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The Effects of Occlusal Trauma on Periapical Healing Following Endodontic Therapy in the Rhesus Monkey

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THE EFFECTS OF OCCLUSAL TRAUMA ON PERiapICAL HEALING
FOLLOWING ENDODONTIC THERAPY
IN THE RHESUS MONKEY

BY
Dennis McKay Byrne D.M.D.

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
1981
DEDICATION

To my parents, Jack and Mary, whose steadfast support over the years has enabled me to learn.
ACKNOWLEDGMENTS

To Dr. Franklin Weine, my friend and teacher, I thank you for the tools with which I might attain excellence in the field of endodontics.

To Dr. Marshall Smulson, I express my appreciation for teaching me how to teach.

To Dr. Patrick Toto, I thank you for your help in examining and evaluating the histologic sections for this study.

To Dr. James Koelbl, I extend my gratitude for your guidance in conducting a scientific research project.

To Dr. Scott Shellhammer, my friend and colleague, I offer my thanks for your technical assistance which made this project possible.
VITA

The author, Dennis McKay Byrne, was born in Salisbury, Maryland on the thirteenth of September, 1950.

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In September of 1978 he entered the Loyola University School of Dentistry and began a dual course of study leading to the degree of Master of Science in Oral Biology and a Certificate of Specialty Training in Endodontics.
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CHAPTER I

INTRODUCTION

The failure of a periapical lesion to heal after exacting and thorough conservative endodontic therapy is one of the more frustrating occurrences in clinical dentistry. The endodontist's knowledge and understanding of root canal anatomy is now greater than ever before. The various techniques available for the cleansing, shaping and subsequent obliteration of the root canal space allow the operator to obtain a result which is not only satisfactory as viewed on radiographs but is truly adequate on a three dimensional level. Traditionally recognized materials and methods used to irrigate and fill the root canal space have been shown to cause minimal postoperative inflammation of consequence in the periapical tissues. Yet the endodontist still encounters the situation where a periapical lesion persists well beyond the time required for evidence of healing despite the delivery of sound endodontic therapy and the absence of any systemic problems.

Possible explanations for such lack of healing range from simply poor endodontic treatment to anachoretic infection from another tooth (32). Another possibility is the existence of trauma from occlusion which might prolong the periapical inflammatory reaction of an endodontically involved tooth, hence potentiating pain and delaying or preventing healing following completion of therapy. Such trauma could arise from
occlusal discrepancies in the natural dentition or the restoration of an endodontically treated tooth with a "high" amalgam or cast restoration. Even forces exerted by clasp arms of removable partial dentures could traumatize an involved abutment tooth.

Most clinicians circumvent occlusal complications by reducing cuspal height and increasing central fossa depth of the tooth undergoing endodontic treatment. A tooth treated in this manner would presumably have no contact with opposing teeth during centric occlusion or during excursive mandibular movements. Such a procedure may eliminate occlusal trauma during the phase of endodontic therapy but trauma might be introduced during the restorative phase of treatment - before the periapical tissues have healed completely. The purpose of this study is to examine the histologic effects of induced occlusal trauma on periapical lesions following endodontic treatment.
CHAPTER II

REVIEW OF THE LITERATURE

EFFECTS OF OCCLUSAL TRAUMA ON THE PERIODONTIUM

The realization that the forces of occlusion can, in certain instances, become destructive to the teeth and their supporting structures is not a new one. The term "occlusal trauma" was introduced by Karolyi (37) in 1902 to describe the destructive effects of chewing forces in the periodontally involved mouth. Although some portions of the dental literature have dealt with the effects of occlusal trauma on the healthy as well as the infected dental pulp a larger number of studies have examined the effects of occlusal trauma on the periodontium. The literature is practically void of any information dealing with the relationship between occlusion and the healing of pulpoperiapical lesions.

In 1924 Grove (33) discussed occlusal trauma in terms of the functional anatomy of the periodontium. He pointed out that the fibers of the periodontal ligament (P.D.L.) were arranged so as to withstand forces along the long axis of the tooth. Occlusal wear increased the masticatory force necessary for chewing and placed horizontal stress on the tooth. This caused tension of the P.D.L. on one side of the root and compression of the P.D.L. on the other side. By placing a high crown on an unspecified dog's tooth, Grove was able to demonstrate injury in the occlusal third of the P.D.L. Microscopic sections of this area showed destruction of the cribriform plate and cortical bone along with detachment of periodontal fibers from cementum and bone. He
suggested that healing of such a lesion would occur readily provided that no infection existed and that the tooth was restored to normal occlusion.

Four years later, Orban (54) reported a human post-mortem study where conditions of occlusal trauma had existed at time of death. In posterior segments the premature loss of several teeth had led to the mesial inclination of the remaining teeth (i.e. posterior bite collapse). Histologic sections of the latter showed that the most destruction had occurred at the fulcrum (middle) of the root, the furcation, and at the apices. Along the mesial aspect of the roots he found necrosis of the compressed P.D.L. with minimal resorption, whereas the distal aspects displayed more resorption and peripheral osteogenesis. He also noted that the cementum and dentin of the inclined teeth showed areas of resorption whereas nonfunctioning teeth showed a generally thicker cementum layer, a narrower P.D.L., and no resorptions. Orban theorized that occlusion may play a role in (sic) pyorrhea but that it was not the etiologic factor. He also postulated that the systemic status of the patient may influence the final healing or destruction of the periodontium.

Kronfeld (39) examined the post-mortem occlusion and histologic sections of a 38 year old man with posterior bite collapse and loss of vertical dimension of occlusion. Areas where vertical occlusal trauma predominated showed hypertrophied P.D.L. fibers around root apices and reinforcement of spongy bone with dense compact bone. Areas under horizontal stress showed a widening of the P.D.L. at the cervical level of the root. Teeth that were completely out of function showed a much
narrower P.D.L. and resorption of cementum and dentin. Kronfeld pointed out that this hypertrophy of the P.D.L. under stress was desirable and that such an adaptive response may not occur in all patients.

Gottlieb and Orban (31) compared the histologic responses of young dogs with old dogs under conditions of occlusal trauma. After six days, bone resorption was much greater than root resorption in the younger animals. Older animals showed equal resorptions of bone and root. The researchers postulated that as the tooth was intruded, the P.D.L. was destroyed due to pressure between the root and bone. Repair proceeded only after bone and necrotic tissue were resorbed. Generally, resorption of cementum occurred later than resorption of bone but the authors pointed out that variations in this sequence may be seen.

The late 1930's was particularly fruitful with regards to the understanding of the pathologic process associated with occlusal trauma. In 1937 Coolidge (11) examined the width of the P.D.L. of 172 teeth from fifteen human jaws. He concluded that the space was generally narrower in older people and that teeth in heavy function will have a P.D.L. of greater width than nonfunctioning teeth. Teeth subject to greater stresses had an average space width of 0.19 mm.

Coolidge (12) also evaluated histologically the periradicular tissues from fifteen human jaws whose occlusal relationships were known. Injury seen was of two types: (1) thrombosis, hemorrhage and hyalinization of the P.D.L. with bone resorption, and (2) necrosis of the P.D.L. with root resorption and cemental tearing. In the latter type, resorptive cells were destroyed by necrosis and the necrotic tissue was removed by
undermining resorption from adjacent healthy tissue. Repair was seen to occur more readily on the tension side rather than on the compression side of the root. In teeth where trauma had been present and then removed, repair had led to a decrease in thickness of the P.D.L. and the deposition of new cementum along sites of cementum fracture.

In 1938 Stones (71) placed amalgam fillings in supraocclusion in posterior teeth of seven monkeys. The animals were sacrificed after ten to 43 weeks and sections were evaluated histologically. Stones noted that most of the teeth had become intruded as a result of the high restorations. Lymphocytes and plasma cells were seen in the periradicular connective tissues and proliferation of epithelium along the root surfaces was noted. Stones concluded that occlusal stress could be a predisposing factor to "paradontal" disease regardless of the presence or absence of gingival infection.

Kronfeld (41) discussed the morphologic changes of the periodontium associated with trauma from occlusion. He pointed out that in the traumatized tooth the fibers of the P.D.L. were well developed and ran perpendicular to the tooth. In the nonfunctional tooth, the P.D.L. became atrophic and the fibers tended to run parallel to the root surface. Bone around teeth in trauma was usually well developed with an increase in trabeculation size and number. Kronfeld also stated that in situations where the occlusal stress was such that the cellular response was one of construction rather than destruction, a decrease in the width of the P.D.L. might have been seen as a result of the apposition of new bone and cementum. Hence constitutional factors and the type of stress involved led to
variations in the response seen on the histologic level. Over a long period of time the response of the periodontal tissues was usually one of adaptation.

In 1939 Box (4) induced occlusal stress in a sheep by placing a gold crown on a lower incisor. Due to the peculiar occlusion normally found in the sheep, the incisor articulated with the maxillary "gum pad". After fourteen weeks the incisor displayed a marked increase in mobility and a crevicular depth increase of one millimeter on the lingual surface. Although the crown margins were supragingival and caused an obvious build-up of calculus, Box concluded that the increase in crevicular depth was due to occlusal trauma.

That same year Orban (55) reported a study where high crowns were placed on posterior teeth in dogs. The crowns were so constructed that as the animal occluded the maxillary teeth were forced to the buccal and the mandibular teeth were forced to the lingual. A similar study was conducted simultaneously using monkeys and sections from both studies were examined histologically. In areas of mild compression necrosed P.D.L. tissue was resorbed by giant cells and osteoclasts. Where compression was most severe necrotic tissue persisted as resorptive cells had difficulty reaching the site. When compression occurred on the buccal aspect of the root, a compensating deposition of bone was evident on the external surface of the buccal plate. After 48 hours, areas of tension showed a widened periodontal space and bone formation. As teeth moved out of occlusion healing occurred. Orban concluded that the inflammatory lesion of the gingiva and the lesion of trauma were two different entities which might sometimes be superimposed.
There appears to be a ten to twelve year hiatus where no discussions or studies of any significance dealing with trauma from occlusion were published. The subject seems to have disappeared from the literature until 1952 when Schaffer (64) discussed the role of occlusal relations and occlusal trauma in periodontal disease. He pointed out that masticatory forces generated in centric closure were much greater than those generated during lateral or protrusive movements and that premature contacts in centric could be the cause of vertical periodontal pockets. According to Schaffer, resistance to periodontal disease was determined by age, sex, heredity, nutrition, and metabolism. He also postulated that the production of adreno-corticotropic hormone could affect the periodontium due to a resultant destruction of protein, increase in fat utilization, decrease in carbohydrate utilization, and decrease in blood ascorbic acid.

Weinmann (80) produced experimental occlusal trauma in rats by placing rubber dam material interproximally in posterior teeth. He found that destruction progressed apically via the P.D.L. rather than via the alveolar bone. Since fibers from bone and cementum join in the center of the P.D.L. forming an intermediate layer, Weinmann theorized that trauma caused these fibers to "unravel" and thus open a path for inflammation.

In probably one of the most classic studies dealing with occlusal trauma, Bhaskar and Orban (1) placed cast gold MOD inlays on maxillary bicuspid s of Rhesus macacus monkeys. The inlays were so constructed that vertical and horizontal stress were placed on experimental as well as opposing teeth. Animals were sacrificed at three days, three weeks,
three months and six months and histologic sections were made. At three
days, areas of compression showed hyalinization of the P.D.L., thrombosis
of vessels, and clastic activity in adjacent bone. Areas of tension
showed stretched P.D.L. fibers and an osteoid layer along adjacent bone.
At three weeks the experimental teeth had moved almost completely out of
trauma. Compression areas showed resorption of cementum and dentin along
the root surface with evidence of bone apposition. Necrosis of fibers
had occurred in areas of intense compression but much of the necrotic
tissue had been resorbed. Areas of tension showed widening of the P.D.L.
and resorption of the root surface. At three and six months experimental
teeth were completely out of trauma and the picture was generally one of
repair. Root resorptions had been filled in by cellular cementum and
areas of bone resorption were restored with new bundle bone with fibers
of the P.D.L. attached. The P.D.L. was of normal width and necrotic con­
nective tissue had been replaced by dense collagen bundles. The re­
searchers concluded that the changes induced by trauma from occlusion
were reversible and that inflammation - as evidenced by the migration of
cells and exudation - did not occur as a result of such trauma.

A similar study was conducted by Waerhaug (76) which appeared in the
same journal and also the same issue as Bhaskar's and Orban's work. Waerhaug
produced occlusal trauma in dogs by placing cast crowns with buccal shelves
on mandibular first molars creating a seven millimeter open bit. The sit­
uation was such that experimental teeth could not move out of occlusion
without intruding completely below the alveolar crest. Histologic sec­
tions revealed a progression of destructive events similar to that reported
by Bhaskar and Orban. When the crowns were removed healing proceeded in a fashion similar to that seen in teeth which have moved out of occlusion, i.e., the P.D.L. width returned to normal and resorbed areas of cementum and dentin were repaired with new cementum. Waerhaug noted that during intrusion the most apical portion of the sulcular junction became necrotic. He speculated that continued intrusion with a lateral component of trauma might lead to the destruction of the principle fiber group just apical to the epithelial cuff and allow epithelial downgrowth. Waerhaug stated that such an occurrence would cause irreversible damage to the P.D.L.

Glickman and Weiss (27) placed cast onlays on anterior teeth in dogs, thereby producing an edge-to-edge relationship. The animals were sacrificed at three to 110 days and histologic sections were prepared. The workers described the P.D.L.'s of intruded experimental teeth as having two layers. One narrow layer, extending along adjacent bone, was devoid of fibers and consisted of polyhedral cells, osteoclasts and dilated blood vessels. A second broader layer extended along the root surface and was composed of dense fiber bundles separated by dilated blood vessels. The bundle bone normally adjacent to the P.D.L. was absent and there was a direct communication between the P.D.L. and marrow spaces. On no experimental tooth was pocket formation present. Zander and Muhlemann (86) exposed Rhesus monkeys to "stressful" systemic situations while placing orthodontic forces on maxillary and mandibular incisors via an intricate spring loaded, screw-type force applicator. The systemic stresses included simulated altitude, radiant heat, typhoid vaccine, meningococcic toxin, "pyromen" and artificial hypoxia. Areas of tension showed hyalinization of the P.D.L.
whereas areas of compression showed P.D.L. necrosis and osteoclastic removal of necrotic tissue from along the periphery. Such resorption of necrotic tissue only occurred when some vital P.D.L. tissue remained. Generally there was no clearcut difference with respect to bone loss between stressed animals and unstressed control animals.

At this time it was known that the gingiva receives its blood supply from vessels located on the outer surface of the alveolar process and also from vessels running parallel to the root surface within the P.D.L. Goldman (29) placed high silver crown on premolars of dogs to determine whether or not these secondary vessels from the P.D.L. could be occluded by trauma from occlusion. At five days, one group of animals was injected with 10% gelatin mercury mass and a second group with India ink. Histologic sections revealed that the blood supply to the P.D.L. was entirely occluded but that the marginal gingiva remained healthy due to the continued blood supply from supraalveolar vessels.

In a similar study Cohen, et al., (7) placed gold crowns on posterior teeth of Mangabey monkeys and perfused the animals with India ink prior to sacrifice at 30 hours, three days, five days, two weeks, two months, and four to eighteen months. In no specimen was the gingival blood supply found to be diminished as a result of the induced trauma.

Posselt and Maunsbach (61) surveyed 97 young patients to determine the normal amount of tooth mobility and its correlation with radiographic evidence of occlusal trauma. The authors concluded that mobility is not always indicative of occlusal trauma. Some mobile teeth showed no radiographic evidence of the widened P.D.L., thickened lamina dura, hypercementosis and root resorption that is usually associated with trauma.
Sometimes these entities were found when mobility was minimal.

In 1958 Wentz, et al., (83) defined occlusal "jiggling" as an alternation of stressful forces from one direction and then another opposite direction. They claimed that such forces, when created experimentally, were more analogous to those present during occlusal traumatism as it occurred naturally. Orthodontic appliances were fabricated which placed tension on crowned maxillary posterior teeth during jaw opening in Rhesus monkeys. Histologic sections at three days to six months showed various stages of repair. At fourteen days the researchers found undermining resorption of bone in apical areas and a generalized progressive widening of the P.D.L. space. At three months connective tissue was more cellular but neither necrosis nor thrombosis were present. Only the bifurcation area continued to show signs of a persisting resorptive process.

Ramfjord (62) reported a study of the labio-cervical and coronal one half of roots from humans where a known functional increase and decrease in occlusal stress was present. He found that during healing the reorganization of P.D.L. fibers was most evident at the crestal area. Apposition of bone often occurred on the labial aspect of the alveolar crest regardless of patient age. This cervical region appeared to be more stable under stress as opposed to the midroot area which showed early hyalinization and vascular changes. Ramfjord speculated that cervical fibers were strong enough to resist epithelial downgrowth during trauma.

In 1961 Muhlemann and Herzog (52) pointed out that static interferences can be functionally avoided by proprioceptive reflexes. In
order to circumvent this reflex action they produced occlusal stress in a fourteen year old girl by placing a gold inlay on a maxillary bicuspid. The inlay was constructed such that one of two secondary inlays could be inserted and thereby produce an interference on the lingual aspect of the buccal cusp or the buccal aspect of the lingual cusp, depending on which secondary inlay was in place. Thus by interchanging the secondary inlays a "jiggling" effect could be produced. When inlays were interchanged every other day no difference in mobility was produced between the experimental tooth and normally occluding control teeth. When interchanges were made four times per day there was a marked increase in mobility which decreased over a four month period to a level comparable to that of the control teeth. The trauma was renewed by adding solder to the inlay and the experimental tooth was removed via block section. Histologic examination revealed osteoblastic activity and no root resorption. The authors concluded that adaptation to trauma occurred via cuspal wear and the reorganization of periodontal structures. After adaptation, stress may continue but mobility would be decreased and a renewal of acute trauma would not induce a resorptive response.

Ewen and Stahl (19) studied the combined effects of occlusal trauma and gingival inflammation. They extracted incisors from dogs and initiated movement of adjacent teeth toward the resulting edentulous spaces via orthodontic appliances. Since the appliances created food impaction areas around the experimental teeth, a severe gingival inflammatory reaction was produced. At one year histologic examination revealed spread
of the inflammatory lesion to the crestal alveolar bone and the initiation of infrabony pocketting.

In 1963 Itoiz, Carranza, and Cabrini (35) experimented with three techniques for creating occlusal trauma in rats. They found that cross arch palatal wires cemented between maxillary first molars produced a profound classic lesion (i.e. necrosis of the P.D.L., bone and cementum resorption) after one week whereas "high" occlusal amalgams in maxillary molars required two weeks to produce a similar lesion. The third technique whereby a head pin was cemented in the occlusal aspect of posterior teeth produced lesions similar to those seen in animals with palatal wires. The workers pointed out that when an operative procedure was necessary to create occlusal trauma the experimental tooth will usually undergo pulpal changes due to the procedure itself. In such a situation it would be wise to study sections of the opposing tooth in which no iatrogenic lesion would have been produced.

Throughout the 1960's a considerable amount of research dealing with trauma from occlusion was reported by Glickman (21, 22, 23, 24, 25, 26) of Tufts University. In 1962 Glickman and Smulow (23) created abnormal functional relationships by placing gold crowns on posterior teeth of adult Rhesus monkeys. In uncrowned control teeth inflammation progressed along blood vessels extending from the gingiva into alveolar bone interproximally and lateral to the alveolar plate buccally and lingu­ally. On pressure sides of experimental teeth, inflammation passed directly into the P.D.L. whereas on sides of tension this trend was not so noticeable. Hence the direction and severity of occlusal forces could
affect the pathway of inflammation.

In another discussion Glickman (21) referred to the periodontium as having two zones with respect to occlusal trauma. One, the zone of irritation, included the marginal gingiva and inter-dental papillae bounded by the gingival fibers. This zone was affected by local irritants, not by trauma. A second, zone of co-destruction, included the supporting periodontal tissues, i.e., the P.D.L., alveolar bone and cementum. This zone was bounded occlusally by the transseptal and alveolar crest fibers and its condition was constantly regulated by occlusal forces. Once the inflammatory process broke through the barrier of crestal fibers it worked concomitantly with the occlusal forces causing breakdown of this second zone.

Glickman and Smulow (24) reported another study in which post-mortem sections of teeth were taken from Rhesus monkeys and humans where conditions of occlusal stress were known to have existed. They noted that in areas of tension, when located buccally, new bone was laid down on the labial surface of the alveolar plate presumably to buttress against occlusal forces. When areas of tension were located lingually, this buttress effect occurred along the outside of the P.D.L. A similar phenomenon was seen in areas of compression. When present on the buccal side of the tooth new bone was found on the labial surface of the buccal plate. When compression was toward the lingual, new bone was layed down in the marrow spaces. When forces were generally from a vertical direction, this buttressing occurred along endosteal surfaces of marrow spaces. The authors described this phenomenon as the body's attempt to maintain a
normal width of P.D.L. under varying occlusal forces.

In 1966, Glickman, et al., (26) attempted to determine the effects of occlusal hyperfunction on healing after mucogingival surgery. The researchers performed split thickness flap and repositioned flap procedures on anterior segments in dogs. Some of the teeth were simultaneously placed in hyperfunction via placement of cast splints while others were placed in hypofunction via extraction of opposing teeth. The animals were sacrificed two weeks later and histologic sections were made. The teeth in hyperocclusion showed classic signs of trauma, i.e., widened P.D.L. with dilation of blood vessels and apposition of bone along the labial plate. Teeth in hypofunction showed a reduction in the number of P.D.L. fibers and an orientation of these fibers parallel to the root surface. The healing process of the gingiva itself appeared normal and unaffected by the hyper- or hypofunction.

Glickman and Smulow (25) demonstrated how the morphology of the P.D.L. changes to accommodate chronic stressful occlusal forces. High crowns were placed on maxillary premolars of Rhesus monkeys. Orthodontic springs attached to other teeth in the same quadrant prevented the experimental teeth from undergoing intrusion. Animals were sacrificed after six months and histologic sections were made. Although no migration of the epithelial attachment was noted, the authors did describe a "V-pattern" or splitting of the P.D.L. in the crestal region. It was stated that this change in morphology occurred along with widening of the P.D.L. and an angular resorption of bone in order to establish a state of equilibrium in response to chronic trauma.
In a later discussion, Glickman (22) summarized his concept of the relationship between traumatic occlusion and the periodontium. He stated that although trauma would cause a widening of the P.D.L. at the expense of bone leading to the formation of angular bony defects and tooth mobility, it was not an etiologic factor in periodontal pocketing. He emphasized that the most effective way to eliminate the lesion of trauma was to establish proper occlusal relationships via occlusal adjustment.

Most of the research since the mid 1960's has been in agreement with and expanded upon the concepts of Glickman. Waerhaug and Hansen (77) studied the effects of long term repeated trauma from occlusion in monkeys. They placed high gold crowns on mandibular first molars which opened the bite five millimeters and resulted in excess vertical and horizontal stress. As the experimental teeth moved out of trauma the crowns were removed, the teeth allowed to extrude and the crowns replaced. Some animals were sacrificed after one such extrusion and the rest were sacrificed after several extrusions. Histologic sections showed initially the classic lesions of trauma followed by healing with no destructive inflammation. Epithelial downgrowth was less than one millimeter in all animals except for one which had become systemically ill prior to termination of the experiment. The authors concluded that occlusal overload plus poor systemic conditions might lead to pocket formation.

Kemper, et al., (38) placed stainless steel crowns and orthodontic appliances to create occlusal stress in dogs. Since no inflammatory exudate appeared along with the traumatic lesion in the P.D.L., the workers
stated that the term "trauma-togenic" was inappropriate for artificially created forces. They also emphasized that the term "periodontitis" should not be used to describe the lesion of trauma.

In 1969, Comar, Kollar, and Gargiulo (8) studied the inflammatory process in Rhesus monkeys after placing high cast crowns with overhangs. Histologic sections showed that interdental fibers may orient themselves slightly more obliquely but generally remain intact. Changes involving the alveolar crest were determined only by changes occurring in the interdental fibers. Due to the local irritation some inflammatory cells were seen in the connective tissue and P.D.L. but there was no epithelial migration.

Carranza, et al., (5) studied the combined effects of marginal irritation, occlusal trauma, and total body radiation on periodontal bone in rats. The marginal irritation was created by placing nylon ligature interproximally and the trauma was induced via placement of palatal arch wires. The three treatments were applied in eight different combinations, each on a different group of rats and results were evaluated histometrically. The greatest amount of furcal bone was lost as a result of combined marginal irritation and radiation. Although occlusal trauma seemed to potentiate effects produced by marginal irritation, the bone loss due to trauma alone was negligible.

Lindhe and Svanberg (45) compared the effects of marginal irritation with combined marginal irritation and trauma from occlusion in beagle dogs. Surgical defects were created in crestal bone and copper bands were seated which extended into the defects. Other dogs received
the same treatment and also had a cap splint with occlusal bar appliance inserted to induce trauma. After 180 days the animals were sacrificed and histologic sections were made. Although both groups showed pronounced horizontal bone loss, the group receiving combined treatment showed development of crestal angular defects and also periradicular lesions in the P.D.L. The latter group also showed epithelial downgrowth and an apical extension of an inflammatory infiltrate.

In a similar study using dogs, Svanberg and Lindhe (72) induced trauma in one group of animals and a combined, traumatic-irritational lesion in another group. Both groups received injections of colloidal carbon I.V. so that an indication of vascular labelling, leukocytic infiltration and osteoclastic activity might be obtained. Where trauma alone was present an initial (seven to 30 days) exudation and leukocytic influx gave way to compensatory changes with eventually no inflammatory infiltrate present (180 days). In the combined lesion, however, the initial leukocytic migration was eclipsed by the appearance of lymphocytes and persistent osteoclastic activity. Hence no constructive compensatory changes were seen with the combined lesion.

Meitner (50) combined a mesial-distal "jiggling" type trauma with a preexisting induced marginal periodontitis in adult squirrel monkeys. After ten weeks experimental teeth showed minimal loss of connective tissue attachment as compared with teeth in which only a marginal periodontitis existed. This finding was not in agreement with the co-destructive theory of Glickman and also conflicted with the findings of Svanberg
and Lindhe. However, experimental teeth did show a significant amount of bone loss at the crestal height without epithelial downgrowth. Meitner suggested that such bone loss could be an irreversible effect of the co-destructive insult or possibly a functional adaptation to tooth movement in the presence of marginal periodontitis.

In 1976 Polson, Meitner, and Zander (59, 60) reported studies dealing with the response of interproximal bone to jiggling forces in squirrel monkeys. When such forces were induced and teeth remained free of marginal periodontitis the clinical picture was one of angular bone loss, widened P.D.L. space but no loss of connective tissue attachment. Histologic sections showed islands of osseous tissue along the periphery of the P.D.L. surrounded by highly cellular and vascular connective tissue. Resultant mobility of such traumatized teeth proved to be reversible when the jiggling was stopped. After the cessation of jiggling the osseous islands were surrounded by new bone and the crestal bone was restored to its original height. This reversibility was not seen in animals where the traumatic insult was superimposed over a preexisting marginal periodontitis.

In a similar study Kantor, Polson, and Zander (36) induced a combined traumatic-irritational lesion with jiggling forces in squirrel monkeys. Ten weeks prior to sacrifice the jiggling forces were stopped and the teeth were cleaned every other day, thereby removing the irritational insult. The researchers noted that although bone regeneration was significant and the vertical defects were repaired, the bone was not restored to its original horizontal level. In other words the bone was restored in volume but not in height and there was no coronal
regeneration of the connective tissue attachment. The authors concluded that an interaction of the two insults leads to irreversible crestal bone loss and both insults must be removed to gain optimal healing.

Lindhe and Ericsson (44) created marginal inflammation in dogs and later induced occlusal trauma via cap splint and bar devices. After 280 days all periodontal pockets were surgically removed and half the teeth were taken out of trauma. From this point on teeth were brushed, thus minimizing the recurrence of marginal inflammation. Clinical observation revealed that the healing pattern proceeded normally in all experimental teeth either "in" or "out" of occlusal trauma. Hence trauma from occlusion did not seem to interfere with the regeneration and reorganization of epithelial and connective tissue components.

Zander and Polson (87) summarized the role of occlusal trauma in periodontics by observing that the resorptive, reparative and adaptive reactions induced by these forces were confined to the subcrestal tissues, leaving the tissues of the supracrestal zone intact. Changes occurring as a result of traumatic forces were found to be reversible and did not include periodontal pocketting. Treatment of co-destructive lesions should seek to eliminate inflammation primarily and trauma secondarily.

Nyman, Lindhe, and Ericsson (53) produced co-destructive lesions in dogs and concluded that trauma from occlusion increased the mobility, alveolar bone loss, and apical epithelial migration initially occurring due to periodontitis. The authors made a distinction between "increased mobility" as opposed to "increasing mobility". In the former, the
pathologic processes in the periodontium had proceeded to a certain point and stopped, whereas in the latter these processes were ongoing entities.

"Increased mobility" was not necessarily a bad finding but "increasing mobility" was definitely an undesirable situation.

EFFECTS OF OCCLUSAL TRAUMA ON THE PULP AND PERIAPEX

Over the years there have been relatively few studies dealing with the effects of occlusal trauma on the dental pulp. Box (4) reported finding an increased number of plasma cells and lymphocytes in pulps from occlusally stressed sheep teeth. McCall (48) pointed out that more severe masticatory pressures could disturb apical vessels entering the pulp thereby disrupting pulpal circulation. Furthermore, he stated that trauma induced by a "high" restoration could lead to hyperemia and, eventually, to pulpitis.

In the first of two studies dealing solely with the effects of occlusal force on the healthy pulp, Landay, Nazimov, and Seltzer (42) placed high amalgam restorations in the maxillary first molar of rats. The animals were sacrificed after two to 35 days, and three to seven months. Histologic studies were performed on pulps from opposing, non-restored teeth. Although no abnormal pulpal changes were seen, there were also no long term abnormal changes seen in the periodontal tissues. The authors concluded that due to amalgam wear and fracture of the restored teeth, a sufficiently severe trauma was not induced over a long period of time.

In an attempt to rectify the problems of the previous study, Cooper, Landay, and Seltzer (13) created trauma by cementing stainless
steel pins with circular caps into prepared rat molars. Again, histologic sections of pulps from opposing teeth were examined. The only teeth showing pulpal changes were those which had been in traumatic occlusion for a year or more. These pulps showed a central influx of macrophages and lymphocytes with odontoblastic disruption and reparative dentin deposition over the furcation area. Generally pulps did not show destructive changes until the P.D.L. tissues were nearly repaired. The authors speculated that short-term trauma may initiate pulpal change on an ultrastructural or biochemical level.

Stahl, Miller, and Goldsmith (69) exposed pulps and placed "high" amalgam restorations in rats. One group of animals was also placed on a protein-free diet. After twelve weeks histologic sections showed an inflammatory infiltrate in the periapical area as well as destruction of the inter-radicular septum. In teeth which had not been placed in occlusal trauma there was evidence of osteogenic repair in the inter-radicular region. Animals placed on the protein-free diet presented with minimal fibrous encapsulation of the localized infiltrate and marked resorption of the inter-radicular septum. Hence, occlusal trauma in the presence of periapical inflammation led to a greater resorption of inter-radicular bone than that seen when one of these entities was present alone. Furthermore, protein deprivation led to a delay in the repair process and a failure to localize the inflammatory infiltrate.

EFFECTS OF ENDOdontIC PROCEDURES ON THE PERiapICAL TISSUES

There has been a substantial amount of research dealing with the reactions of the root, periodontal ligament, and the periapical tissues
to endodontic procedures. Prior to the 1950's most of the human studies utilized endodontically treated teeth which were later extracted for one reason or another.

In 1929, Blayney (3) pointed out that teeth prepared to a position within the apical one third and filled with gutta percha showed repair which began at the apex and moved into the unprepared portion of the canal. Teeth with inflamed pulps showed a similar phenomenon with resorptions filled in with a cementum-like material. Teeth that had been overfilled showed delayed healing with extensive resorptions and fibrous encapsulation of the filling material.

In the same year, Hatton, Skillen, and Moen (34) described three stages in the healing of the unfilled portion of a root canal. An initial "tearing down" process involved the invagination of blood vessels into the apical foramen. The rebuilding stage, involving the deposition of osteoid cementum complete with lacunae and canaliculi, gave way to the terminal or resting condition in which a final layer of fibrous tissue was produced. Moen (51) reported that tissue left in main and accessory canals of teeth with vital pulps was of an atrophic or hypertrophic fibroid nature whereas all tissue remaining in teeth with necrotic pulps was destroyed.

Coolidge (9) stated that vital pulp removal gave rise to a transitory "reactive inflammation" in remaining vital tissue. Hyperemia of the periodontal tissues led to resorption and eventual repair by cementum. In infected teeth, the author described the localization of a granuloma at the periapex with cemental repair occurring at mid root, at orifices
of accessory canals, and over dentinal tubules.

In 1933, Coolidge (10) described the healing sequence as involving resorption and inflammatory exudation which was gradually replaced by connective tissue, osteoblasts, scar tissue, and new bone. Overextension of filling material was seen to cause resorption and eventual isolation of filling material by connective tissue. Resorptions filled in with cementum and fibers of the P.D.L. reoriented themselves across the root end.

Kronfeld (40) described granulation tissue as consisting of fibroblasts, capillaries, inflammatory cells and giant cells. He pointed out that the fibroblasts did not take part in the inflammatory process but were responsible for the proliferation of connective tissue from the periphery of a periapical lesion towards the center. Hence, the granulation tissue was transformed into bone and/or scar tissue. The author emphasized that such a progression would not occur unless the infection within the root canal was controlled (presumably by endodontic therapy).

In 1947, Weaver (78) pointed out that if the root canal was sterilized and filled properly, then a periapical lesion would heal by itself. He stated that in the absence of infection the P.D.L. would form new cementum and also regenerate periodontal fibers which would run parallel to the root surface.

One of the first studies of healing after endodontic treatment in experimental animals was conducted by Matsumiya and Kitamura (47) in 1960. They infected 215 teeth in dogs, removed the necrotic pulpal tissue and placed antibiotics in the canals. Histologic sections revealed that after instrumentation the number of bacteria within the canal
decreased with time but there was no relationship between the number of bacteria present and the degree of periapical healing. The authors concluded that healing occurred despite the presence of bacteria in the canal and as healing progressed the number of bacteria present decreased.

Ostby (56) reported the healing sequence observed after endodontic treatment in dogs and humans. In teeth filled to a position "short" of the apical foramen, the author observed that bleeding in the unfilled portion gave way to an ingrowth of fibrin which was soon replaced by granulation tissue and eventually by fibrous connective tissue. In many instances, the P.D.L. extended right into the root canal space. Dessication of the apical fibrin clot often left a space between the filling material and the final connective tissue plug.

Erausquin, et al., (18) overfilled root canals with various filling materials in rats and studied the effects on the periapical tissues. Pressure from the filling material caused necrosis of the P.D.L. within 24 hours. Blockage and infarcts of periapical blood vessels led to necrosis of adjacent bone and cementum. Healing of the periapical tissues occurred only after necrotic material had been resorbed and the P.D.L. was restored to a normal condition. The researchers concluded that periapical destruction could result from overfilling as well as overinstrumentation of root canals.

In 1967 the first of a series of articles dealing with the effects of endodontic treatment on the periapical tissues in teeth with vital pulps appeared by Sinai et al., (68) Pulpal extirpations were performed on monkeys and humans and access cavities were sealed with amalgam.
Block sections were taken at various times and histologic studies were performed. At one month, an acute infiltrate had been replaced by chronic inflammation in the pulp stumps and periapical tissues in the monkeys. After three months the inflammatory infiltrate had been replaced by normal or repairing periapical tissue. One and three month specimens from humans showed necrosis of remaining pulp tissue with granuloma formation. The healing in monkeys occurred without placement of any root canal filling material.

Seltzer, et al., (66) purposely overinstrumented a number of teeth and instrumented other teeth short of their apical foramina in monkeys and humans. In general they found that teeth instrumented short of the foramen showed an initial acute inflammatory reaction which was eclipsed by repair after six months. Overinstrumentation led to a chronic infiltrate and granuloma formation. Some specimens showed epithelial proliferation in periapical areas.

In the final study of the series, Seltzer, et al., (67) again using monkey and human models, filled one group of teeth short of the radiographic apex and, in another group, purposely forced material beyond the apical foramen. Initially both groups showed an acute inflammatory response in the remaining pulpal and the periapical tissues which was soon replaced by a chronic infiltrate. After three to six months, the "short" teeth showed repair of resorptions and generalized healing. Overfilled specimens demonstrated delayed healing with a persisting inflammatory infiltrate and epithelial proliferation. The overextended filling material was eventually encapsulated by fibrous tissue. The authors recommended that teeth with vital pulps be filled several millimeters
"short" after pulpal extirpation.

In a similar study, Bhaskar and Rappaport (2) overfilled and underfilled teeth with gutta percha and silver points in dogs. This study was unique in that experimental teeth were infected prior to pulpal extirpation and periapical lesions were present at the time of root canal fill. Here again the inflammatory response was somewhat greater in teeth which had been overfilled.

Davis, Joseph, and Bucher (15) compared the healing attained when instrumentation as well as filling was carried out at various distances from the radiographic apex of teeth with vital pulps in dogs. Healing was more complete in teeth which had been instrumented to the radiographic apex (i.e. slightly overinstrumented) and filled three millimeters short of that point. The unfilled portions of such teeth were found to be either plugged with dentin chips, filled in with bone, or showed regeneration of a complete attachment apparatus.

In a more recent primate study, Tronstad (74) prepared teeth with vital pulps one millimeter short of the radiographic apex and attempted to plug the remaining pulp stump with dentin chips. Teeth treated in this manner showed formation of a hard tissue matrix between the pulp and the chips while the pulp tissue itself remained vital. Control teeth which had no apical dentinal plug showed pulpal necrosis and inflammatory cells in the periapical tissues.

Malooley, Patterson, and Kafrawy (46) performed endodontic treatment on infected teeth in monkeys. Teeth in which the root canal fillings terminated short of the apical foramen showed the greatest evidence of healing. Although the teeth were not filled until negative cultures
were obtained, roughly one third of the teeth showed bacteria in the apical portion of the root canal. Bacteria present in the periapical tissues were localized at a point in the P.D.L. just opposite the apical foramen. The authors concluded that the periapical response found was related to the apical extent of the root canal filling, the time lapse between treatment and sacrifice, and the presence of bacteria in the apical portion of the root canal.

THE HEALING PROCESS

Information dealing with the "hows" and "whys" of the healing process itself is rather sparse in the dental literature. Many of the theories and mechanisms of histodifferentiation have been extrapolated from studies conducted by embryologists in the field of morphogenesis. More recently orthopedic studies of the so-called "induction phenomenon" have shed some light on this otherwise nebulous topic.

In order for the healing process to begin, the insulting entity or invading agents must be removed or neutralized. The role of the inflammatory response in this neutralization process cannot be overemphasized. Ross (63) and Peacock and Van Winkle (57) described a localization of neutrophils and, later, macrophages at the site of injury in the early stages of wound repair. Chemotactic substances released by these cells invoked the movement of undifferentiated mesenchymal cells into the area. The authors described the fibroblast, which originated from the mesenchymal cells, as being the "workhorse" of the healing process. Fibrin production was rather unique in that the otherwise time consuming step of protein processing by the fibroblast Golgi apparatus
was skipped. Concurrent with the production of fibrin was the proliferation of capillaries, bone formation and, in the case of teeth, cementum deposition. Penick (58) described the formation of a scar composed of dense fibrous connective tissue after endodontic treatment.

The various cells which are produced during the healing process arise from the undifferentiated mesenchymal cells present. According to Weinmann and Sicher (81) heterogenous bone developed from young connective tissue which previously arose from mesenchyme. The cells of this connective tissue were not as pluripotent as those of embryonic tissue or mesenchymal tissue but when exposed to the proper stimulus (i.e. calcium salts) they differentiate into osteoblasts.

Toto and Abati (73) injected tritiated thymidine into albino rats in order to trace the development of "undifferentiated reserve connective tissue cells". They found that such cells differentiated into fibroblasts, capillary endothelial cells, and even muscle fibers. Fibrin was produced before reserve cells were fully differentiated into fibroblasts. The authors concluded that such undifferentiated cells were found all over the body and were potential precursors of hemocytoblasts, lymphocytes, plasma cells, phagocytic cells, cells of Haversian canals, periosteum and endosteum.

Goldin and Joseph (28) followed the responses of connective tissue ground substance in rats via an electrometric technique. They found that a rapid disaggregation of ground substance occurred immediately after injury, with reaggregation occurring within four days. This re-aggregation appeared to be a prerequisite for collagen formation.
In 1950, Weiss (82) discussed the various factors which could influence the differentiation of embryonic cells. He theorized that a progenitor cell would take on different functions depending on the orientation and alignment of molecular groups present within the cell and along its outer membrane. This alignment would be determined by the types of cells and the chemical nature of the media with which the progenitor cell was in contact. Membrane molecules bonded and reacted with surrounding molecules in accordance with their stereochemistry as in an antigen-antibody bonding. Weiss described the state whereby a cell was in complete equilibrium with the chemical state along its surface as "coaptation". Hence the mobilization and path of differentiation seen in wound healing was a result of a change in this "coaptative" state. The influence of the cell's surrounding environment during differentiation was described as the "induction" phenomenon.

Urist, et al. (75) discussed the induction phenomenon with regard to bone formation. In bone induction systems, responding cells—usually perivascular hypertrophied mesenchymal cells—were induced to differentiate into osteoblasts, chondroblasts, and/or hematocytoblasts. Once a group of responding cells was induced and became established, they became inducing cells for the next layer of responding cells. In studies using decalcified lyophilized bone matrix as inducing substance, Urist and his associates were able to observe the entire sequence of events from the initial influx of leukocytes and histiocytes to the formation of bone. In every case the process was greatly retarded by any continuation of the inflammatory cell influx.

Young (85) studied cell specialization in bone formation by
injecting rats initially with para-thyroid extract and later with one of thirteen different tritiated amino acids. Autoradiograms were then made from rib and tibia sections. The parathyroid extract caused an alteration of osteoblast morphology to that of osteoprogenitor cells. In animals that were allowed to recover from the extract injections, osteoclasts were observed to revert to progenitor cell morphology. Young concluded that the specialized bone cells were simply different functional states of the same cell. The specialization was determined by the cell's physical and chemical relationship to its microenvironment and limited by the cell's genetic code. Moreover cell specialization could have varying degrees of permanence.
CHAPTER III

METHODS AND MATERIALS

One male and one female Macaca mulatta monkey, weighing 8.0 and 5.4 kilograms respectively, were used in this study. Although the exact ages of the animals were unknown a survey of their dentitions indicated that they were between four and six years of age. The animals were purchased, treated and maintained at the Loyola University Medical Center Animal Research Facility. The monkeys were kept in separate cages and were under continuous veterinary supervision. They were maintained on a diet of Purina Monkey Chow in the form of an expanded biscuit and water ad libitum, except on the days of treatment, at which time they received water only.

RADIOGRAPHIC SURVEYS

Preoperative radiographs were taken to verify that apices of teeth to be used in the study were fully formed and that no periapical pathosis was present. Standard size periapical radiographs were taken with a portable hand-held X-ray machine with settings of 60 kvp. at 20 milli-amperes and an exposure time of 0.2 seconds. Exposures were made with periapical ultra-speed dental X-ray film* which was, in turn, developed for 15 seconds in Insta-Neg** solution, fixed for 30 seconds in Insta-Fix solution and washed with water in a portable developing box.

* Kodak DF-58, Eastman Kodak Company, Rochester, New York, 14650
** Microcopy, P.O. Box 977, Newbury Park, California, 91320
SEDATION AND ANESTHESIA

At the beginning of each operative session each animal was sedated by an intramuscular injection of phencyclidine hydrochloride.* A dosage of 0.5cc was sufficient to allow removal of the female from her cage and the completion of all operative procedures. The male required the supplementation of an initial dose of 0.8cc of phencyclidine hydrochloride with 1.0 to 1.5cc of pentobarbital sodium** given intravenously in order to carry out the operative procedures. The barbiturate was administered in the posterior vein of the hind leg until adequate anesthesia was obtained as determined by the absence of voluntary muscle movement and the loss of the eyelid reflex. A total working time of from one to one and a half hours was obtained by both of these techniques with each animal.

The experiment was divided into two separate phases: 1) pulpal inoculation with a pure culture of Streptococcus faecalis in order to produce periapical lesions, and 2) endodontic treatment of the infected teeth followed by placement of an amalgam restoration. In experimental teeth this final amalgam restoration was undercarved so that when the animals closed into centric occlusion the experimental teeth occluded with their opposing teeth before all other teeth. In the control teeth amalgam restorations were carved so as to allow complete closure into centric occlusion. Phase two followed phase one by 13 to 24 1/2 weeks.

* Sernylan, Bio-Centric Laboratories Incorporated, St. Joseph, Missouri, 64502.
** W.A. Butler Company, Columbus, Ohio.
PHASE ONE

A human isolate of *S. faecalis* was obtained from the Loyola University School of Dentistry Department of Microbiology. The organisms had been cultured on a solid media slant of trypticase soy agar and kept in a refrigerated state. The organisms had been previously identified as a gamma hemolytic strain (20). An inoculate of the slant was transferred to a flask containing Brain-Heart infusion broth and incubated for 14 hours at 37°C. An inoculum was then transferred from the flask to a trypticase soy agar plate and maintained until needed.

The day before the initial inoculation procedure on the experimental animals, a flask containing Brain-Heart infusion broth was inoculated with *S. faecalis* from the agar plate and incubated for 14 hours at 37°C. The broth culture was then centrifuged for 15 minutes at 10,000 RPM and the resulting pellet of microorganisms was washed in 50 ml. of sterile saline. This procedure was repeated two more times and the organisms were suspended in saline. In a previous study using this same culture of *S. faecalis*, McCormick (49) determined that a suspended culture with an optical density of 0.24 as compared to a saline blank contained $2.63 \times 10^8$ organisms per milliliter. In the present study the suspended culture was diluted with saline until an optical density of 0.24 was obtained. The culture was then used to inoculate the experimental animals. In order to ensure that the culture had not become contaminated by different organisms, a gram stain was conducted. *S. faecalis* is a gram positive coccus that appears singly, in chains, or in small clusters.
The animals were sedated, removed from their cages, weighed and taken to the operating room. Clinical and radiographic oral exams were conducted. Both animals displayed minimal to moderate occlusal wear and a mild degree of marginal gingivitis. The occlusal relationship in the Rhesus monkey dentition is very similar to that seen in humans, with the lingual cusps of maxillary posterior teeth resting in the central fossae of the mandibular teeth. The occlusions of both animals could have been considered Angle Class I. The teeth selected for inoculation in both animals included the mandibular first and second premolars and the first molar on both sides of the mouth.

The animal's mandible was stabilized in an open position with a spring loaded mouth prop. Each tooth to be inoculated was isolated via rubber dam and an Ivory #12 rubber dam clamp.* An occlusal access preparation was produced with a 557 fissure bur in the highspeed hand-piece. Once the roof of the pulp chamber was removed the coronal pulp tissue was removed with a spoon excavator. Style B standardized #10 and #15 root canal files** were sterilized in a salt sterilizer and inserted into the root canals. The pulpal tissue was then macerated and, at the same time, an attempt was made to leave the pulp tissue in the apical one quarter of the root canal intact. Twenty-five microliters of the _S. faecalis_ broth culture were drawn into a 50 μl pipette and inserted into the pulp chamber. Hence each tooth was inoculated with

* Columbus Dental Company, 634 Water St., Columbus, Ohio, 43206.
** Star Dental Manufacturing Company, Incorporated, Conshohocken, Pennsylvania, 19428.
approximately 675 microorganisms. The root canal files were reinserted and the tissue was macerated further in an attempt to distribute the microorganisms apically. A dry sterile cotton pellet was then placed into the pulp chamber and the tooth was closed with a double seal of base plate gutta-percha and amalgam. After all teeth to be studied were so inoculated, the animals were returned to their cages and placed on a water-only diet for 24 hours.

**PHASE TWO**

At 13 weeks a radiographic survey showed evidence of periapical radiolucencies in all previously inoculated teeth. Under rubber dam isolation endodontic treatment was initiated on either both the first or both the second premolars in each animal (see Table 1). One of the first premolars in the female monkey showed evidence of a sinus tract draining through the buccal mucosa adjacent to the root apices. Working lengths were verified radiographically and canals were irrigated with a 5.25% sodium hypochlorite solution. Due to the narrow diameter and curved configuration of the root canals, instrumentation was terminated at file size #25. Canals were then dried with sterile paper points, a cotton pellet was placed into the pulp chamber and the teeth were closed with the double seal previously described. In each animal one side of the mandible was designated the experimental side while the other was designated the control side. The seal of the tooth on the experimental side included an amalgam filling that was overcontoured. In this way it was hoped to produce an occlusal interference that would
open the bite approximately one to two millimeters. The amalgam in the tooth on the opposite or control side was carved to its natural form. Three weeks later (16 weeks after the inoculation procedure) the animals were again sedated and the endodontic treatment in progress was completed. Burnish marks were evident on the occlusal surfaces of the experimental teeth. In the female monkey the sinus tract which was present at the initiation of endodontic treatment was no longer evident. The teeth were appropriately isolated and the amalgam and gutta-percha seals were removed with a high speed handpiece. Some canals had remained dry whereas others showed the presence of a hemorrhagic exudate. The root canals were irrigated and the walls were freshened with the last size file used in each. The canals were irrigated again and dried with sterile paper points. A mix of Proco-Sol root canal sealer* was prepared and the canals were filled with standardized gutta-percha points and sealer via a lateral condensation technique (79). The excess gutta-percha was seared from each orifice with a hot instrument and the entire access cavity was filled with amalgam. As before, the predesignated experimental teeth were given high restorations whereas the control teeth received restorations in harmony with their occlusal surfaces.

During this same operative session endodontic treatment was initiated on the other infected premolar teeth in the female and on the infected lower first molars in the male. After the root canals were

cleansed and shaped, the experimental teeth were closed with a high amalgam restoration. An attempt was made to carve the restoration such that the tooth would be allowed to contact its antagonist at the same time as the previously filled experimental tooth contacted its antagonist. In this manner a state of trauma could be maintained in all treated teeth on the experimental side of the jaw.

After three weeks (19 weeks after inoculation) the endodontic treatment begun during the previous operative session was completed. Clinical examination revealed occlusal burnish marks on all experimental teeth. The control teeth showed no such marks.

Twenty four weeks and four days after the inoculation procedure, endodontic treatment was begun on the remaining infected teeth. At this time a sinus tract was noted in the buccal mucosa adjacent to the apices of one of the as yet untreated mandibular molars in the female. Again, the canals were cleansed and shaped to a #25 file, irrigated, dried and closed. No root canal filling material was placed in these teeth.

Twenty five weeks after inoculation, the animals were sacrificed. This was accomplished by giving an intravenous injection of five cc. of a highly concentrated solution of pentobarbital sodium* which is available specifically for this purpose. The soft tissue was immediately dissected away from the maxilla and mandible and block jaw sections containing the experimental and control teeth and their antagonists

* Buthanasia-D, Burns-Biotec Laboratories Division, Chromalloy Pharmaceutical, Incorporated, Oakland, California.
were removed with a reciprocating surgical saw. The sections were placed in a solution of 10% neutral buffered formalin for fixation and removed from the operatory.

**HISTOLOGIC PREPARATION**

Remaining soft tissue was dissected away from the jaw sections and extraneous hard tissue was removed with a high speed bur. The formalin solutions were changed after the first twenty four hours and again after one week. After two weeks the specimens were removed from the fixative and rinsed under running water for 24 hours.

The jaw sections were decalcified in a solution of equal parts of 20% sodium citrate and 50% formic acid. After four weeks the block sections were trimmed and cut in half in a mesio-distal dimension with a razor blade and immersed in the decalcifying solution for five more weeks. The blocks were then dehydrated in increasing concentrations of alcohol, embedded in paraffin and sectioned at seven microns. The sections were then mounted on glass slides, deparaffinized, hydrated and stained alternately with hematoxylin and eosin, Masson's trichrome for connective tissue, Van Gieson's stain for collagen, and Brown and Brenn stain. Stained sections were examined under the light microscope and evaluated in terms of the degree of inflammation and resorption, cemental and osseous repair, and the presence of microorganisms.
CHAPTER IV

RESULTS

The animals tolerated all operative procedures very well and remained healthy throughout the study. At time of sacrifice the male weighed 7.9 kilograms and the female weighed 5.5 kilograms.

The short-term healing of a periapical lesion is a difficult entity to quantify. Therefore healing at the time of sacrifice was evaluated with respect to three parameters:

1. the extent of each lesion radiographically with respect to the root apex;
2. the various cell types present in each lesion as seen under the light microscope;
3. the amount of connective tissue proliferation and bone apposition around the periphery of each lesion.

Using this information the healing displayed by each tooth was ranked on a scale of one to four with one representing an acute destructive lesion and four representing obvious repair with no evidence of continued bone, cementum, or dentin destruction. These assigned values were then used to conduct a statistical analysis.

Endodontic treatment performed at 13 weeks post inoculation

This category included the mandibular second premolars of the female and the mandibular first premolars of the male. Sacrifice came 12 weeks after endodontic treatment was initiated and experimental occlusal trauma was created.
Radiographically the experimental tooth of the female showed a radiolucency at the apex of the distal root measuring 1.5 x 1.5 millimeters (see Figure 7). The mesial root showed minimal evidence of a periapical radiolucency despite an endodontic fill that terminated three to four millimeters from the apex. The control tooth of the female showed almost no evidence of rarefaction or widening of the apical periodontal ligament (P.D.L.) on either root (see Figure 8). A comparison of the final radiographs with radiographs taken at the time of initiation of endodontic therapy indicated that there had been significant improvement in both teeth.

Histologic examination of the experimental tooth revealed well defined lesions around both apices consisting of polymorphonuclear leukocytes (PMN's) and a small to moderate number of macrophages and plasma cells. A wide band of cellular cementum extended down the inner aspect of both roots from the furcation to the apices. There was minimal evidence of root resorption but the lamina dura appeared moth-eaten with osteoblastic activity along the inner aspects of both roots (see Figure 11 and 22). Masson's trichrome and Van Gieson stains showed a moderately wide band of connective tissue surrounding both lesions (see Figure 12). There was also evidence of connective tissue proliferation within the lesions. Little if any osteoblastic activity was seen in the periphery of the lesions.

Brown and Brenn stain showed particulate matter and microorganisms present in the root canal spaces but no microorganisms were seen in the periapical tissues. This finding was very consistent with regard to all the remaining sections examined.
The control tooth showed several apical and lateral lesions around both roots. The lateral lesions consisted predominantly of PMN's but the apical lesions contained moderate to large numbers of plasma cells and lymphocytes (see Figure 13). The main lesion on the distal root extended apically into the marrow spaces of surrounding bone. The lateral lesions on the distal root surrounded foreign matter – possibly sealer. The P.D.L. and lamina dura appeared intact but the distal root had been resorbed from an apical direction. Connective tissue capsules surrounding the main apical lesions were fairly thick and there was evidence of connective tissue infiltration throughout the lesions.

In light of the overall trend towards healing and the absence of destructive processes both the experimental and control teeth were assigned values of three.

The experimental tooth of the male showed a large radiolucency measuring 3.0 x 1.5 millimeters at the apex of the mesial root (see Figure 10). The root canal filling terminated three to four millimeters from the apical foramen. The distal root had been slightly overfilled and showed some widening of the apical P.D.L. The control tooth showed some widening of the periodontal ligament at the apex of the mesial root but no obvious periapical radiolucency (see Figure 9). The distal root, however, had been ledged three to four millimeters short of the apical foramen and showed a periapical rarefaction measuring 5 x 4 millimeters. The radiographic appearances of both teeth were generally worse than they had been at the time of endodontic treatment.

A large portion of the mesial root of the experimental tooth was
lost during sectioning but a large lesion was present consisting of mostly PMN's with some widely scattered plasma cells and lymphocytes. The lesion extended out into the marrow spaces of the surrounding bone and several areas of osteoclastic activity were present. It was impossible to determine if any root resorption had occurred. Some parts of the lesion were very well encapsulated by fibrous connective tissue but there was little fibrous infiltration of the lesion proper. Inflammation around the distal root was minimal but the root apex may not have been included in the section.

The control tooth showed several well defined lesions apical to the distal root. Since the mesial root had such a severe distal curvature and was rather long the lesions were actually situated between the two roots. The lesions had a moderate to high concentration of plasma cells and lymphocytes and appeared well encapsulated. There was obvious osteoblastic and osteoclastic activity involving the surrounding bone trabeculae but there seemed to be no pattern as to the location of these cells. There was no evidence of root resorption. The experimental tooth was assigned a healing value of two and the control tooth a value of three.

**Endodontic treatment performed at 16 weeks post inoculation**

Teeth in this category included the mandibular first premolars of the female and the mandibular first molars of the male. Sacrifice came nine weeks after endodontic treatment was begun and experimental occlusal trauma was created.

Radiographically the experimental tooth of the female showed some widening of the P.D.L. around the apex of the mesial root but no other
obvious radiolucencies (see Figure 7). This was a significant improvement over the situation at the time endodontic treatment was begun. At that time obvious periapical radiolucencies were associated with both roots. In the control tooth root canal filling material had been overextended three to four millimeters on the distal root (see Figure 8). It appeared that this root had been resorbed on its mesial aspect by a lesion that was present at the time endodontic therapy was initiated. At that time the radiolucency had extended from the apex to the furcation. At the time of sacrifice the furcation lesion was persisting but the overall size of the radiolucency was much less than at 16 weeks. The mesial root showed a periapical radiolucency measuring 1.5 x 1.5 millimeters. This was a significant improvement over the situation at the time endodontic treatment was begun.

Histologic sections of the experimental tooth did not include the apex of the mesial root. Nonetheless granulomatous tissue surrounded that portion of the mesial root which was included in the section and a small well defined lesion was present at the apex of the distal root. The lesions consisted almost entirely of PMN's. Masson's trichrome showed some fibrous encapsulation and infiltration but this was not as extensive as that seen in the experimental second premolar. There was no evidence of osteoblastic activity in the surrounding bone. The P.D.L. fibers were generally oriented parallel to the root surfaces and some apical cemental tearing and minor cemental resorptions were visible.

The lesion surrounding the extruded filling material on the distal root of the control tooth consisted of PMN's and a moderate number of
plasma cells and macrophages. There were extensions of the lesion out into the surrounding marrow spaces but there was also a wide band of fibrous connective tissue adjacent to the extruded filling material (see Figure 16). Concentrations of inflammatory cells were seen surrounding blood vessels in marrow spaces which were not in direct contact with the lesion itself. The special stains showed that some fibrous infiltration of the lesion had occurred but there was minimal evidence of peripheral osteoblastic activity. On this basis both the experimental and control teeth were assigned healing values of two.

The radiographic survey of the experimental tooth in the male showed a large periapical radiolucency on the distal root measuring 5 x 4 millimeters and extending coronally along the mesial and distal aspects of the root (see Figure 10). This lesion was present despite a radiographically acceptable root canal fill. The mesial root displayed a similar lesion but of much smaller dimensions. The periapical status of the tooth was significantly worse than it had been at the time endodontic therapy was begun. The control tooth showed radiolucencies on both the mesial and distal roots measuring 2 x 2 and 4 x 4 millimeters respectively (see Figure 11). Here again the root canal fills appeared to be clinically acceptable on the radiograph. The periapical lesions appeared to be worse than they had at 16 weeks post inoculation.

Histologic sections of the experimental tooth showed well defined apical and lateral lesions on both roots consisting almost entirely of plasma cells (see Figure 15). The lesions were encapsulated by relatively
thin bands of fibrous connective tissue and showed little connective tissue infiltration. Peripheral bone trabeculae were surrounded by lines of osteoclasts and osteoblasts (see Figure 14). This phenomenon was seen extending along the lamina dura towards the furcation on both roots. The P.D.L. fibers in the furcation area appeared to be compressed against the alveolar crest and undergoing necrosis. Apically both roots showed evidence of cemental resorptions which were being filled in with new cementum.

The control tooth showed multiple well encapsulated lesions around both apices. The largest lesion on the mesial consisted of PMN's and a large number of macrophages (see Figure 19). The main lesion on the distal was composed of PMN's and plasma cells. The lesions were well defined with few or no extensions into surrounding bone marrow spaces (see Figures 17 and 18). There was osteoblastic activity involving peripheral bone on the distal lesion whereas the mesial lesion showed obvious osteoclastic activity in a coronal direction along the distal aspect of the mesial root. There was minimal cemental and dentinal resorption on both roots. In light of evidence of bone apposition both experimental and control teeth were assigned healing values of three.

Endodontic treatment performed at 24 weeks, 4 days, post inoculation.

This group included the mandibular first molars of the female and the mandibular second premolars of the male. The root canals were not filled after the cleanse and shape procedure. Sacrifice came three days after the endodontic procedure and initiation of experimental occlusal trauma.
Radiographs showed that both experimental and control teeth in the female had developed radiolucencies on the distal aspects of all roots (see Figures 7 and 8). On the mesial roots these rarefactions extended from apex to the furcation areas. All roots showed severe resorption from an apical and distal (lateral) direction. The radiographic situation was identical to that seen at the time endodontic treatment was begun.

Histologic sections of the experimental tooth showed large localized lesions consisting of PMN's and a small to moderate number of plasma cells and macrophages. The apical portion of the distal root was lost during sectioning but the coronal extension of the distal lesion remained. Some portions of the lesions were surrounded by a heavy band of fibrous connective tissue whereas other portions displayed a very thin fibrous capsule. There was evidence of osteoblastic-osteoclastic activity around peripheral bone trabeculae but there was no predominance of one cell type over the other. The distal aspect of the mesial root was severely resorbed with no evidence of cemental repair. The portion of the distal root that was included in the section was similarly resorbed along its distal aspect. The special stains revealed little or no connective tissue infiltration of the lesions.

The control tooth showed lesions composed of PMN's and a small to moderate number of plasma cells and macrophages extending along the resorbed aspects of both roots. All lesions were surrounded by a heavy band of connective tissue. The resorbed surface of the mesial root was relatively smooth and regular with no evidence of cemental repair. The resorption
of the distal root was more irregular but cementoblasts were seen in close apposition to the root surface with evidence of cemental repair. Apical to the distal lesion osteoblasts arising from the proliferating connective tissue could be seen encircling bone trabeculae. There were few, if any, inflammatory cells in this area. Due to the destructive nature of the associated lesions the experimental tooth was assigned a value of one. The control tooth was assigned a three.

The radiographic survey of the experimental tooth in the male showed a slight widening of the apical P.D.L. on the mesial root and evidence of apical resorption on the distal root (see Figure 10). No large radiolucencies were present. The control tooth displayed periapical radiolucencies on both roots measuring 2 x 3 millimeters (see Figure 11). The mesial lesion extended halfway up the distal aspect of the root towards the furcation. The radiographic appearances of both teeth were identical to those at the time endodontic treatment was initiated. Comparison with radiographs taken 13 weeks after inoculation, however, showed that an improvement had occurred in the experimental tooth even before endodontic treatment was begun.

Histologically the experimental tooth showed periapical lesions composed mostly of fibrous connective tissue and a small number of PMN's (see Figure 20). The lesions extended into surrounding marrow spaces but not to any great extent. There were apical cemental resorptions with repair on both roots. Osteoblastic activity was present all along the borders of the peripheral bone trabeculae. A heavy concentration of PMN's on the distal aspect of the mesial root showed osteoclastic
resorption of the lamina dura along the lesion's periphery. The P.D.L. in the furcation area was normal. Masson's trichrome and Van Gieson's stains showed a considerable amount of fibrous infiltration of the lesions.

The control tooth displayed small multiple lesions extending into the surrounding marrow spaces on both roots. Around the apices there was a considerable amount of fibrosis with higher concentrations of PMN's and osteoclasts in the peripheral areas of the main lesions. The lesions were encapsulated by a thin connective tissue band. Connective tissue infiltration of the lesions which extended into surrounding bone was very minimal. There was evidence of significant clastic activity in the marrow spaces between the roots at the level of the apices and also apical to the lesions. The P.D.L. and alveolar bone in the furcation area was normal (see Figure 21). Since the healing process in the experimental tooth appeared to be so advanced the tooth was assigned a value of four. The control tooth was assigned a one in light of the high level of osteoclastic activity.

Table II shows the assigned healing values for all teeth studied. An analysis of variance block design test was conducted to determine any differences between the healing values of experimental and control teeth (i.e. differences between treatments).
<table>
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<th>Mean Squares</th>
<th>F Value</th>
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<td>0</td>
<td>0</td>
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<tr>
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<td>0</td>
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<tr>
<td>Interaction Between Times and Treatments</td>
<td>2</td>
<td>0.5</td>
<td>0.25</td>
<td>0</td>
</tr>
</tbody>
</table>

Zero F value calculations indicate no difference between treatments.

There was no statistical difference in the progression of healing between the teeth placed in occlusal trauma and the control teeth.
It has been pointed out in a review of the literature that the lesion of occlusal trauma is a reversible one (1,5,38,44,52,59,60). Once the lesion of trauma is induced it may be reversed by eliminating the excessive forces or by the movement of the tooth into such a position that the excessive forces are no longer present.

In the present study the experimental teeth were left with high occlusal fillings for periods of twelve weeks, nine weeks, and three days. In the twelve and nine week teeth the healing values assigned were practically identical to those values assigned to the corresponding control teeth (see Table II). In the three day teeth, however, there was a considerable difference between experimentals and controls. Assuming that the healing seen in the male experimental second premolar was due to the animal's extraordinary recuperative power (this phenomenon is examined more closely further on in the discussion) there is a significant difference between the female first molars. The control tooth showed signs of obvious healing whereas the experimental tooth displayed an acute destructive pattern. This could indicate that occlusal trauma does have a retarding effect upon healing initially in the periapical area but such an effect may only exist for a relatively short period of time (three days to one week). In theory, the sudden intrusive
force on the tooth could cause pressure necrosis of the connective tissue fibers at the interface of the periodontal ligament (P.D.L.) and the pre-existing periapical granuloma. The organization of connective tissue fibers in the periphery and interior of the endodontic lesion would be disrupted by an influx of polymorphonuclear leukocytes (P.M.N.'s) and a localized exudate. This sequence of events would alter the "inductive" pattern as described by Weiss (82) and Urist, et al., (75). The chemical nature of the environment surrounding the fibroblasts, osteoblasts, and undifferentiated mesenchymal cells would be changed, hence altering or delaying the healing process. Osteoblasts might revert to the morphology of progenitor cells, as described by Young (85) or even to osteoclasts. Once the intrusive forces became negligible, the microenvironment of these cells would return to a state more conducive to organization - i.e. chronic inflammation (Figure 15 and 20) and eventual healing. In the nine and twelve week teeth enough tooth movement may have occurred so that the experimental teeth were no longer in a state of trauma and healing had progressed to a level comparable to that in the control teeth.

The use of six pairs of teeth in the study allows conclusive statements regarding the long-term relationship between trauma and healing. But only two pairs of the six were evaluated on a short-term basis. Of these two pairs only one pair (the female first molars) showed a significant difference between experimental and control. Obviously the small number of animals and teeth used in the study precludes drawing any
definite conclusions concerning the short-term effects of occlusal trauma and therein lies a weakness of the study. Only two animals were used since it was thought that the endodontic and restorative procedures involved would require a significant amount of time on the part of the operator. This was, in fact, the case. However, any future studies dealing with this topic should include more pairs of teeth evaluated on a short-term basis (i.e. three days to one week).

Another factor that may have influenced the results of the study was the necessity of removing all endodontic sources of periapical irritation prior to the evaluation of the periapical effects of trauma. Perhaps the healing in the first premolar control of the male would have been more advanced had the distal canal not been ledged during preparation (see Figure 9). Similarly, the retarded healing in the first premolar control of the female was undoubtedly due to the over-extension of filling material into the periapical area (see Figure 8 and 16). The mandibular first premolar of the Macaca mulatta monkey is very difficult to treat endodontically due to the considerable length and severe distal curvature of the mesial root. A recommendation that can be made concerning future studies of this type is to use teeth distal to the first premolar so that more acceptable endodontic treatment can be performed.

The criteria used in this study to evaluate the degree of healing are classic and have been used in previous studies (2,15,18,46,66,67). In this study healing was evaluated after three days, nine weeks, and
twelve weeks. The degree of healing after such relatively short periods of time is still a very difficult entity to quantify and may be open to error. The cell types associated with a particular periapical lesion may vary depending on where within the lesion the section is taken. In addition the presence of osteoblasts in the periphery of a lesion may not indicate healing at all but may simply be part of an appositional response to osteoclastic activity elsewhere in the lesion. An aid that might make the interpretation of healing more objective in future studies is the use of Procion Brilliant Red dye. A multiple injection sequence would allow easier visualization of hard tissue deposition under the light microscope and give more credence to the assigned healing values.

The most plausible explanation for the lack of difference in the healing patterns between the experimental and control teeth is that the pathologic effects of occlusal stresses are borne by the entire periodontal ligament as a unit and not just by isolated segments of the P.D.L. In the experimental teeth retrogressive changes were evident at the bony crest in the furcation areas (Figures 21 and 22) as well as along the lateral lamina duras (Figure 11). The fibers of the P.D.L. were often reoriented so as to run parallel to the root surface indicating that compensatory changes had occurred. With occlusal stress so uniformly distributed over the entire tooth-P.D.L. interface the destruction that occurred in the periapical area was minimal. Most of the burden seems to have been undertaken by the horizontal fiber group, which runs perpendicularly between the cementum and alveolar bone, and by the oblique group,
which includes about two thirds of all the P.D.L. fibers (30). Because the forces of occlusion become distributed uniformly along the P.D.L. and the teeth tend to move out of trauma (previously discussed) it can be concluded that long-term occlusal trauma has little or no effect on the healing of a periapical lesion.

The amalgam restorations appeared to withstand the excessive occlusal forces very well (see Figures 3 and 6). There was no clinical evidence of cracking or fracture of the restorations and the burnish marks generated were clear signs of the excessive forces at work. This is contrary to the findings of Landay, Nazimov, and Seltzer (42) who reported fracturing of amalgam restorations in rat teeth. Since teeth in the Rhesus monkey are much larger than those in rats, in this study it was possible to use an adequate bulk of restorative material in each occlusal preparation. It was desirable to use amalgam as the restorative material rather than cemented occlusal pins or orthodontic appliances because amalgam is more likely to be used in a clinical situation. Future studies of this type may even consider using cast restorations although the impression, waxing, and casting procedures involved would require an increased amount of time on the part of the operator.

In several experimental and control teeth there appeared to be inconsistencies between the radiographic appearance of the root canal treatment and the radiographic and histologic evidence of healing. The mesial root of the experimental second premolar of the female showed improvement radiographically despite an endodontic fill that terminated three to four millimeters from the apex (Figure 7). A plausible explanation would be
that a significant portion of the necrotic debris had been removed from the root canal at the time of preparation and that some semblance of an apical seal had been affected by dentinal shavings. The debridement and sealing was enough to cause a significant improvement of the destructive lesion.

Conversely the increase in the destructive lesions in the experimental and control first premolars of the male was due to the fact that not enough debridement of the root canals had been performed (Figures 9 and 10). The distal root of the control tooth had been ledged in the coronal third so there had been no debridement of this root in the apical portion. In addition no apical seal of dentinal shavings had been created and the inadequate root canal fill did not improve the situation. The only reason the tooth was given a relatively high healing value (three) was due to the high level of osteoblastic activity in the periphery of the lesion.

There were also inconsistencies between the degree of healing as determined radiographically versus the histologic evidence of healing. The radiographic appearance of the lesions in both the experimental and control first molars of the male was poor despite the clinically (radiographically) acceptable root canal fills. However, the histologic picture was one of repair of cemental resorptions and osteoblastic activity involving peripheral bone trabeculae - obvious repair (Figures 14, 17, 18). Seltzer et al., (66) pointed out that failure of a periapical lesion to improve radiographically after endodontic therapy might be due to the
presence of a radicular cyst. Penick (58) maintained that the formation of a fibrous connective tissue scar might curtail the process of complete healing. The present results indicate that healing may begin long before there is radiographic evidence of healing. Perhaps the radiograph should not be relied upon so much as an indicator of early healing.

Studies of sections treated with Brown and Brenn stain indicated that microorganisms, when present, were confined to the root canal space. This was true even in the 24 1/2 week teeth which were debrided only three days prior to sacrifice. This finding supports the so called "Mountain Pass Concept" of Kronfeld (40) which states that the periapical granuloma is not an environment conducive to bacterial growth.

THE MONKEY AS AN EXPERIMENTAL ANIMAL

The *Macaca mulatta* monkey was selected as an experimental animal due to its evolutionary and anatomical similarity to man. Steiner and Van Hassel (70) stated that the use of the monkey allows the extrapolation of experimental results to the clinical results as would be found in the human. The only shortcoming in the use of the monkey might be that its recuperative power of the monkey is greater than that of the human. Citrome (6) noted that periapical repair in the form of dentin, cementum, and bone could occur despite the presence of an active inflammatory infiltrate. This ability could explain the radiographic improvement and histologic evidence of repair in the experimental second premolar of the male. The improvement appears to have occurred even before any debride-ment of the root canals had been carried out and indicates that healing
may have proceeded without endodontic therapy.

THE USE OF STREPTOCOCCUS FAECALIS

In 1958 Leavitt, et al., (43) studied cultures from root canals of teeth with vital and nonvital pulps. They concluded that the streptococci comprised the largest group of all microorganisms isolated.

Winkler and van Amerongen (84) examined 4,000 cultures taken at successive points throughout the root canal treatment of teeth with vital and nonvital pulps. They found that initially the levels of Streptococcus mitis were quite high but that as treatment progressed the levels of S. faecalis became high. They also found that the levels of S. faecalis and S. liquefaciens tended to persist longer than levels of other microorganisms and where, therefore, implicated as possible pulpal pathogens.

Engstrom and Frostell (17) studied the microflora of teeth with necrotic pulps which had not been open to the oral environment. They found Streptococci in 38% of the anaerobic cultures and in 38% of the aerobic cultures. Similarly Crawford and Shankle (14) compared cultures taken from 57 teeth and examined them via phase-contrast, dark-field, and light-field microscopy and aerobic and anaerobic cultures. In all cases the streptococci were the most common organisms found.

In 1964 Engstrom (16) examined the frequency of enterococci in 233 teeth which had either been previously treated endodontically or had nonvital pulps. Of these 14.9% showed the presence of enterococci upon initial culture. At the second visit 65% of this group showed a positive culture indicating that enterococci are difficult to eliminate from the
root canal.

Felder (20), in monkeys, and McCormick (49), in dogs, inoculated macerated dental pulps with a pure culture of \textit{S. faecalis} and obtained radiographic evidence of periapical lesions from within two weeks to three months.

In light of these studies, \textit{S. faecalis} was chosen as the means of creating periapical pathosis in the present study. All teeth inoculated in both animals developed radiographic evidence of periapical lesions within three months.
CHAPTER VI

SUMMARY AND CONCLUSIONS

In order to examine and describe the effects of occlusal trauma on periapical healing after endodontic therapy in monkeys, the pulps of twelve mandibular teeth were partially extirpated in two adult Rhesus monkeys - one male and one female. A standardized inoculum of a pure culture of *Streptococcus faecalis* was placed into the partially macerated pulps of each tooth and the access cavities were sealed. After thirteen weeks a radiographic survey revealed the presence of periapical radiolucencies on each tooth. Endodontic treatment was begun on the mandibular second premolars of the female and the mandibular first premolars of the male. In each animal the tooth on one side of the mandible was sealed with an overcontoured occlusal amalgam. The tooth on the opposite or control side was sealed with a normally contoured amalgam. At sixteen weeks canal fillings were placed in the thirteen week teeth and the same procedure was begun on the first premolars of the female and the first molars of the male. Canal fillings were placed in these teeth at nineteen weeks. At twenty-four weeks, four days, endodontic therapy was begun on the remaining teeth - the first molars of the female and the second premolars of the male - and the appropriate amalgam restorations were placed. The canals of these teeth were not filled. Both animals were sacrificed at twenty-five weeks after a final
radiographic survey was conducted. The jaws were removed at necropsy and following fixation, decalcification and histologic preparation, the teeth and surrounding structures were examined microscopically.

The conclusions drawn from this study should be considered in light of the fact that the endodontic therapy performed on the experimental and control teeth was somewhat less than ideal. It is possible that, in certain teeth, periapical pathosis persisted as a result of an unresolving endodontic lesion and not strictly as a result of the occlusal trauma.

Thus on the basis of this study the following statements could be made:

a) Occlusal trauma has little or no effect on long-term periapical healing after endodontic therapy.

b) *S. faecalis* is capable of producing pathologic periapical lesions in the Rhesus monkey.

c) Amalgam is a satisfactory restorative material that can be used in studies involving excessive occlusal forces in the Rhesus monkey.

Under the conditions of this experiment, the following impressions could be drawn:

a) Occlusal trauma may delay periapical healing after endodontic therapy on a short-term basis (i.e. three days to one week), but due to the low number of teeth used no definite statement can be made concerning these short-term effects.

b) Histologic evidence of healing of periapical lesions as determined by peripheral osteoblastic proliferation, the presence of
chronic inflammatory cells, and infiltration by fibrous connective tissue, precedes any radiographic evidence of healing.

c) For experimental purposes, the mandibular first premolar of the Rhesus monkey is a poor candidate for endodontic therapy. This is due mainly to the considerable length and severe distal curvature of the mesial root.
CHAPTER VII

REFERENCES


CHAPTER VIII

TABLES AND FIGURES
### TABLE I

**EXPERIMENTAL TIMETABLE:**
**EXPRESSED IN WEEKS AFTER INOCULATION**

<table>
<thead>
<tr>
<th>Tooth (Palmer Notation)</th>
<th>Animal Number One (Female)</th>
<th>Animal Number Two (Male)</th>
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<td></td>
<td>61</td>
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<td>Placement of over-contoured occlusal amalgams (initiating occlusal trauma)</td>
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<tr>
<td>Obliteration of root canals</td>
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<tr>
<td>Sacrifice</td>
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<td>Amount of time in trauma (unrelated to inoculation)</td>
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<tr>
<td>Amount of time from cleanse and shape to sacrifice (unrelated to inoculation)</td>
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<td>3 days 12 weeks 9 weeks 12 weeks 3 days 9 weeks 3 days 12 weeks</td>
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# TABLE II

**ASSIGNED HEALING VALUES* AS RELATED TO HEALING TIME**

<table>
<thead>
<tr>
<th>Amount of time from cleanse and shape to sacrifice:</th>
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</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

| Animal Number Two (Male) | Trauma | 2 | 3 | 4 |
| Control | 3 | 3 | 1 |

* Assigned healing values range from 0 to 4 with 0 indicating no evidence of healing and 4 indicating obvious healing with no signs of an active destructive process.*
Figure 1: Mandibular first molar of male monkey under rubber dam isolation. The Ivory #12 clamp was used due to the small size of the animal teeth as compared to those in the human. Orifices to mesial and distal canals can be viewed through the small access preparation.

Figure 2: File in place in the distal canal of the mandibular first molar. Canals were enlarged to a size #25. Mouth prop can be viewed in the lower right portion of the picture.
Figure 3: Overcontoured occlusal amalgam restorations in mandibular left quadrant of the female animal just prior to sacrifice. Note burnish marks (arrows) indicating the involvement of excessive occlusal forces.

Figure 4: Occlusal amalgam restorations in the control teeth of the female animal just prior to sacrifice. The burnish marks noted in the experimental teeth are absent here. Note scar tissue in buccal mucosa adjacent to first molar indicating closure of sinus tract.
Figure 5: Amalgam restorations in the control teeth of the male monkey just prior to sacrifice. Restorations were carved to natural form. Burnish marks are absent.

Figure 6: Experimental side of male animal. Burnish marks are clearly seen (arrows) in overcontoured amalgams.
Figure 7: Radiograph of the experimental side in the female at time of sacrifice. Note the absence of radiolucencies at the apices of the first premolar and the mesial root of the second premolar. The radiolucency on the mesial root of the first molar extends up into the furcation area (arrows).

Figure 8: Radiograph of the control side in the female at time of sacrifice. Radiolucencies associated with the second premolar are almost negligible. Note the perforation of the distal root of the first premolar and the extrusion of filling material (arrows). The roots of the first molar are severely resorbed.
Figure 9: Radiograph of the control side in the male at time of sacrifice. Note the ledged distal canal (arrow) of the first premolar and the large peri-apical radiolucency of the same. Radiolucencies persist on both roots of the first molar despite relatively acceptable root canal fills. The considerable length and severe distal curvature of the mesial root of the first premolar makes this tooth a poor candidate for endodontic therapy.

Figure 10: Radiograph of the experimental side in the male at time of sacrifice. A small amount of extruded sealer can be seen at the apex of the distal root in the first premolar (arrow). Some rarefaction has occurred in this area. Radiolucencies associated with the second premolar are minimal. The radiolucency on the distal root of the first molar extends into the furcation area (arrows).
Figure 11: Periodontal ligament (P.D.L) along mesial aspect of distal root in experimental second premolar of female. Note dilated blood vessels - present in response to occlusal trauma. Osteoblasts and occasional osteoclasts (arrows) indicate remodeling of alveolar bone. Cementum (C), P.D.L (P), alveolar bone (B). (Hematoxylin and eosin stain, original magnification X40).

Figure 12: Periapical area of mesial root in experimental second premolar of female animal. Van Gieson's stain allows visualization of connective tissue fibers (arrows) infiltrating lesion. Lesion is seen extending into surrounding marrow spaces in lower left portion of the picture. Root apex (A), alveolar bone (B). (Van Gieson's stain, original magnification X40).
Figure 13: Periapical lesion along lateral aspect of mesial root in control second premolar of the female. Lesion is composed predominantly of polymorphonuclear leukocytes (PMN's). Foreign matter (arrows) - possibly sealer - is seen towards the center of the lesion. Root apex (A), alveolar bone (B). (Hematoxylin and eosin stain, original magnification X40).

Figure 14: Bone trabeculae in periapical area of experimental first molar of the male animal. Increased blastic activity (arrows) may be due to the presence of a periapical lesion (PL) or to increased occlusal forces or both. Alveolar bone (B). (Hematoxylin and eosin stain, original magnification X100).
Figure 15: Periapical lesion (PL) consisting mostly of plasma cells in the experimental first molar of the male. Band of fibrous connective tissue can be seen (arrows). Alveolar bone (B). (Hematoxylin and eosin stain, original magnification X100).

Figure 16: Fibrous tissue capsule (FC) surrounding extruded root canal filling material (S) on the distal root of the control first premolar in the female animal. Extravasated blood cells (arrows) are still present in the area. Most of the filling material has been lost due to sectioning. Alveolar bone (B) (Hematoxylin and eosin stain, original magnification X40).
Figure 17: Masson's trichrome stain of periapical lesion on the control first molar in the male. A heavy band of fibrous connective tissue surrounds the lesion. Osteoblastic proliferation adjacent to the peripheral bone (arrows) indicates an obvious attempt at healing. Periapical lesion (PL), fibrous capsule (FC). (Masson's trichrome stain, original magnification X40).

Figure 18: Higher magnification of periapical area in Figure 17 showing fibrous connective tissue proliferation (FC) and osteoblastic activity (arrows). Evidence of capillary proliferation is seen in the top, center and bottom, right portions of the picture. (Masson's trichrome stain, original magnification X100).
Figure 19: High magnification of periapical lesion on mesial root of control first molar in the male. Lesion contains a high number of macrophages (arrows) which have engulfed lysed PMN's. PMN's (PM). (Hematoxylin and eosin stain, original magnification X100).

Figure 20: Periapical lesion on mesial root of experimental second premolar in the male. Arrows indicate osteoclasts (here, cementoclasts) resorbing the root surface. Tooth was assigned a high healing value due to increased infiltration of the lesion by fibrous connective tissue. Root apex (A), alveolar bone (B), encapsulated lesion (PL). (Hematoxylin and eosin stain, original magnification X40).
Figure 21: Healthy furcation of control second premolar in the male animal. Note intact P.D.L. fibers extending from the radicular surface to the alveolar crest. There is a distinct absence of osteoblastic and/or osteoclastic activity. Cementum (C), alveolar bone (B), P.D.L. (P). (Hematoxylin and eosin stain, original magnification X40).

Figure 22: Furcation of experimental second premolar in the female. Note thrombosis of blood vessels in the P.D.L. and osteoclastic remodeling of the alveolar crest (arrows). Such findings indicate the role played by the entire supporting apparatus in response to increased occlusal forces. The clear areas are sectioning artifacts. Cementum (C), alveolar bone (B), P.D.L. (P). (Hematoxylin and eosin stain, original magnification X40).
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