The Effect of the Absence of a Coronal Seal on Periapical Tissues Following Endodontic Therapy

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THE EFFECT OF THE ABSENCE OF A CORONAL SEAL
ON PERiapICAL TISSUES FOLLOWING ENDOdONTIC THERAPY

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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
January
1987
DEDICATION

To Dr. Marshall Smulson, in appreciation of his thirty-one years of dedicated service to the students of Loyola University, School of Dentistry.
ACKNOWLEDGEMENTS

To Dr. Franklin Weine who gave me the opportunity to train under one of the leaders in the field of endodontics, for his lasting friendship and assistance in helping shape my career.

To Dr. Marshall Smulson who first ignited the spark of interest in endodontics for me through his tireless devotion to education.

To Dr. Hal McReynolds who helped stimulate ideas throughout my professional training, and whose friendship and assistance helped make this study possible.

To Dr. James Sandrik whose expertise in dental materials and scientific method are well acknowledged, and whose friendship and interest has added to my professional development.

To Dr. Gary Taylor who brought me into his practice and helped me develop and refine my clinical skills, and whose friendship I will always value.
VITA

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CHAPTER I
INTRODUCTION

Statistically, endodontics is highly predictable, realizing a success rate approximating 95% of all cases treated. Predictability of this magnitude may be attributed to a meticulous conformation to three basic principles known as the endodontic triad. Accurate diagnosis of the disease process, biomechanical preparation and obliteration comprise the three phases of the endodontic triad. Failure to properly execute any one of these procedures might severely compromise the prognosis.

Emphasis in the literature seems to have been placed on obliteration techniques and materials. The goal of the third phase of the endodontic triad is to hermetically seal the root canal system, with an inert substance, from the interstitial fluids circulating in the periradicular region. Studies abound as to the sealing effectiveness of various endodontic materials and techniques, all substantiating the necessity of attaining an apical seal. Loss of, or failure to have achieved an apical seal will allow apical interstitial fluid to percolate into the root canal system. The exact mechanism of how fluid percolation is capable of producing apical inflammation has yet to be elucidated. It is speculated that the exudate found leaking into the canal system is derived from the blood serum. This material, once isolated within a canal space, may undergo degradation and diffuse back into the periapical region, producing an inflammatory reaction. Thus, the import of attaining and securing an apical seal must be realized.
Following completion of successful endodontic therapy, a restoration is fabricated to bind the residual tooth structure together and return the treated tooth to occlusal function. At times, the final restoration may fail in that the marginal integrity is lost, or the patient may have entirely neglected further dental treatment with the eventual demise of the temporary filling. In either case, salivary and bacterial contaminants might be allowed access to the root canal system from the coronal aspect, possibly jeopardizing the endodontic prognosis due to breakdown of the hermetic seal with resultant periapical changes.

The literature is deficient concerning loss of the coronal seal and its effect on periapical tissues following endodontic treatment. It is apparent that further research into the effectiveness of an intraradicular filling in the presence of a failing coronal seal is warranted. Therefore, this study was initiated to evaluate whether loss of a coronal seal would adversely affect a minimal apical seal within a root canal space, especially following post room preparation, by investigating periapical reaction both radiographically and histologically. It is hoped that this study will give insight into the import of the temporary intertreatment coronal seal following root canal therapy and the necessity for placement of a permanent restoration thereby preventing leakage from the oral environment after completion of endodontic therapy.
CHAPTER II
REVIEW OF THE LITERATURE

A. HISTORY

Endodontics was first recognized as a separate clinical specialty by the American Dental Association in 1963. Although seemingly in its infancy in the mid-twentieth century, early Egyptian records dating back to 3700 B.C. show the existence of dental prescriptions as remedies for gingival abscesses and/or sinus tracts arising from nonvital teeth. Evidence of a crude form of root canal therapy presents in the first century A.D. when trephines were used to establish drainage from the pulp cavity. Arabian surgeons were also noted to be performing some type of endodontic therapy. In 1757, Dr. Bourdet was practicing pulp extirpations and filling roots of anterior teeth to the apex with gold. Dr. Hudson, a Philadelphian dentist, introduced this technique in the United States in 1809. Various filling materials were advocated to seal root canal spaces including cotton and other fibers, copper, plaster of Paris and even sterile sparrow droppings. Sharpened wood sticks were utilized in one technique to remove and destroy the canal contents, later filling the space with the same stick. Frequent root fractures shortened the longevity of this method. In 1839, Dr. Baker recommended removing the exposed nerve from the canal, cleaning the canal space and filling the "fang" with gold. This was the first account of pulp extirpation, canal debridement and root filling.

During the 1800's, root canal instruments were crude and not commonly
available. Success frequently depended on the ingenuity and manual dexterity of the operator. Vital or mortal pulp amputation was advocated to circumvent the need for instrumentation and obliteration of the root canal space. For these reasons, an era of chemical sterilization was embarked upon. Various chemical and medicinal agents were utilized to treat root canal spaces. Flagg and Koeker, in 1825, recommended treating the dental pulp with oil of cloves, oil of cajuput, camphor, opium, alum and myrrh. Dr. John Hunter, in 1835, advocated burning the nerve of the tooth with strong acids and alkalies. Arsenic was introduced by Dr. Spooner in 1836 as another method for devitalizing the pulp. Thomas Rogers reviewed 220 cases of pulp capping procedures in which various caustic agents were used, in which 202 were claimed successful. Five conditions were enumerated for success, but he proceeded to discredit this scientific observation by prescribing three leeches and a smart aperient if the pulp capping failed.

The practice of using a mummifying paste was introduced by Dr. W.D. Miller about 1893. Mercuric chloride was used for this purpose, but alum, thymol and formalin came into favor later.

The mid 1800's saw the introduction of instruments used for the total removal of pulp tissue. Thomas Bell suggested that the tissue be burned with a cauterizing wire at white heat. In 1836, Dr. Edward Maynard barbed one side of an untempered steel watch spring and also made reamers from piano wire. Dr. Fauchard described instruments made from annealed piano wire, cut into proper lengths, filed down to various diameters and burnished. Small barbs were then cut along the length of the instrument. In 1852, Arthur developed broaches with cutting barbs that were designed to grasp
the organic contents of the canal space. Bennett also describes broaches, barbed and spiral, developed primarily to cleanse the canal of tissue debris but seldom used for enlarging and shaping.

Prior to the Second International Conference on Endodontics in 1958, endodontic instruments were unstandardized. Instruments were haphazardly numbered from one to twelve, each manufacturer having their own specifications. Ingle and Levine presented a plan where endodontic instruments would have the same taper of shaft, a definite increase in size from one instrument to another and a universal numbering system with plastic color coded handles. Standardization was approved and accepted by the Conference and is the guide used by manufacturers today.

B. FOCAL INFECTION THEORY

Advances in instrument design, instrumentation and endodontic techniques for the pulpally involved tooth were being introduced and perfected at the turn of the twentieth century. The application of x-rays as a diagnostic tool in dentistry was also being utilized.

In 1888, Dr. W.D. Miller demonstrated the presence of bacteria within the structures of the teeth and on the tissues adjacent to them. He later showed that teeth with necrotic pulps were generally infected and explained the formation of dento-alveolar abscesses as the spread of pulpal infection into the periapical region. A relationship was alluded to between infections of the mouth and many general constitutional diseases. As early as 1898, William Hunter introduced the term "oral sepsis" and suggested a link between dental infections and various maladies. In 1910, Hunter delivered
an address at McGill University entitled "The Role of Sepsis and Antisepsis in Medicine" expounding that prosthetic dentistry was harboring and propagating areas of frank infection. The stage was now set for one of the most disastrous eras in modern dentistry. Two physicians, Rosenow and Billings, applied Hunter's theories to the situation of the pulpless tooth and developed the "Theory of Focal Infection". In addresses before the American Medical Association, Rosenow and Billings implicated oral infections in the production of systemic diseases. Non-vital and pulpless teeth, irregardless of their radiographic patterns, were assumed to be dead and infected or easily infected by anachoresis, presenting a source or foci of suppuration which could exude itself throughout the body. Thusly, alveolar focal infections and abscesses could act as a dominant, if not the sole factor in the production of systemic disease. The list of diseases associated with root abscesses, pus pockets and pulpless teeth included chronic rheumatism, neuritis, appendicitis, goiter, hay fever, asthma, ulcers, Hodgkin's disease, endocarditis and mental illness. Said diseases could only be arrested by extracting the offending tooth or teeth propagating the focus of infection. By 1920, the crusade against the pulpless tooth had reached such catastrophic proportions that the dental profession could no longer justify the devitalization of teeth. Needless extractions of pulpless and vital teeth were performed to placate the medical profession.

It was not until the 1930's that serious doubt arose as to the validity of the Focal Infection Theory. Bacteriologists, attempting to replicate Rosenow's research, were frequently unable to reproduce his results. A discrepancy also existed between the findings of bacteriologists and
pathologists as to the frequency of infections found on extracted teeth submitted for cultural and histopathologic evaluation. Rosenow's experimental protocol allowed for immediate culturing of extracted root surfaces or placement of extracted teeth into sterile test tubes for later bacteriologic or histopathologic evaluation. Bacteriologists were identifying a high proportion of extracted pulpless teeth with bacterial growth on their root surfaces and condemned them as foci of infection. Vital teeth with intact periapical regions were also noted to produce instances of bacterial growth. Pathologists, however, were not identifying microscopic evidence of infection as frequently on extracted pulpless teeth and found complete absence of infection on extracted vital teeth.15

As early as 1917, Meyer criticized the culturing of extracted root surfaces due to the possibility of normal flora contamination during extraction.16 Gunter and others17 cultured dentinal dust and pulpal tissue of teeth sterilized with tincture of iodine prior to removal, followed by an apical culture after extraction. Bacterial growth was noted on only one dentinal culture, but all apical cultures were positive, casting grave doubt on the clinical significance of positive apical cultures from extracted teeth.

Vital periapical tissue had been shown to be devoid of bacteria histologically, yet their presence confirmed bacteriologically. Fish and MacLean were puzzled by these observations and theorized that bacteria remaining within the gingival sulcus following chemical sterilization could be pumped along the root surface during extraction, providing the source for positive bacterial root cultures. Fish and MacLean18 demonstrated, in
1936, that cauterization of the gingival sulcus prior to extraction, eliminated the evidence of any bacterial organisms from root surface cultures. Blood cultures were also shown to be sterile, disputing to a degree, Okell and Elliott's earlier observation of transient bacteremia following extraction of healthy vital teeth. Therefore, conclusions based upon bacteriologic studies utilizing root surface cultures were totally negated, as was the essential foundation of the Focal Infection Theory. Fish and Mac-Lean's research had such a profound effect that Grossman later noted that "almost all investigations of the pulpless tooth prior to 1936 were invalid". During this same time, numerous articles and case histories were appearing in the dental literature supporting the fact that nonvital teeth could be successfully treated and retained.

C. CANAL MORPHOLOGY AND INSTRUMENTATION

Following repudiation of the Focal Infection Theory, it became increasingly evident and desirable that pulpally involved teeth be treated and retained. Endodontic therapy was performed with increasing frequency and became viewed as an acceptable treatment modality.

According to Ingle, root canal therapy consisted of a triad of procedures: canal enlargement, canal sterilization and canal obliteration, with each phase given equal emphasis in achieving success.

Early investigators noted enormous anatomic and morphologic variations within the unprepared root canal. Numerous lateral and accessory canals were found, along with weblike communications between canals in multirooted teeth. More recent studies by Meyer and Skidmore and Bjorndal, using wax and plastic models respectively to reconstruct the canal
morphology, demonstrated again the complex nature of the root canal system. The existence of these apical and coronal ramifications however, had largely been ignored. Prior to Kuttler's article, in 1955, it was generally agreed that the root canal space was a uniformly tapered, cylindrical channel "following in its apical third the same direction as the middle and cervical thirds, ending in the extreme apex with a very narrow foramen". It was assumed that standardized instruments were capable of producing a canal preparation conforming in shape and size to standardized filling materials. Seidler described the ideal instrumented canal as being round and tapering, with a minute opening at the apex. Luks and Bolatin stated however, that this ideal was too simplistic, the root canal system was composed of tortuous turns, apical foramina and accessory canals. To attempt to employ standardized instruments in a system incapable of being standardized was a "whim of the imagination".

Numerous authorities have regarded the thorough debridement and elimination of pulpal and dentinal contaminants from the root canal system as the primary goal of endodontic therapy. Mechanical instrumentation, generally with the aid of irrigating solutions, substantially reduces the quantity of injurious agents within the canal, thereby enabling the natural defense mechanisms of the body to maintain or repair the periradicular environment. Even though instrumentation was at a crude stage of development, Hatton et al., in 1928, evaluated the effectiveness of canal debridement by examining histologic sections of enlarged pulp canals. Results indicated that canals were only superficially cleansed and much of the pulpal tissue remained. Masterton observed, in 1965, that even after careful canal
preparation, small irregularities which may harbor bacteria and debris can exist. Haga\textsuperscript{37} was among the first investigators to show that canal instrumentation left a surprisingly high percentage of voids and irregularities along prepared canal walls. In numerous cases, the instrument cut only three of the canal walls, leaving the fourth wall untouched throughout the entire length of the root segment. Gutierrez and Garcia\textsuperscript{38} reconfirmed the presence of irregularities, termed prolongations or fins, in instrumented mandibular incisors and canines. They further remarked that it was not even feasible to expect complete canal negotiation due to the prolongations, considering that instrumentation left a path through the geometric center of the root canal space. Brayton, Davis and Goldman,\textsuperscript{39} utilizing injected silicone rubber impression material, demonstrated the vast number of morphologic irregularities and variations still existing following the debridement procedure. Biomechanical instrumentation was generally observed only along one wall of the canal with as much as one-half of the surface area untouched by the debridement procedure. More recently, Moodnick et al.\textsuperscript{40} evaluated the efficiency of K files versus Hedstroem files in a scanning electron microscopic study. No difference was found between the cleaning ability of either file type but it was noted that the root canal walls even after thorough debridement contained many irregularities that trapped debris and harbored pulp tissue, with no canal level being found cleaner than another.

Numerous investigators utilizing simulated canal models,\textsuperscript{41} histologic evaluation,\textsuperscript{42} scanning electron microscopic studies\textsuperscript{43} and various other methods, have further demonstrated the limitations of present day
biomechanical techniques. The general consensus of recent studies, conclude that all hand and mechanical instrumentation, and irrigants utilized during the cleansing process, leave debris, both organic and inorganic within the canal system. The recent introduction of sonic and ultrasonic instrumentation promises to provide a more thorough means of canal debridement. Cunningham and Martin have shown the endosonic ultrasonic system of root canal preparation to be "superior" to hand-filing techniques. Further research into the true efficacy of sonic and ultrasonic instrumentation however, needs to be performed as evidenced by an investigation by Langeland et. al. showing that neither hand nor sonic/ultrasonic instrumentation totally cleaned curved or irregular canals better than the other method.

The above studies not only obviate the need for as complete a debridement of the canal system as possible, but also the necessity for an obliteration technique that will successfully seal the root canal system from the periapical region.

D. SUCCESS VERSUS FAILURE

Root canal therapy has become a highly predictable procedure evidenced by reported success rates of 95 percent as found by Ingle. There does not appear to be any clear definition or agreement however, as to what constitutes an endodontic success or failure.

Some experts feel that as long as an endodontically treated tooth is retained, it is successful. A number of early investigators established the validity of utilizing radiographs as a criterion of success. Bender et al. defined a case successful if: (1) the periapical region appeared
normal initially and no areas of rarefaction were observed six months and
two years following obturation of the canal(s) or (2) radiolucent areas
which existed prior to treatment, decreased in size six months and two
years after filling. When doubt existed as to whether an area became
smaller or not, they were classified as unsuccessful. Results of this study
were dependent upon radiographic interpretation alone, without considering
clinical factors and demonstrated a success rate of 82 percent. Strinberg
claimed that a case could not be considered successful until a pre-
existant periapical radiolucency had completely healed, with the possible
exception of a slightly thickened periodontal ligament space. Those lesions
which decreased in size but had not entirely resolved would be classified
as doubtful or uncertain. Nicholls has reviewed the literature and has
concluded that a two year postoperative observation period is sufficient
time for complete resolution of any periapical pathology. Conversely, Seltzer et al. have found that the majority of endodontic failures occur within twenty-four months of the completion of treatment.

Even though success or failure in endodontics is determined primarily
by radiographic findings, radiographic evaluation is not an exact science in
that observer interpretation must be introduced. Variations in success
rates can depend on interpretation of the radiographic evidence and upon
the definition of success. Goldman, Pearson and Darzenta illustrated that
the reliability of radiographic interpretation, in determining success or
failure of a case, is questionable at best. Five of six examiners agreed
independently upon the radiographic diagnosis of success versus failure
only 67 percent of the time. When all six evaluators were tabulated in the
results, the agreement rate dropped to 47 percent. Bender and Seltzer\textsuperscript{54} demonstrated that unless bony destruction encroaches on the junctional area of cancellous and cortical bone, a periapical radiolucency will not be perceived. In addition, differences in vertical and/or horizontal angulation can lead to different radiographic imagery in that lesions may appear larger or smaller. Bender, Seltzer and Soltanoff\textsuperscript{55} warn of the inadequacies in using radiographs as the sole criteria in determining endodontic success. They demonstrated that teeth with a normal periapical radiographic appearance were frequently found histologically to have chronic inflammatory cell infiltration and granulomatous tissue in the periapical region. There was no definite correlation found between a negative radiographic image and the periapical histologic findings in endodontically treated teeth. There was however a correlation between teeth with radiolucent areas in that the histologic sections revealed the presence of granulomatous tissue or cysts. Their histologic studies indicate the inability to use radiographs as a sole criteria of treatment success. They concluded that clinical observations should be further examined as additional evidence of endodontic success.

Clinical symptoms were evaluated along with radiographic evidence by Grossman, Shephard and Pearson\textsuperscript{56} who proposed that if symptoms developed or persisted following root canal therapy, the case should be classified as a failure, despite negative radiographic findings. Those cases in which an area of rarefaction decreased in size, but had not resolved entirely, were classified as doubtful. Also included were situations where a thickened periodontal ligament space had formed. A study was performed by Harty et.
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to determine the success rate in root canal therapy where successful
treatment was not attained unless the tooth remained clinically asymptomat-
ic and functional for two or more years and the radiographic pattern either
remained normal or returned to normality by complete healing of the bony
radiolucency and the periodontal ligament space. Their study was limited
to anterior teeth with single canals and found an overall success rate of
ninety percent. Heling and Tamshe\textsuperscript{58} included posterior teeth in a similar
study, maintaining the same rigid guidelines for success and evidenced a
drop in the success rate to 70 percent. Seltzer et. al.\textsuperscript{59} performing a
clinical, radiographic and histologic evaluation of endodontic failures
made the conclusion that no agreement exists as to the correct definition of
success or failure. They suggested that regardless of the radiographic in-
terpretation, endodontically treated teeth which were functioning and with-
out adverse clinical symptoms, be regarded as successful. Those teeth with
adverse clinical symptoms, such as pain, swelling, persistent sinus tract,
etc., should be regarded as treatment failures irrespective of the absence
of periapical rarefactions.

Due to the difficulty of objectively defining the parameters of suc-
cess in root canal therapy, it might be more prudent to investigate and
identify the reasons for failure. As early as 1931, Rickert and Dixon\textsuperscript{60}
were stressing the importance of root canal obliteration techniques, stat-
ing that after instrumentation, the canal space should be filled completely
with a material well tolerated by the periapical tissue. Their experiments
demonstrated through macroscopic examination, that while sterile solid im-
plants were well tolerated by rabbit subcutaneous connective tissue, sterile
hollow steel and platinum tubes elicited a severe inflammatory reaction around the open ends. These results formed the basis for the hypothesized "Hollow Tube Theory". This theory postulated that voids remaining following obliteration, may become filled with periradicular tissue fluids, isolated from the blood stream, which could then undergo degradation through enzymatic breakdown, even in the absence of microorganisms. These breakdown products could then flow back or diffuse out through the apical foramen into the periapical region producing an inflammatory response. It was realized therefore, that many factors, other than bacteria alone, could be contributory to root canal failures.

Dow and Ingle,\textsuperscript{61} in 1955, stated that the single largest factor in endodontic failures was the poorly obturated root canal. Using radioactive iodine and an autoradiographic technique to trace apical leakage, it was shown that considerable percolation of the isotope occurred from the apex into the canal in poorly obturated samples, while no leakage was evident in the well filled population. Their results tended to support those of Rickert and Dixon and in their conclusion stated that fluid circulating into the interstices of the poorly filled root canal could lead to periapical inflammation. Kalnins, Masin and Kisis\textsuperscript{62} stated that organic matter might pool in the "dead space" of a hollow tube, such as an unfilled or underfilled root canal and subsequently lead to infection.

To determine the rate of success in endodontic therapy, an extensive study was undertaken at the University of Washington Dental School in 1955 by Ingle.\textsuperscript{1} Over twelve hundred patients were recalled for a two year postoperative evaluation demonstrating a success rate approximating 95 percent.
More importantly, the rate of failure was also examined and the causes of said failures investigated. Analysis of these cases disclosed that 63.46 percent of the failures were due to apical percolation, resulting from improper or incomplete obliteration of the root canal space. The next most frequent reason for failure was inadvertant root perforation, accounting for 9.61 percent of the unsuccessful cases. Ingle\textsuperscript{24} reiterated the view of the hollow tube concept, stating that breakdown or lack of a good apical seal invites failure due to apical percolation of periapical fluids with subsequent diffusion stasis into the canal space. Tissue fluid products, i.e. water soluble proteins, enzymes and salts are capable of diffusing through defects in the apical seal, undergo degradative changes and again diffuse out initiating a periapical inflammatory response. These physiochemical products might act as a constant irritant in the periapical region irregardless of the presence or absence of bacteria. Periapical inflammation would persist as long as some noxious stimulus existed.

Goldman and Pearson,\textsuperscript{63} using hollow teflon tube implants, showed that tissue fluids were able to exchange freely through the core without producing inflammatory changes at either open end of the tube. Further doubt regarding the validity of the hollow tube theory has been shed by the studies of Seyle\textsuperscript{64} and Torneck\textsuperscript{65} studying glass and polyethylene tube implants respectively. Both substantiated Goldman and Pearson's results noting little or no inflammation at the open ends of the tubes and in some instances found fibrous tissue bridges growing into or through the tube openings. A later study performed by Torneck,\textsuperscript{66} in 1967, illustrated the inflammatory potential of implanted sterile polyethylene tubes filled with sterile autoclaved
muscle and bacterial contaminated autoclaved muscle. The tissue response in both instances was considerably more severe than that of empty polyethylene tubing, with the most intense reaction found in the bacterial contaminated sampling. In summation, the most favorable conditions found for healing and repair were when the lumen of the tubes were sterile and clean, emphasizing the debridement process.

The above studies were conducted in soft tissues. It remained for Hodosh, Povar and Shklar to implant plastic teeth with holes drilled in them into fresh sockets. Inflammation about the open ends was not evidenced and in some cases the holes were filled with fibrous tissue or bone. They concluded that hollow tubes were not destined to create tissue destruction or even inflammatory changes by virtue of their existence but may in fact be associated with a repair mechanism.

E. RESTORATION MICROLEAKAGE

One of the primary objectives in restorative dentistry is to recapitulate tooth anatomy and function with a proper restorative material and attain a hermetic seal at the tooth/restoration interface. Phillips states that, with the possible exception of the polyacrylic acids, none of the materials used in the restoration of carious lesions actually seal the cavity preparation. A microscopic space always exists between the restoration and the tooth. Fluids, microorganisms and debris from the mouth may penetrate the outer margins of the restoration and progress down the walls of the cavity preparation. This phenomenon is referred to as microleakage. Axim attributes the formation of this space to several factors including the linear coefficients of thermal expansion, modulus of elasticity, material solubility,
volumetric changes of the restorative material, permeability of the involved tooth structure and influence of oral body fluid.

As early as 1929, research was performed as to the efficiency of dental fillings in achieving marginal seals. Fraser, using bacterial cultures, evaluated whether various materials would remain impermeable to bacterial penetration. Copper cements and copper amalgam formed an efficient barrier, while gutta-percha stopping and silicate cements were questionable. Grossman evaluated the adequacy of temporary filling materials in forming a hermetic seal during root canal treatment. Glass capillary tubes were filled at one end with 2-3 mm of each test filling material and suspended in either an aqueous dye, dye colored saliva or a bacterial suspension to determine their permeability or lack thereof. Temporary stopping, base plate gutta-percha, zinc oxyphosphate and zinc oxide-eugenol cements were examined, but only zinc oxide-eugenol was noted to be "leak-proof without exception". Massler and Ostrovsky confirmed the results obtained by Grossman in a separate dye penetration study. Zinc oxide and eugenol and amalgam showed little or no leakage after more than four months. Fischer studied the sealing properties of zinc oxyphosphate, copper and silicate cements, amalgam, inlays and foils and found that all filling materials tested allowed some degree of fluid penetration along the restoration margins.

Nelsen, Wolcott and Paffenbarger demonstrated the role of thermal expansion in causing fluid exchange between tooth structure and dental restoration margins. Simulated cavities were made in extracted teeth, filled with various restorative materials, including amalgam and zinc oxide-eugenol
cement, chilled in ice water for thirty seconds, wiped dry and viewed under a binocular microscope. Droplets of water were found to exude from the margins of the restorations as the teeth were warmed. They concluded that marginal percolation was caused by differences in thermal expansion between the tooth and restoration and by thermal expansion of the fluid occupying the crevice. They computed that a channel ten microns in diameter could develop at the junction of the filling and tooth structure during a cooling cycle. Since the limit of visual acuity is fifty microns, this defect would be imperceptible to the naked eye. Common oral bacteria, bacterial produced acids and oral enzymes are all capable of percolating through these spaces during thermal changes, illustrating the importance of these marginal flaws.

Realizing the necessity of obtaining and preserving as complete a marginal seal as possible, researchers have continued their evaluation of the marginal integrity attained by temporary and permanent restorative materials. The most popular technique continues to be dye penetration, while bacterial, air pressure and radioisotope studies have also been conducted.

F. OBLITERATION

The final objective of endodontic therapy is the total obliteration of the root canal space. Although thorough debridement will reduce or eliminate the presence of protein degradation products, bacteria, bacterial toxins and necrotic tissue, according to Schilder, it is the sealing off of the complex root canal system which ensures the health of the attachment apparatus against periapical breakdown of endodontic origin. Therefore, considerable attention must be given the attempt at achieving a fluid tight
seal at the apical foramen. Ingle stated that "anything short of total obturation is not to be tolerated if a high level of success is to be maintained".24

The same techniques used to investigate restorative marginal seals were being applied to evaluate the adequacy of apical seals in root canal therapy. In an earlier study by Ingle,1 the greatest single cause of endodontic failure was found to be the poorly or incompletely obturated root canal space. Dow and Ingle61 tested the effectiveness of root canal filling methods. Several anterior teeth were studied, half were filled carefully with gutta-percha and sealer using lateral condensation, while the other teeth were filled without great effort. The specimens were coated with sticky wax, leaving the apex uncovered, and then immersed in radioactive iodine for five days. Using an autoradiographic technique, they were able to demonstrate that apical leakage occurred only in poorly obturated teeth. They concluded that this same leakage would ultimately lead to periapical inflammation and endodontic failure.

Nicholls88 states that a poor apical seal may allow for voids in the apical region of the root canal space where stagnation of tissue fluid might occur. Proteolysis and irritation may cause persistence of an existing periapical lesion or formation of a new one. In addition, microorganisms may lodge in such an area during a transient bacteremia according to Grossman.71 Of further consequence is the possibility of microorganisms remaining within the canal following cleansing and shaping. As demonstrated by Shovelton,89 bacteria found in a root canal following pulpal necrosis were located primarily just within the dentin surrounding the canal space.
Mechanical preparation would remove most of the organisms from the tooth, but those existing deep in the dentin would remain, even after vigorous cleansing. Therefore, an inadequate apical seal may expose any persistent bacteria and possibly propagate further periapical disease. Kakehashi et al., 90 were able to show the influence of viable oral organisms on surgically exposed dental pulp tissue. Pulp exposures of the maxillary first molars were performed in both conventional and germ-free laboratory rats and left open to observe the response. Conventional animals generally developed complete pulpal necrosis with chronic inflammatory reactions and apical abscess formation. The germ-free animals, despite the pulpal exposures, indicated minimal inflammatory responses with reparative dentinal bridge formation over the remaining vital pulp tissue. Of significance is the observation of the irritational effect of microorganisms causing a progressive deterioration of the pulpal tissue. The same sequence might occur periapically as bacteria trapped within a canal space become exposed to percolating tissue fluids from the apical region.

Since it was generally agreed that every effort should be made to completely and permanently obturate the root canal space, a further question presented as to the effectiveness of the filling materials and sealers used for that purpose. Marshall and Massler 91 performed a sweeping study investigating the marginal seal attained by various root canal filling techniques and materials. Single cone gutta-percha and silver points were used as filling materials and four sealers: Rickert's, Wach's, Klora Perko NO and Grossman's, were selected for a total of eleven different root canal filling methods. Radioactive isotopes of sulfur, iodine, sodium, phosphorous
and calcium were placed either inside the tooth above the root canal filling or the apex of the tooth immersed in the tracer solution immediately after filling for a period of twenty-four hours to test the root canal seal. Radioactive sulfur was found to produce the sharpest autoradiographic images and had the best marginal penetration along the root canal fillings. Results showed that gutta-percha points with sealer permitted less isotope penetration than silver points with the same sealer. There were only minor differences in efficiency between most of the sealers, with Grossman's permitting slightly greater penetration than the others. Obturating the canal space without sealer did not produce an adequate seal and allowed complete penetration of the isotope in fifty percent of the test population and partial penetration in the remaining samples. It was concluded that sealer is essential for effective root canal obturation and of the sealers tested, little clinical difference in sealing efficiency was found between them. The isotope tracers were found to penetrate equally well from either the apical or coronal direction.

Polar and nonpolar radioactive isotopes were used by Kapsimalis and Evans\textsuperscript{92} to measure sealing properties of commonly used endodontic filling materials. Teeth filled with single silver cones and laterally condensed gutta-percha, both with and without sealer, were immersed in baths of radio-sulfur, tritiated glucose and tritiated proline for forty-eight hours. Those specimens filled with a single silver cone or laterally condensed gutta-percha without sealer all showed gross radioactive isotope leakage, re-emphasizing the need for sealer as previously shown by Marshall and Massler. Of the eight root canal cements tested, only Proco-Sol and AH-26 showed no
leakage. Varied results were obtained with Wach's and Biotech sealers while Kloroperka NO, Diaket, PCA and Kerr all exhibited patterns of leakage.

Grossman, listing eleven qualities of an ideal sealer, noted that the sealer should be impervious to moisture, thereby ensuring a well sealed canal space. Stewart evaluated the permeability of three root canal sealing agents including Kerr, New Grossman's and Diaket. Extracted teeth were prepared and then obturated with either silver points or laterally condensed gutta-percha in conjunction with the various sealers and then immersed in methylene blue dye for six months. Several batches of sealer were also mixed and placed in the dye solution for the same time period. Results showed that methylene blue did penetrate through the dentinal tubules toward the root canal space but no evidence of permeability through the root canal filling materials were noted. The sealer specimens showed a surface penetration of 0.5 mm for both the Kerr and New Grossman's sealers with no permeability seen in the Diaket material. Performing a similar study, McElroy found that Wach's sealer was the least porous of nine materials tested while Chloropercha was the most permeable. Concern was expressed in that if a sealer proved to be porous, tissue fluids and bacteria could freely enter the material and possibly create a source of inflammation or infection.

Schroeder, using 210 formalin-fixed teeth and 90 freshly extracted teeth, took a unique approach to investigate the permeability of various root canal filling materials. Canals were prepared and sealed and then subjected to a dye penetration study using methylene blue, however, some of the specimens were centrifuged with a reservoir of the dye sealed over the crown. Both zinc phosphate cement and gutta-percha sealed with chloropercha
leaked with and without centrifuging while AH-26 and silver amalgam retained their seal even with centrifuging. Diaket showed no leakage before centrifuging but did leak when centrifugal forces were applied.

Ten different sealing agents were investigated by Curson and Kirk\textsuperscript{96} using methylene blue for their dye penetration study. The cements were introduced into clean, dry, glass tubing, the ends of which were then immersed in dye for varying periods of between twenty-four hours to thirty days, at which time the depth of penetration was measured. Results showed that zinc phosphate and Bioxol did not form a satisfactory seal while a number of zinc oxide and eugenol cements, Ricker's sealer, Diaket, Tubliseal and AH-26 performed well. Grossman's new sealer gave a good initial seal but was found to deteriorate over the thirty day period, attributed to possible dimensional changes in the material.

In a further dye penetration study, Grieve and Parkholm\textsuperscript{97} evaluated the sealing ability of eight different sealers used in conjunction with silver points. Kerr's sealer, Diaket A, N2 Normal and Stailine Super all showed minimal leakage patterns while AH-26, Tubliseal and Endomethasone gave somewhat higher leakage values. All were considered however capable of producing satisfactory clinical results. Only Grossman's sealer demonstrated a gross unacceptable leakage pattern.

Messing\textsuperscript{98} studied the sealing properties of chloropercha, Rickert's paste and AH-26 in conjunction with both silver points and gutta-percha from the coronal and apical aspects with conventional and fluorescent dyes. It was concluded that all methods tested provided an adequate seal as long as a careful debridement and filling technique were employed. Yates and
Hembree evaluated N2, Diaket and Tubliseal with either gutta-percha or silver points for a period of one year using an autoradiographic technique with radioactive calcium. In each instance, gutta-percha provided a greater sealing ability than the silver point fill and of the three sealers, Tubliseal performed the best.

Most of the early investigations of various apical sealing techniques used dye penetration, radioisotopes or bacterial methods. These studies have provided a broad measure of agreement but have an inherent possibility of subjective bias. To overcome these methods, a number of objective, low-variable investigative techniques have been introduced.

Jacobson and von Fraunhofer performed a quantitative study in which periapical leakage in a coronal direction was evaluated using an electrochemical technique. Extracted teeth were filled with vertically condensed gutta-percha and Rickert's sealant, allowing for post room. The root surfaces were coated with an inert impermeable medium from the gingival enamel to the apex, leaving the apical 3 mm uncoated to permit functioning of both the apical foramen and any accessory canals. A mild steel rod was placed 2 mm below the cemento-enamel junction and then immersed in an electrolytic solution of one percent potassium chloride completing the galvanic cell. A galvanic corrosion current would only occur once there had been leakage into the root canal space. The time elapsed between immersion and current flow would denote the penetration rate, while the magnitude of the current would indicate the degree of penetration. Current first appeared on the seventh day for one tooth, the eighth day for two teeth and the ninth day for the remaining test teeth. A constant current was not observed until the
eleventh day. Continuing immersion for an additional ten days, the mean current found after twenty-one days was 2.0 microamperes showing a tenfold increase over the initial current density. Significance of the magnitude of the current was not immediately apparent to the investigators, but the method permits an accurate detection of the onset of leakage.

Another quantitative effort was made by Ainley using Rhodamine B dye for a fluorescent assay. Single-rooted anterior teeth were debrided and filled with gutta-percha and Diaket sealer and both sealed and unsealed split silver points. The coronal half of each canal was grossly enlarged as a dye reservoir. Samples were suspended in 5 ml of distilled water and analyzed for microleakage of dye on a fluorometer after forty-eight hours and again after two weeks. The samples were then centrifuged for thirty minutes to determine if increased intracanal fluid pressure would result in additional leakage. Gutta-percha and Diaket had the lowest mean leakage values while the unsealed silver cones had the highest. Approximately half the total leakage occurred within the first forty-eight hours, while centrifuging resulted in slight increases in leakage when sealer was employed and much greater leakage values in the unsealed groups.

Attempting to achieve an effective apical seal often produces varying and conflicting evidence which can only lead to the conclusion that no method or material is effective in all cases. This has stimulated research into the area of alternatives to the classical obturating materials. The use of injectable materials may enable complete obturation of irregularities found in the root canal system.

Spalding and Senia compared the sealing ability of four types of
syringeable paste filling materials to that of laterally condensed gutta-percha with Tancredi sealant. PCA sealer, zinc oxide-eugenol, Endo Fill and Hydron specimens were immersed in methylene blue dye for seven days to investigate the material permeabilities. Only Hydron was shown to have a significantly better sealing ability than laterally condensed gutta-percha. In an isotope study of Hydron, Rhome et al.\textsuperscript{103} using radioactively labeled carbon human serum albumin, evaluated the apical seal attained by laterally and vertically condensed gutta-percha with Grossman's sealer relating to that of Hydron. Radioactive carbon was injected into each canal and the amount of penetration of the isotope into the suspension media noted over a six month period. Hydron showed a significantly greater amount of leakage in the range of 30-40 percent as compared to between 10-17 percent for laterally and vertically condensed gutta-percha. Murrin et al.\textsuperscript{104} again found Hydron to be significantly more permeable than laterally condensed gutta-percha with Grossman's sealer.

Jones\textsuperscript{105} investigated the use of Silastic, a silicone rubber polymer, as an injectable root canal obturating material. Thirty-five single-rooted teeth were prepared endodontically, twenty-eight were filled with injected Silastic while seven controls were obturated with gutta-percha and Proco-Sol sealer with either vertical or lateral condensation. The specimens were then suspended in radiosulfur, sectioned and autoradiographed. Both experimental and control groups evidenced leakage with neither significantly better than the other. Using Silastic as a sealant in concert with gutta-percha or silver points as a core material was investigated by Nathanson et al.\textsuperscript{106} The penetration of radioactive sulfur as determined by
autoradiographs was found to be significantly less with Silastic acting as a sealer than those results obtained with Grossman's sealer.

Investigating the possibility of using a dentinal bonding agent as a sealant, Zidan and El Deeb\textsuperscript{107} compared Tubliseal and Scotchbond dentinal bonding agent in a dye penetration study. Instrumented teeth were obturated in both instances with laterally condensed gutta-percha, the only difference being the sealer used. The quality of the apical seal achieved by Scotchbond was significantly better than that of Tubliseal, supposedly due to the primary bonding between Scotchbond and the inner canal walls.

Another new technique for obturation is that of injection molded thermoplasticized gutta-percha. El Deeb\textsuperscript{108} evaluated the sealing ability of injection molded thermoplasticized gutta-percha both with and without sealer and laterally condensed gutta-percha with sealer. Performing a dye penetration study, it was concluded that sealer was the significant factor determining whether leakage occurred or not. There were no differences in leakage patterns obtained between laterally condensed and thermoplasticized gutta-percha provided a sealer was used. Significantly greater leakage patterns developed when sealer was not used. When tested for leakage with radioisotopes, Czonstkowsky et al.\textsuperscript{109} found that injected thermoplasticized low-temperature gutta-percha created an apical seal comparable to that of laterally condensed gutta-percha when both methods were used in conjunction with sealer. In a scanning electron microscopic investigation, Michanowicz et al.\textsuperscript{110} evaluated the adaptation of low-temperature injected gutta-percha to the dentinal walls of the root canal space. The low-temperature gutta-percha technique when used without sealer was shown to reproduce the shape
and irregularities of the root canal walls. Gutta-percha projections were even noted extending into the dentinal tubules, though this finding was only apparent in the middle and coronal thirds of the root canal. Teeth obturated with the low-temperature injection technique with sealer also reproduced microscopic irregularities of the root canal walls but neither sealer nor gutta-percha was observed projecting into the dentinal tubules. Rather the sealer appeared as a homogeneous layer between the gutta-percha and the dentin. Laterally condensed gutta-percha with sealer was found closely adapted to the root canal walls but no microscopic ridges or other surface features could be demonstrated. It was concluded that low-temperature injected gutta-percha with sealer could obturate the root canal system as well or better than laterally condensed gutta-percha with sealer.

Performing an ink penetration study, Evans and Simon\textsuperscript{111} demonstrated that injected thermoplasticized gutta-percha does not provide an adequate apical seal when used without a root canal sealer. An effective seal was obtained with either laterally condensed or injected thermoplasticized gutta-percha only when a sealer was used.
CHAPTER III

MATERIALS AND METHODS

Histologic evaluation of periradicular human tissue following endodontic therapy, with other than limited surgical specimens, is virtually impossible. Therefore, research conducted to further our knowledge and understanding necessitates the use of animal experimentation. Animal models are utilized in an attempt to simulate, as closely as possible, true clinical conditions.

Primate research, although closest in evolutionary standards to man, remains cost inhibitive, and handling difficulties exist. These factors can be minimized using smaller animals, i.e. rats, guinea pigs or rabbits. For these reasons, rodents have frequently been selected for endodontic research. These animals, however, are not ideal in that access to the pulp chambers proves difficult at best. In addition to the size and position of the teeth, molar root anatomy also severely limits the range of endodontic procedures available. Thus, larger animals would be better suited, providing they possessed a dentition similar to that of humans, were readily available at a moderate cost and were easily maintained. Beagle dogs were chosen for this study having matched the above qualifications. Beagles also exhibit an even temperament, adapt well to a kennel environment and have an excellent disposition making special handling and restraints unnecessary.112

Dogs possess three incisors, a canine and seven cheek teeth per
mandibular quadrant. The seven cheek teeth consist of four premolars and three molars. The five middle cheek teeth all have two roots (mesial and distal), with the first molar (fifth cheek tooth) being the largest. The canal spaces are sufficiently wide to allow instrumentation to the apex. The pulp chamber consists of a prominent central horn with smaller mesial and distal horns. The pulp tissue is found to be morphologically similar to that of the human dental pulp. The dog root apex, however, does have a distinctive anatomy. The main root canal terminates in a complexity of fine peripherally radiating canals, all confined to the cementum. The mandibular second, third and fourth premolars are readily accessible and have been recommended for endodontic research by Barker and Lockett.\textsuperscript{113} This study utilized the second, third and fourth mandibular premolars and the mandibular first molars.

Four adult Beagle type dogs, of approximately two years of age, were selected for this study. The animals were procured by the Animal Research Facility (ARF) at the Loyola University Medical Center. Upon their arrival at ARF, the dogs were inoculated and observed for a minimum of ten days to ensure their health and suitability as research animals. The dogs weighed between 7.5 and 11.5 kilograms and were identified by numbered collar tags. These tags were tied around their necks and the numbers thereafter recorded on all experimental data that pertained to each animal. The dogs were kept in separate cages and fed and cared for by personnel at ARF.

A. ANESTHETIC PROCEDURE

The experimental design required two surgical procedures per dog, scheduled at two week intervals. Twelve hours prior to any surgical
procedure, the dog was not fed and water was withheld to prevent complications during general anesthesia. General anesthesia was attained via intravenous injection of sodium pentobarbital* into the cephalic vein, being the only superficial vein of the thoracic limb. The third plane of stage three anesthesia (stage of surgical anesthesia) was attained, at which time autonomic breathing still occurs, but the respiratory rate increases as the depth of respiration decreases with a noticeable pause interceding between inspiration and expiration. The eyeballs become central and the pedal reflex (retraction of the limb when the web between the digits is pinched) disappears. One cubic centimeter (cc) of sodium pentobarbital was administered per two kilograms of body weight. According to the manufacturer, one cc contains 65 milligrams (mg) of the barbiturate. Sodium pentobarbital is a long acting barbiturate, whose principal action is depression of the central nervous system. The drug rapidly reaches the central nervous system and its effects become apparent within thirty seconds of injection, with surgical anesthesia generally attained within two to four minutes. Anesthetic induction was uncomplicated in all cases. Anesthesia was supplemented during the surgical procedure by additional one cc doses as required. After induction, 2 cc of atropine sulfate** (concentration of 0.5 mg/ml) were also administered subcutaneously to inhibit salivary flow. Atropine sulfate inhibits transmission of post ganglionic cholinergic nerve impulses, having its primary effect upon the heart and salivary

* W.A. Butler Co., Columbus, Ohio
** Wyeth Laboratories, Philadelphia, Pennsylvania
Having attained surgical anesthesia, the dog was secured to the operating table using surgical tape, to facilitate operative and radiographic procedures. The mandible was retracted utilizing a spring-loaded device attached to the maxillary and mandibular canines on the side opposite the surgical area. The mandibular second, third and fourth premolars and the first molar were selected for experimental purposes in each quadrant, allowing for a total of eight teeth per dog, with each tooth possessing a mesial and distal root.

B. RADIOGRAPHIC PROCEDURE

Preoperative radiographs were taken of the experimental and control teeth to evaluate canal configuration, patency and preoperative periapical anatomy. Kodak ultraspeed single exposure radiographic film packets* were held in place utilizing a hemostat and modeling clay (Fig. 1). X-rays were taken with a portable hand-held General Electric X-ray generator supplying 60 KVP at 20 ma with an exposure time of 0.2 seconds (Fig. 2). Exposed radiographs were developed in a portable dark box equipped with rapid developer and fixer**, allowing viewing of the radiographs within three minutes.

C. OPERATIVE PROCEDURE

Due to the xerostomia produced by combination of the general

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* Kodak DF-58, Eastman Kodak Company, Rochester, New York
** Insta-Neg and Insta-Fix, Micro-Copy, Buffalo, New York
anesthesia and the atropine sulfate, a rubber dam was not required. The teeth operated on were isolated with 4 x 4 inch cotton gauze pads, both buccally and lingually.

Eight teeth per animal were selected for root canal therapy, divided into paired samples: four experimental and four control teeth, each quadrant containing two experimental and two control teeth respectively, being adjacently alternated (Table 1). Both control and experimental teeth were treated identically during the first experimental session, with the exception of one control tooth per animal. A single canal in a randomly selected control tooth in each animal was instrumented and left unobliterated, sealing the access as will be discussed later. This was done to determine the effects of instrumentation alone on the periapical tissue. A control involving the instrumentation of a tooth without obliteration and its direct exposure to the oral environment was not provided for since this has been adequately documented in the literature as causing formation of periapical pathosis.116

Initial access into each tooth was made with a heatless stone, using a low speed handpiece powered by a carbon dioxide cannister (Fig. 3). The occlusal surface was reduced until the mesial, central and distal pulp horns were exposed. The remainder of the pulp chamber roof was then removed with a number four round bur, sufficiently opening the chamber for direct access and instrumentation.

Initial lengths were determined with 25 mm standard K-type files*

* Union Broach Company, Inc., Long Island City, New York
equipped with silicone stops. The files were inserted into each canal with a vaiven motion until a definite apical stop was reached. This apical position was easily perceived tactilely due to the unique apical morphology present in the dog root apex. Radiographs were taken to confirm the proper apical position of the initial file lengths. The canals were then prepared using a circumferential filing action, aided by copious amounts of 5.25% sodium hypochlorite irrigation as originally recommended by Grossman. \cite{117} Master apical file (MAF) lengths were again verified radiographically (Fig. 4). Upon completion of the cleansing and shaping process, each canal was thoroughly irrigated and then dried with paper points.

General anesthesia always carries a risk factor in that the vital functions of the subject may not respond favorably to the induction process. To minimize the potential risks, it was decided to perform one-appointment endodontic therapy and complete the root canal treatment in one operative session. Soltanoff, \cite{118} Oliet \cite{119} and Pekruhn, \cite{120} evaluating the incidence of failure following single-visit endodontics, found comparable failure rates between single and multiple-visit cases.

Each canal, with the exception of one control canal per animal, was immediately obliterated following biomechanical preparation. Non-staining Proco-Sol sealer* (primarily a zinc oxide and eugenol sealer) was introduced along the canal walls with a K-type file one size smaller than the MAF and with the master cone. The canals were obliterated with Kerr gutta-percha**

\* Star Dental, Valley Forge, Pennsylvania
\** Sybron/Kerr, Romulus, Michigan
performed with a lateral condensation technique. Post room was prepared in each canal by warming a hand Luks plugger (Fig. 5), retaining approximately three millimeters of gutta-percha to seal the apex of each canal. Kwan and Harrington\textsuperscript{121} have shown that immediate post room preparation with a heated instrument, following obliteration, had no significant effect on the apical seal. Neagley,\textsuperscript{122} investigating the effect of dowel space preparation when varying amounts of filling material remained sealing the apical region, demonstrated that laterally condensed gutta-percha showed no trend toward increased leakage over the control population, even when dowel space was made to a depth of within 4 mm from the apex. Successive K-type files were introduced to clear the post room space until a size 100 file was reached. A dry cotton pellet was placed in the chamber and an IRM temporary* inserted. The experimental animal was then returned to its cage.

The second operative session was performed exactly two weeks after the first surgical session. This time span was required to allow the Proco-Sol sealer sufficient time to set and more accurately reproduce a true clinical situation. The experimental animal was again anesthetized following the same induction technique. The IRM temporary fillings were removed from both the control and experimental teeth. Control teeth were sealed with a dry cotton pellet and an occlusal silver amalgam** while the experimental teeth were allowed to remain open to the oral environment (Fig. 6).

\begin{itemize}
\item [*] IRM, L.W. Caulk Company, Milford, Delaware
\item [**] Tytin, S.S. White, Philadelphia, Pennsylvania
\end{itemize}
D. SACRIFICE PROCEDURE

Three dogs were sacrificed after six months and one dog after one year through intravenous injection of Beuthanasia-D*. The active ingredients of this preparation are sodium pentobarbital (195 mg/ml) and sodium phenytoin (25 mg/ml), with a recommended dosage of 1 ml/2 kg. The body of the mandible was quickly dissected, the segments sectioned with a reciprocating surgical saw and immediately submerged into separately labelled jars containing approximately 500 cc of 10% neutral buffered formalin solution for 21 days. After an initial period of fixation, the buccal and lingual cortical plates were reduced with an acrylic bur in a lowspeed handpiece. The specimens were then replaced in 10% neutral buffered formalin for an additional 21 days after which time they were rinsed for twenty-four hours under running water.

E. HISTOLOGIC PREPARATION AND EVALUATION

The specimens were decalcified by placing 5% formic acid in each of the labelled jars. After sufficient decalcification had occurred, the specimens were further trimmed with a razor blade into blocks containing individual roots and their associated periradicular tissue. These specimens were randomly coded and recorded, wrapped in a 2 x 2 inch gauze along with the corresponding label and immersed in a large container of 5% formic acid for further decalcification.

Following decalcification, the blocks were rinsed in running water for six hours and placed in increasing concentrations of alcohol over a

* Burns-Biotech Laboratory, Chromalloy Pharmaceutical Inc., Oakland, CA.
two day period. The blocks were cleared in xylol and embedded in paraffin. The block segments were cut parallel with the long axis of the tooth in sections of six microns with each twentieth section mounted on a slide, de-paraffinated and stained with hematoxylin and eosin for light microscopic examination. A histologic evaluation of the periapical regions of the experimental and control teeth was then conducted.

Each histologic section was examined under light microscopy by three evaluators, assessing the degree of periapical inflammation in a manner similar to that used by Guttuso,123 Rappaport et al.,124 and Deemer and Tsaknis.125 The periapical inflammatory response was arbitrarily classified as either normal (score=0), minimal (score=1), mild (score=2), moderate (score=3) or severe (score=4) depending on the number and type of inflammatory cells in the periapical region, the presence or absence of a fibrous connective tissue capsule and the extent of vascularity and osteolytic activity. Photomicrographs of the varying degrees of inflammation were reviewed by each evaluator, prior to examining the slides to establish interrater reliability (Fig. 7, 9, 10, 11 and 12).

F. RADIOGRAPHIC EVALUATION

Preoperative and postoperative radiographs of both experimental and control teeth were projected on a viewing screen and compared by three evaluators concurrently and a consensus reached as to whether the periapical region had remained normal radiographically (-) or had developed a periapical radiolucency (+).
G. STATISTICAL ANALYSIS

Following microscopic observation by each of the three evaluators, a mean periapical histologic score was calculated for each root of each tooth. These mean scores were then combined to determine the mean experimental and mean control values per dog for comparison. The periapical histologic score of the single unfilled control root per dog was not included in the computation of the control value calculated for each dog. The mean experimental, mean control and mean unfilled control values were also computed for all four dogs. Due to the small sample size, descriptive statistics were employed to evaluate the results.

A contingency table was constructed to determine whether a relationship existed between the radiographic images and the degree of inflammation noted histologically.
CHAPTER IV

RESULTS

All the animals were reexamined at varying intervals from the time of operation to the time of sacrifice, and remained in good physical condition. Neither the experimental procedure nor the anesthesia seemed to create any adverse effects.

Preoperatively, all dogs were examined and found to have intact and caries free teeth except for dog #4499, which was missing the mandibular left second premolar. Periodontal characteristics were considered within-normal-limits and noncontributory to the experimental results.

Radiographic evaluation preoperatively, revealed a normal bony pattern with no evidence of any periapical pathology. All the obliterated canals appeared adequately filled upon radiographic evaluation, with three to four millimeters of gutta-percha and sealer. Due to the unique apical anatomic structure, all canals were filled to the apical terminus, with no instances of short or overextended filling material.

Dog #4450

This dog was a male weighing 10.5 kg. The experimental period was one year. The experimental teeth were the mandibular left second (LL2) and fourth (LL4) premolars and the right third premolar (LR3) and first molar (LR1). The control teeth were the mandibular left third premolar (LL3) and first molar (LL1) and the right second (LR2) and fourth (LR4) premolars.

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The mesial root of LR4 (LR4-mesial) served as the unfilled control root (Table 1).

Clinical Findings

There was no clinical evidence of periapical pathosis.

Radiographic Findings

A slight periapical radiolucency with poorly defined borders was found in the apical region of LL1-mesial, a moderately sized periapical radiolucency with poorly defined borders was located at the apex of LL4-distal and a large periapical rarefaction was associated with LR4-mesial having a well-defined border (Table 3).

Histologic Findings

Two control teeth, LL3-distal and LL1-distal did not show any inflammatory changes in the periapical region. Two control teeth, LR3-mesial and LL1-mesial displayed minimal inflammatory changes while LR2 demonstrated a mild periapical inflammatory reaction. Control tooth LR4-distal, which was filled with gutta-percha and sealer, showed no inflammatory changes while the unfilled control root, LR4-mesial, had a moderate inflammatory reaction. Experimental teeth LL2-distal and LR3 all appeared normal histologically while experimental tooth LR1 displayed minimal inflammation of the periapical region. Experimental teeth LL4 and LL2-mesial showed a mild degree of periapical inflammation (Table 3).

Dog #4451

This dog was a male, weighing 11.5 kg. The experimental period was six months. The experimental teeth were the mandibular left third premolar
(LL3), left first molar (LL1) and the right second and fourth premolars (LR2 and LR4 respectively). The control teeth were the mandibular left second (LL2) and fourth (LL4) premolars and the right third premolar (LR3) and first molar (LR1). The mesial root of control tooth LL2 (LL2-mesial) was left unfilled after cleansing and shaping (Table 1).

Clinical Findings

There was no clinical evidence of periapical pathosis.

Radiographic Findings

Slight periapical radiolucencies were noted in the apical regions of LL3, LL4-mesial, LL1, LR3, LR4 and LR1 with either poorly defined borders or fair definition to the periapical radiolucencies (Table 3).

Histologic Findings

The periapical region of control tooth LL4-mesial had a minimal periapical inflammatory response histologically, while LL4-distal had a severe inflammatory periapical reaction. Control tooth LR3 demonstrated moderate periapical inflammation, while LR1 showed moderate to severe inflammatory changes. Control tooth LL2-distal showed a moderate periapical response in the obliterated root, while the unfilled control root (LL2-mesial) displayed mild inflammatory changes. Experimental tooth LR4 had a severe inflammatory reaction histologically while LL1 demonstrated a moderate inflammatory response. Tooth LR2-mesial had mild to moderate inflammatory changes, while LR2-distal showed minimal histologic changes. Tooth LL3 evidenced mild inflammatory changes microscopically (Table 3).
Dog #4582

This dog was a male weighing 10.0 kg. The experimental period was six months. The experimental teeth were the mandibular left second (LL2) and fourth (LL4) premolars and the right third premolar (LR3) and first molar (LR1). Control teeth included the mandibular left third premolar (LL3) and first molar (LL1) and the mandibular right second (LR2) and fourth (LR4) premolars. The mesial root of LL3 (LL3-mesial) remained unfilled following the cleansing and shaping procedure (Table 1).

Clinical Findings

There was no clinical evidence of periapical pathosis.

Radiographic Findings

Slight periapical radiolucencies were found associated with the root apices of LR1, LR4-distal, LL4-mesial and LL3-mesial whose borders were either of fair or poor definition (Table 3).

Histologic Findings

All experimental teeth, with the exception of LL2-mesial and LR3-distal, demonstrated signs of minimal periapical inflammation. LL2-mesial and LR3-distal showed signs of a normal periapical histologic pattern. Control tooth LR2 was also normal histologically. Control teeth LL1 and LR4 had minimal to mild inflammatory changes evident periapically. Control tooth LL3-distal demonstrated a normal periapical pattern, while the unfilled control root, LL3-mesial, had minimal to mild inflammatory changes (Table 3).

Dog #4499

This male beagle weighed 7.5 kg. The experimental period was six
months. The mandibular left second premolar was found to be missing and thereby excluded from experimentation. The experimental teeth were the mandibular left third premolar (LL3) and first molar (LL1) and the mandibular right second (LR2) and fourth (LR4) premolars. Control teeth were the mandibular left fourth premolar (LL4) and the mandibular right third premolar (LR3) and first molar (LR1). The mesial root of LR1 was chosen as the unfilled control root (Table 1).

Clinical Findings

There was no clinical evidence of periapical pathosis.

Radiographic Findings

Moderately sized periapical radiolucencies were found associated with the root apices of LR4 and LL4, both exhibiting fairly well defined borders. Apices of LL1-mesial, LR1-mesial and LR3-distal all showed evidence of slight periapical radiolucencies with either poor or fair definition to the radiolucent borders (Table 3).

Histologic Findings

The periapical region of experimental tooth LR2-distal appeared normal histologically. Experimental teeth LL3, LR2-mesial and LR4-distal had minimal periapical inflammation present histologically, while tooth LR4-mesial demonstrated a minimal to mild periapical inflammatory reaction. Experimental tooth LL1 had a mild to moderate inflammatory response in the periapical region of the distal root (LL1-distal), while the mesial root (LL1-mesial) displayed a mild inflammatory reaction. Control tooth LR3 showed minimal inflammatory changes while LR4 showed a mild to moderate
inflammatory reaction. Control tooth LR1-distal, which was filled with gutta-percha and sealer, evidenced only minimal inflammatory changes, while the unfilled control root, LR1-mesial, appeared normal histologically. Control tooth LL2 was missing (Table 3).

Statistical Results: Histologic

Histologic scores from all three evaluators are listed in Table 2. The graded inflammatory response for individual roots ranged in value from 0 to 4 in both experimental and control teeth. The histologic values for each experimental root were averaged per dog and ranged from 0.66 to 2.70 with two intermediate values of 1.08 and a mean score of 1.38 for all four dogs (Table 4). Histologic values were averaged for all control roots per dog, filled with gutta-percha and sealer, ranging from 0.66 to 3.09 with intermediate scores of 0.71 and 1.20 and a mean score of 1.43 for all four dogs (Table 4). The average histologic score for the single unfilled control root per dog ranged from 0 to 3.33 with an overall mean value of 1.66 (Table 4). Results for each individual dog and averaged values for all four dogs were plotted on a bar graph (Table 5) for comparative purposes.

Statistical Results: Radiographic

A contingency table was constructed (Table 6), plotting the 62 histologic specimens according to the presence or absence of radiographic periapical changes and the degree of histologic variation. Periapical radiolucencies developed in 26 (42%) of the 62 specimens, while 36 (58%) root apices appeared normal radiographically. Only one of the root apices associated with a periapical radiolucency showed a normal histologic pattern,
while two of the apices demonstrated minimal inflammatory changes. Twenty-three of the twenty-six specimens with periapical radiolucencies showed a mild to severe inflammatory reaction histologically. Thirty-six specimens had no evidence of any periapical pathology radiographically of which 12 (33.3%) specimens appeared normal histologically. Another 22 (61.1%) specimens had only a minimal to mild inflammatory reaction, while 2 (5.6%) specimens showed moderate to severe inflammatory changes.
Table 1
Teeth Used in Study and Experimental Periods

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</tr>
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<td>R</td>
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<td></td>
<td>LR1 LR4 LR3 LR2</td>
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<td></td>
<td></td>
<td>C E C E</td>
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<td>C E C E</td>
</tr>
<tr>
<td></td>
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</table>

LR mandibular right
LL mandibular left
C control - sealed with silver amalgam
E experimental - left open to oral environment
* control tooth with one canal unobliterated
X missing tooth
### Table 2

**Histologic Scores for Evaluator #1, Evaluator #2 and Evaluator #3**

Periapical Reaction - Histologic

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</table>

C control
E experimental
* control with unfilled root
X missing tooth

0 no inflammation
1 minimal inflammation
2 mild inflammation
3 moderate inflammation
4 severe inflammation
### Table 3

Averaged Histologic Scores and Periapical Radiographic Evaluation

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<th>Dog Number/Period to Sacrifice</th>
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</table>

- **C**: control
- **E**: experimental
- ***: control with unfilled root
- **X**: missing tooth

Periapical Radiolucentcy:
- **Size:** Sl slight
- **Definition:** Poor Mod moderate Fair Lg large Well
<table>
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<tr>
<th>Dog Number</th>
<th>Experimental Teeth (E)</th>
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<th>Control Teeth (C*)</th>
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<td><strong>Average</strong></td>
<td><strong>1.38</strong></td>
<td><strong>1.43</strong></td>
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<td>#1 to #4</td>
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Table 5

Bar Graph - Averaged Histologic Scores

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E Experimental
C Control
C* Control without fill
Table 6

Contingency Table - Radiographic versus Histologic

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CHAPTER V
DISCUSSION

Success in endodontic therapy is heavily dependent upon as thorough a debridement process as possible, followed by obliteration of the root canal space to attain an apical seal. Failure of this seal, for whatever reason, may result in periapical inflammation, infection, symptomology or even loss of the tooth.

The literature is replete with studies substantiating the presence of marginal percolation, microleakage, fluid exchange or diffusion and capillary penetration with a majority of presently used restorative materials. The same phenomenon has been investigated as applied to the apical seal achieved during root canal therapy. Depending upon the article reviewed, different sealers will have different properties and sealing abilities attributed to them, with no one particular sealer repeatedly selected as ideal. The only point of general consensus appears to be that some form of sealer is required to effect an apical seal. As stated by Marshall and Massler, "the skill of the operator is perhaps more important to successful obturation than the materials used".

Periapical problems can still arise, even through our best efforts. Breakdown of, or failure to even attain an apical seal would open a pathway for fluid and particle interchange between the periapical region and the canal interstices. Ingle speculated that a constantly circulating periapical
exudate could percolate through apical defects into existing intracanal interstices. Fluid and fluid products, trapped within these canal spaces, could undergo degradation and slowly diffuse back into the apical region. These enzymatic breakdown products might act as periapical irritants, causing persistence of an existing periapical lesion or formation of new pathology. This sequence of events, proposed by Ingle, seems to parallel Rickert and Dixon's Hollow Tube Theory, later disproved by Torneck using sterile polyethylene hollow tube implants. Torneck demonstrated that even though a serous type of fluid may fill the lumen of hollow tube implants, their fluid would not induce inflammatory changes at the open ends of the tubes.

Grossman alludes to the possibility of anachoresis in that microorganisms present during a transient bacteremia might be transported to an inflamed periapical region and become lodged within unfilled portions of the canal system. Of equal or greater importance, as discussed by Shovelton, are those bacteria remaining within the confines of the canal spaces and dentinal tubules following the cleansing and shaping process. Nicholls states that it is feasible for organisms remaining in the deeper parts of the dentinal tubules to grow into unfilled canal spaces. In addition, numerous studies have shown the inability of the debridement process to totally eliminate pulpal remnants. The combination of bacteria, bacterial products, vital and necrotic pulpal debris, all harbored within the root canal system can suddenly become a significant factor in whether or not an endodontic case succeeds. Failure of the apical seal may allow fluid percolation into unfilled canal interstices with subsequent diffusion of
bacteria and bacterial products and tissue products into the periapical region. Conceding that a hermetic seal may not be possible, Ainley postulated the existence of a "critical leakage factor" beyond which biologic repair would not occur. Realizing the infinite number of variables involved in successful root canal therapy, the amount of apical leakage that would have a detrimental effect on healing is purely speculative. The fact that seemingly poorly performed root canal therapy does succeed periodically, can be viewed as a tribute to the healing capacities of the human organism.

Another potential source of periradicular and/or pulpal inflammation is the existence of lateral canals. Rubach and Mitchell and Seltzer et al. investigating human teeth, demonstrated a profusion of lateral canals in the roots and furcation regions of posterior teeth and occasionally in anterior teeth. A complete absence of lateral canals was noted in the present investigation. Histologically, the tissue within lateral canals has been found to be confluent with that of pulp tissue, consisting of capillaries, pulp cells, ground substance and fibers. Simring and Goldberg suggested that lateral canals may act as channels for pathogenic materials to pass either pulpally or towards the periodontium. Their significance as channels of communication may outweigh their importance as harbors of necrotic material. Severely inflamed or necrotic pulp tissue and their toxic products, may emanate through a lateral canal, causing an inflammatory response of the periodontal ligament. Conversely, deep periodontal lesions may expose lateral canals, thereby interfering with the nutritional supply of the pulp. Inflammation, degeneration or even necrosis of an otherwise normal pulp might result.
Due to the form and structure of the root canal system, deliberate debridement and obliteration of a lateral canal is essentially impossible. Weine states that a higher percentage of root canal failures would be seen if the cleaning and filling of lateral canals were critical in attaining success.\textsuperscript{130} Weine further warns that improper sealing of a prepared post room space, or leaving the area vacant for too long, may invite breakdown of the periodontium if a lateral canal of significant size is present.\textsuperscript{130}

Minimizing endodontic failures by assuring the integrity of the apical seal became a focal point for endodontic research. Numerous studies evaluated the apical seal from both an apical and coronal approach, but considered breakdown of the apical seal as critical only from the apical direction in clinical situations. Loss of the occlusal seal seemed inconsequential to the clinical prognosis following completion of root canal therapy. Bhaskar and Rappaport noted in an eighteen month histologic study on dogs that once a canal space had been debrided and an effective occlusal seal placed, an existing periapical radiolucency would cease to enlarge.\textsuperscript{116} Marshall and Massler suspected that a good occlusal seal would improve the apical marginal seal of root canal fillings.\textsuperscript{91} Results illustrated, however, that the degree of apical sealing ability was not altered by an effective occlusal seal. They concluded that a good occlusal seal was essential only during treatment, prior to obliteration.

The rationale of having an occlusal seal during root canal therapy is understood, but the importance of that seal after treatment must be examined. Marshall and Massler demonstrated that a good occlusal seal would not prevent periapical leakage, therefore, it seems reasonable to assume
that a good periapical seal would not prevent occlusal leakage. Ainley stated that "the method of obturating the apical 5 mm may not be critical for biologic success; rather, proper debridement and the sealing off of the periapical tissues from the oral fluids are probably more important". Loss of the occlusal seal could possibly lead to periapical breakdown. When subjected to periapical investigative conditions, research has shown the endodontic filling to be as effective from the coronal aspect as it is apically in sealing ability. The types of contaminants introduced, however, from the oral cavity vary from those found periapically. The purpose of this study was to determine if loss of a coronal seal, with the introduction of saliva and oral organisms, would adversely affect the apical seal and eventual prognosis of a tooth following root canal therapy.

A histologic approach was selected to determine whether the periapical tissues would undergo any inflammatory changes following loss of an occlusal seal after root canal therapy. Sinai et. al. evaluated the histologic reaction of apical pulpal and periapical tissues after partial pulpectomies were performed on 24 Rhesus monkey teeth. An acute inflammatory response was noted for a period of up to one month, followed by a repair process. A total pulpectomy, even in cases of normal pulpal vitality, will generate a certain degree of periapical inflammation. To allow the initial inflammatory reaction to subside, and sufficient clinical exposure for the experimental teeth to the oral environment, research periods of six months and one year were chosen.

Histologic grading was performed by three different evaluators using a scale of 0 to 4 to rank the degree of periapical inflammation. The
scores corresponded to inflammatory responses of none, minimal, mild, moderate and severe respectively. No definitive boundaries between these categories exist, but each evaluator viewed photo-micrographs of the varying degrees of inflammation, prior to examining the slides, to establish interrater reliability. The microscopic slides were then compared and ranked on a relative basis.

To minimize experimental bias, all histologic specimens were randomly coded so microscopic evaluation was performed without insight as to whether the sample was an experimental or control tooth. Results were tabulated and matched with their code numbers only after all three evaluators had viewed the specimens independently.

Results of this research project indicate that loss of an occlusal seal, following root canal therapy, should have little if any effect on the periapical region for a period of six months to one year. Although minor differences existed in the degree of histologically reported periapical inflammation between experimental and control teeth in each dog, averaged values for all four animals (histologic experimental value=1.38 vs. histologic control value=1.43) demonstrated an insignificant variance. These observations substantiate the ability of an endodontic obliteration technique to seal in both a coronal and apical direction. There appeared, however, to be an increased tendency towards periapical inflammation in those specimens left unobliterated, even though the occlusal access was sealed (histologic unobliterated control value=1.66). This observation gives credence to the importance of obturating the root canal space after the debridement process has been completed. Placement of an occlusal seal, in
itself, after cleansing and shaping, will not produce a situation conducive to permanent healing.

Histologic evaluation of the specimens was complicated by the fact that cell nuclei did not stain well with hematoxylin. Resected sections of the mandible were immediately placed in 10% formalin after sacrifice, but the size of the specimens and density of the bone may have prohibited proper fixation. Radiographs were taken of the resected mandibles several days following sacrifice, after which the cortical plates were reduced prior to decalcification. During this time period, cellular detail may have been damaged due to the slow diffusion of the fixative. Smaller sections of the mandible were not made until much later when sufficient decalcification had occurred. Another factor possibly affecting the cellular staining was the lengthy decalcification process. Optimally, a weak organic acid is used to decalcify the hard tissues before sectioning and staining. The specimens were immersed in separate jars containing 5% formic acid for five to six months. The volume of formic acid limited the decalcification process, prolonging the interval until separate sections could be made of individual roots and their associated periapical tissues. Excessive exposure to the formic acid may have damaged the intracellular structures, preventing proper uptake of the dye. In retrospect, better results may have been obtained with a more rapid removal of the cortical plates to reduce the amount of calcified structures and usage of 10% formic acid in larger quantities to speed the decalcification process. Regardless of these problems, inflammatory cells were easily seen microscopically, but identification of individual cell types normally seen in a periapical inflammatory reaction was not
always possible.

Reaction of the periapical region to loss of the occlusal seal was also evaluated radiographically. Preoperative radiographs were compared to those taken post-sacrifice to determine whether periapical radiographic changes had occurred. Due to the high inconsistency found by Goldman et al. in individual interpretation of radiographic images, three evaluators viewed projected preoperative and post-sacrifice radiographs concurrently, reaching a consensus of opinion. A contingency table was then constructed to examine the relationship between the radiographic image and the degree of histologically noted inflammation, if any. Results indicated the existence of a trend towards development of periapical rarefactions in instances of more severe periapical inflammation and a lesser tendency for radiographic change in cases of minimal inflammatory reaction. Histologically, mild to severe periapical inflammatory reactions were found in 88.5% of the root apices associated with periapical rarefactions, while 77.8% of the apices with normal radiographic patterns had no periapical inflammation or minimal to mild inflammatory responses. Separate contingency tables for control and experimental teeth respectively were not included due to a statistical similarity with the combined table.

Bender et al. proposed the lack of correlation between histologic findings and negative radiographic patterns. They demonstrated the frequent existence of histologically verified chronic inflammation despite a normal radiographic image. Conversely, teeth with radiolucent areas had a definite histologic correlation, revealing chronic inflammatory patterns. They also concluded that periapical radiolucencies would always appear smaller than
the histologic lesion due to the necessity of the lesion to encroach upon the junctional area of cancellous and cortical bone for the rarefaction to manifest itself radiographically.

This study substantiates the findings of Bender et. al. by confirming the tendency for more severe periapical histologic reactions to elicit periapical radiographic changes. The larger and more severe the chronic inflammatory reaction, the greater the anticipated periapical bony destruction. Once this bony breakdown encroaches upon the cortical plate, radiographic changes would become evident. A lack of radiographic pathology did not guarantee the existence of a normal histologic periapical pattern, although the tendency was towards a minimal to mild response if inflammatory changes were present. These results support the evidence of milder inflammatory reactions producing diminished periapical responses, insufficient to elicit periapical radiographic changes.

Statistical analysis in this study was limited to descriptive statistics due to the small sample size. In retrospect, an experimental design using a minimum of five animals would have facilitated data analysis by allowing the application of the Wilcoxon signed rank test. It is unlikely, however, that statistical results would have varied and the generalizability of the study would still remain limited.

One aspect of contention is the applicability of the animal model to that of humans. Although the pulp tissue of dogs is histologically compatible with that of humans, the apical anatomy varies immensely. Complexity of the apical delta prohibits instrumentation of the peripherally radiating canals, possibly leaving remnants of pulpal tissue. The potential for this
tissue to undergo degeneration exists and may partially explain the incidence of periapical inflammatory changes.

Results of this study indicate that breakdown or loss of the occlusal seal, following endodontic therapy, would not adversely affect the apical seal for a period of six months to one year. This finding, however, does not condone neglecting a suspicious or faulty occlusal restoration or temporary filling following root canal therapy. Even successful endodontic treatment will not prevent a tooth from succumbing to aggressive recurrent decay.
SUMMARY

1. Loss of an occlusal seal, following root canal therapy, should have little if any effect on the periapical seal for a period of six months to one year.

2. Root canal therapy should be completed soon after the debridement process. An intact occlusal seal will not prevent development of periapical pathosis following the cleansing and shaping procedure.

3. A normal periapical radiographic image, following completion of root canal therapy, does not guarantee the absence of chronic periapical inflammation histologically.

4. The radiographic presence of a periapical radiolucency is generally associated with more severe chronic inflammatory changes histologically.

5. Loss or failure of an occlusal seal should not be neglected, thereby avoiding recurrent decay and possible progression to a nonrestorable situation.
REFERENCES


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Figure 1: X-ray film positioning with hemostat and modeling clay for intraoral radiograph of mandibular teeth.

Figure 2: Radiographic technique with hand-held X-ray unit.
Figure 3: Access technique utilizing a large heatless stone in a low-speed handpiece.

Figure 4: Access cavity complete in mandibular first molar. Number 35 K file in mesial canal with silicone stop for length control.
Figure 5: Post room preparation with heated Luks plugger in mandibular first molar. Silicone stop for length control to assure an apical seal of 3-4 mm of gutta-percha.

Figure 6: Amalgam sealed control tooth (C) flanked by adjacent experimental teeth (E) open to the oral environment.
Figure 7: Apex of control tooth (six month sacrifice). Normal apical tissues. One of the numerous canals in the apical delta (A), dentin (D), cellular cementum (C), periodontal membrane (P), alveolar bone (B). (H&E stain, X10)

Figure 8: Apical area of control tooth (six month sacrifice) demonstrating the interconnection of one of the canals of the apical delta (A) with the main canal (C). (H&E stain, X10)
Figure 9: Minimal inflammatory reaction (six month experimental). Cellular cementum (C), minimal inflammatory exudate (I), alveolar bone (B). (H&E stain, X10)

Figure 10: Mild inflammatory reaction (six month experimental). Mild inflammatory exudate (I), alveolar bone (B). Note complexity of the apical delta (arrows). (H&E stain, X10)
Figure 11: Moderate inflammatory reaction (six month control). Cementum (C), alveolar bone (B). Note increase in number and density of inflammatory cell infiltrate (I) and indications of alveolar bone destruction and resorption (arrows). (H&E stain, X10)

Figure 12. Severe inflammatory reaction (six month control). Note density and expansiveness of inflammatory cell infiltrate (I) and lack of alveolar bone. (H&E stain, X20)
Figure 13: Apical region of control tooth, mesial root, without gutta-percha fill or sealer (one year control). Note the isolating effect of the fibrous connective tissue capsule (C) walling off the chronic inflammatory exudate (I). (H&E stain, X4)

Figure 14: Box area of figure 13 enlarged to 20X. Fibrous connective tissue capsule (C) surrounding an area of chronic inflammation (I). Note abundant vascularity (arrows). (H&E stain, X20)
Figure 15: Box area of figure 13 enlarged to 40X. Note chronic inflammatory cells composed of histiocytes (H), plasma cells (P) and lymphocytes (L), and blood vessels (V) typical of granulation tissue. (H&E stain, X40)
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