue. Undifferentiated mesenchymal cells can be seen around the blood vessels. Collagenolytic and sarcolytic degeneration are seen. The connective tissue contains streaming monocytic-cells probably fibroblasts. There is margination of PMN's around the blood vessels with the connective tissue. Macrophages
are present within the connective tissue.

96 Hour Diabetic

Low Power- This section shows keratinized stratified squamous epithelium with a break in the continuity of the epithelium and connective tissue where the incision was made. Three distinct zones: 1) Necrotic, 2) Leukocytic, 3) Fibrinous can readily be seen. The necrotic layer stains basophilically. The connective tissue and muscle tissue are infiltrated with inflammatory cells. There is a fibrinopurulent exudate at the wound site. Mature connective tissue and muscle tissue are present in this slide.

High Power- This section shows three zones: 1) Necrotic, 2) Leukocytic, and 3) Fibrinous. The fibrinopurulent exudate contains PMN's, monocytes, and mast cells. The inflammatory infiltrate in the muscle and connective tissue contains PMN's, monocytes, and mast cells. There is vasodilatation and extravasation of RBC's in the connective tissue. Undifferentiated mesenchymal cells can be seen around the blood vessels. Collagenolytic and sarcolytic degeneration can be seen on this slide. The connective tissue contains proliferating fibroblasts and mitosis of fibroblasts is seen. There is margination of PMN's around blood vessels in the connective tissue. Macrophages are present in the connective tissue.

96 Hour Non-diabetic

Low Power- This section shows keratinized stratified squamous
epithelium with a break in the continuity of the epithelium and connective tissue where the incision was made. Three distinct zones: 1) Necrotic, 2) Leukocytic, 3) Fibrinous can readily be seen. The necrotic layer stains basophilically. The connective tissue and muscle tissue are infiltrated with inflammatory cells. There is a fibrinopurulent exudate at the wound site. Mature connective tissue and muscle tissue are present in this slide.

High Power- This section shows three zones: 1) Necrotic, 2) Leukocytic, and 3) Fibrinous. The fibrinopurulent exudate contains PMN's, monocytes, and mast cells. The inflammatory infiltrate in the muscle and connective tissue contains PMN's, monocytes, and mast cells. There is vasodilatation and extravasation of RBC's in the connective tissue. Undifferentiated mesenchymal cells can be seen around the blood vessels. Collagenolytic and sarcolytic degeneration can be seen on this slide. The connective tissue contains proliferating fibroblasts and mitosis of fibroblasts is seen. There is margination of PMN's around blood vessels in the connective tissue. Macrophages are present in the connective tissue. There is a flattening of the epithelial cells at the edge of the wound. The epithelial edge is located between two fibrinous zones.

7 Day Diabetic

Low Power- This section shows keratinized stratified squamous epithelium completely covering the wound site. Epithelization is nearly
complete and the stratum granulosum appears very granular. Occlusion of capillaries can be seen. Also present in this slide are dense connective tissue (granulation tissue) and mature muscle tissue.

High Power- Epithelial cells with hyperchromatic nuclei and mitotic figures can be seen in the basal cell layer of the epithelium. Fibroblasts and fibrocytes can be seen—there is much fibroblastic activity with active collagen deposition. Undifferentiated mesenchymal cells can be seen around the capillaries. This section contains mononuclear cells, PMN's, tissue mast cells, and plasma cells. Myotubes are present indicating regenerating muscle tissue.

7 Day Non-diabetic

Low Power- This section shows keratinized stratified squamous epithelium partly covering the wound site (due to sectioning). Epithelization is nearly complete and the stratum granulosum appears very granular. Occlusion of capillaries can be seen. Also present in this slide are dense connective tissue (granulation tissue) and mature muscle tissue.

High Power- Epithelial cells with hyperchromatic nuclei and mitotic figures can be seen in the basal cell layer of the epithelium. Fibroblasts and fibrocytes can be seen—there is much fibroblastic activity with active collagen deposition. Undifferentiated mesenchymal cells can be seen around the capillaries. This section con-
tains mononuclear cells, PMN's, tissue mast cells, and plasma cells. Myotubes are present indicating regenerating muscle tissue.

14 Day Diabetic

Low Power- This section shows keratinized stratified squamous epithelium completely covering the wound site. Epithelization is nearly complete and the stratum granulosum appears very granular. Rete pegs are absent. Occlusion of capillaries can be seen. Also present in this slide are dense connective tissue) granulation tissue) and mature muscle tissue.

High Power- Epithelial cells with hyperchromatic nuclei and mitotic figures can be seen in the basal layer of the epithelium. Fibroblasts and fibrocytes can be seen- there is much fibroblastic activity with active collagen deposition. Undifferentiated mesenchymal cells can be seen around the capillaries. This section contains mononuclear cells, tissue mast cells, and plasma cells. Myotubes are present indicating regenerating muscle tissue. This section also shows a well developed vasculature, subepithelial fibrin, and focal accumulations of PMN's.

14 Day Non-diabetic

Low Power- This section shows keratinized stratified squamous epithelium completely covering the wound site. Epithelization is nearly complete and the stratum granulosum appears very granular. Rete pegs are absent. Occlusion of capillaries can be seen. Also present in this section are dense well organized connective tissue (granulation tissue) and mature muscle tissue.
High Power - Epithelial cells with hyperchromatic nuclei and mitotic figures can be seen in the basal layer of the epithelium. Fibroblasts and fibrocytes can be seen- there is much fibroblastic activity with active collagen deposition. The connective tissue contains well oriented fiber bundles. Mononuclear cells can be seen.

28 Day Non-diabetic

Low Power - This section shows keratinized stratified squamous epithelium completely covering the wound site. "Pseudo rete peges" can be seen. Epithelization is complete and the stratum granulosum appears very granular. Occlusion of capillaries can be seen. Also present in the section are dense connective tissue (granulation tissue) and mature muscle tissue.

High Power - Epithelial cells with hyperchromatic nuclei and mitotic figure can be seen in the basal layer of the epithelium. Fibroblasts and fibrocytes can be seen- there is much fibroblastic activity with active collagen deposition. Final repair is evident at the fibromuscular junction. There are few PMN's, mononuclear cells, mast cells, and plasma cells.

PMN and Monocyte counts

The numbers of PMN's and monocytes per 100 micron grid were counted and are presented in table 1 and graph 1. The counts are meant to be informative and were not subjected to statistical analysis.
DISCUSSION

Streptozotocin causes pancreatic islet beta cell necrosis in rats. The animals develop abnormally high blood and urine sugar levels as measured with Eli Lilly Testape and Dextrostix (Ames). It was found in this investigation that intravenous administration of streptozotocin was a more reliable way to induce diabetes than intraperitoneal administration of streptozotocin.

While insulin deficiency was induced in one half the rats used in this experiment, it is important to note that it was of less than 7 days duration. Therefore, in this study, the wound healing in rats occurred in acute short term diabetes characterized by acute hyperglycemia and impaired capacity to produce insulin. This is in contrast to that which may occur in long term complications of diabetes mellitus.

There is virtually no difference in the rate of healing in wounds of 'diabetic' and normal rats, as at 14 days the two groups of wounds were indistinguishable. It seems that neither the lack of insulin nor the elevated blood sugar levels of short duration affected the healing process. This suggests that delayed healing does not occur in diabetes of short term but may be due to a longer term effect of the diabetic state as noted by Glickman, Smulow, and Moreau in 1967.6

Healing of the secondary intention wounds in both the streptozotocin induced diabetic rats and the non-diabetic rats followed the classical healing sequence as described by Orban and Archer11; Ramfjord and
Costich\textsuperscript{12}; Stahl, Slavkin, Yamada, and Levine\textsuperscript{13}; and others. When the injury occurs, the wound margins retract because of normal skin tension and muscle pull. There is cell destruction at the site of injury, and the physical interruption of blood vessels causes immediate bleeding. After injury, erythrocytes and exudate quickly fill the wound cavity forming a clot which will later contract and dehydrate to form the protective scab. Vasoconstriction and vascular occlusion immediately occur in the arterioles and larger veins so that hemostasis is achieved. Vasodilatation occurs after the transient arteriolar vasoconstriction.

Three hours after injury leukocytes infiltrate the area around the wound (see figures \#1 and 2). Within 12 to 18 hours after injury a compact layer of these cells demarcates the intact dermis from the wound site (see figure \#4). Bundles of fibrin appear in the wound exudate above the leukocytic layer. The acute inflammatory reaction subsides within 3 to 5 days.

Classically epithelization, the first definitive sign of healing, begins during the acute inflammatory reaction. This normally occurs within 24 hours after injury. In our study there is evidence of epithelization in the 24 hour non-diabetic rat (see figure \#5) and there is almost complete epithelization in the 7 day diabetic and non-diabetic rats (see figures \#15 & 16). Classically, there is an increase in mitotic activity of cells in the basal and prickle cell layers near the wound edge. At the margin of the migrating epithelium, a single layer of flattened cells moves across the wound. Upward cell migration
produces stratification. Differentiation and keratinization occur where several layers of cells cover the wound. After ten to twelve days, numerous projections of epithelial cells move downward from the under-surface of the migrating epithelium into the granulation tissue to form "pseudo rete pegs" (see figure #20). In our study "pseudo rete pegs" are visible in the 28 day non-diabetic specimen. Classically these disappear and degenerate at a later date. True rete ridges never develop at the injured site.

Our finding in both the diabetic and non-diabetic rats support the classic studies which report that by two days a network of new capillaries develops (see figure #12). The new capillaries plus immature fibroblasts form granulation tissue. Vascularization increases until the fifth day and then gradually declines, but it is still greater than in normal tissue on the fourteenth day.

Wound contraction begins about the fourth day after injury. The processes of epithelization and wound contraction are independent. During the productive phase leading up to contraction, the fibroblasts proliferate (see figure #12). In this study fibroblast-like cells are visible in the 24 hour non-diabetic rat. The majority of these fibroblasts are probably derived from resting mesenchymal cells and fibrocytes located in the adventitia of small blood vessels in the connective, muscle, or fat tissue immediately under the wound margin.

Collagen synthesis starts as early as the second day, increases rapidly from about the sixteenth day and then gradually declines over
the next two years as some late scar remodeling occurs.

During the course of this investigation the 28 day diabetic rat died at approximately 20 days and was not included in the results. The 28 day non-diabetic rat was included to demonstrate longer term healing.

At 3, 6, 12, 24, 36, 48, and 96 hours the numbers of PMN's and monocytes in the wound areas of the streptozotocin induced diabetic rats and the non-diabetic rats were compared. No comparative conclusions can be drawn between the two groups (see table 1; graph 1). Because of inconsistencies in the counts the data was not subjected to statistical analysis. The results of Perillie, Nolan, and Finch; Brayton, Stokes, Schwartz, and Louria; Bybee and Rogers; Mowat and Baum; Bagdade, Root, and Bulgar; and Bagdade, Stewart, and Walters reported significantly fewer PMN's in diabetic animals due to impaired migration, phagocytosis, and adherence. In this investigation fewer PMN's were counted in the 3 and 6 hour diabetic animals. In the 24, 36, 48, and 96 hour animals fewer PMN's were counted in the non-diabetic animals. In both groups, when numbers of PMN's decreased, numbers of monocytes increased (Graph 1). These observations are not based on statistical analysis.
SUMMARY AND CONCLUSIONS

Nineteen male Sprague Dawley rats were utilized in this study. Nine of the rats were made diabetic chemically with streptozotocin. The diagnosis of diabetes mellitus was confirmed by polydipsia, polyurea, glycosuria, hyperglycemia, and loss of weight. After one week nine of the diabetic rats and nine of the non-diabetic rats were subjected to a gingivectomy surgical procedure. Experimental rats were sacrificed at the time intervals of 3, 6, 24, 36, 48, 96 hours and 7 and 14 days.

Immediately upon sacrifice the wound site was biopsied. The biopsies were fixed in 10% formalin and prepared according to conventional histologic technique utilizing hematoxylin and eosin staining procedures. Descriptions of the histological characteristics of each slide were carried out. PMN's and monocytes were counted near the wound margins.

The following conclusion can be drawn from this study:

Since there is no histologic difference in the rate of healing between streptozotocin induced diabetic rats (less than 7 days) and normal rats, and at 14 days the two groups of wounds were indistinguishable, it seems that neither lack of insulin nor the raised blood sugar levels of short duration affected the healing process.
REFERENCES


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<th>Slide</th>
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<th># Monocytes</th>
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<td>18</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>22 ave.-20</td>
<td>12 ave.-9</td>
</tr>
<tr>
<td></td>
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<td>7</td>
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<td>3</td>
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<tr>
<td></td>
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<td></td>
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Table 1. This table represents the totals from cell counts made within streptozotocin induced diabetic and non-diabetic wound sites in experimental rats. It is meant to be informative and was not subjected to statistical analysis. (#cells/100 micron grid)

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<th>Slide</th>
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<th># Monocytes</th>
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</tr>
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<td>**Dextrostix</td>
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<td>45%</td>
</tr>
<tr>
<td>6 hour diab.</td>
<td>2%</td>
<td>250%+</td>
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<tr>
<td>6 hour non-diab.</td>
<td>0</td>
<td>45%</td>
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<tr>
<td>24 hour diab.</td>
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<tr>
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Table 2. Table of blood and urine glucose levels in experimental animals.

*Testape (Urine sugar semiquantitative analysis paper for in-vitro diagnosis).
By Eli Lilly and Company; 307E McCarty St., Indianapolis, Ind., 46206.

**Dextrostix (Reagent strips for determination of blood-glucose levels qualitatively and quantitatively.)
By Ames Company, Division of Miles Laboratories, Inc. Elkhart, Indiana, 46514.
Graph 1. This graph represents cell counts made within streptozotocin induced diabetic and non-diabetic wound sites in experimental rats. It is meant to be informative and was not subjected to statistical analysis. (# cells/100 micron grid)
Fig. 1. 3 hour diabetic rat. Note vasodilation, cellular debris, red blood cell and leukocytic infiltrate. (Original magnification 100X)

Fig. 2. 3 hour non-diabetic rat. Note cellular debris, vasodilation, loss of blood clot, disruption of epithelium. (Original magnification 100X)
Fig. 3. 24 hour diabetic rat. Note superficial necrosis, leukocytic infiltration, and early fibrin formation. (Original magnification 100X)

Fig. 4. 24 hour non-diabetic rat. Note superficial necrosis and that the leukocytic and fibrinous zones are well demonstrated. (Original magnification 100X)
Fig. 5. 24 hour non-diabetic rat. Note epithelial migration, PMN's in well demarcated fibrin area. (Original magnification 400X)
Fig. 6. 24 hour diabetic rat. Note extravasation of red blood cells, PMN's, monocytes, and fibrin formation. (Original magnification 400X)

Fig. 7. 24 hour diabetic rat. Note tissue basophil, PMN's, and monocytes in a fibrin matrix. (Original magnification 1000X)
Fig. 8. 36 hour diabetic rat. Note fibrinopurulent exudate. (Original magnification 400X)
Fig. 9. 36 hour diabetic rat. Note edge of epithelium, fibrinopurulent exudate, infiltration of primarily PMN's and monocytes. (Original magnification 200X)

Fig. 10. 48 hour non-diabetic rat. Note necrotic layer, leukocytic layer, fibrinous zone, epithelium between the leukocytic and fibrinous zone. (Original magnification 100X)
Fig. 11. 48 hour diabetic rat. Note the vasculature contains primarily leukocytes; indifferentiated mesenchymal cells around the blood vessels; PMN's, monocytes in adjacent tissues; presence of tissue basophils. (Original magnification 400X)
Fig. 12. 48 hour non-diabetic rat. Note vascular dilation and stasis and undifferentiated mesenchymal cells. (Original magnification 1000X)
Fig. 13. 96 hour diabetic rat. Note the dense inflammatory cell infiltrate and the poorly organized fibrin layer. (Original magnification 100X)
Fig. 14. 96 hour non-diabetic rat. Note granulation tissue-like appearance, inflammatory cell infiltrate in fibrin stroma. (Original magnification 100X)
Fig. 15. 7 day diabetic rat. Note 4 layers within the epithelium, densely organized connective tissue, and well vascularized connective tissue. An artifact is visible in this section. (Original magnification 100X)
Fig. 16. 7 day non-diabetic rat. Note deeply basophilic staining basal cell layer; epithelization is almost complete. (Original magnification 100X)
Fig. 17. 7 day non-diabetic rat. Note the myocytes undergoing mitotic activity and myotube formation. (Original magnification 400X)

Fig. 18. 14 day diabetic rat. Note fibroblasts and fibrocytes. (Original magnification 1000X)
Fig. 19. 14 day diabetic rat. Note mitotic activity in fibroblasts, angioblastic activity (endothelial proliferation), and collagen formation. (Original magnification 1000X)

Fig. 20. 28 day non-diabetic. Note pseudo rete peg formation; well organized dense connective tissue fiber orientation. (Original magnification 100X)
The thesis submitted by Steven Segall D.D.S. has been read and approved by the following committee:

Dr. Joseph J. Keene, Jr.
Associate Professor, Periodontics
Loyola University

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

Dr. Anthony W. Gargiulo
Professor and Chairman, Periodontics
Loyola University

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 by the Committee with reference to content and form.

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

3/28/80
Date

Director's Signature