1975

A Study of Host Resistance of the Periapical Tissues of Dogs When Exposed to Streptococcus faecalis

Ronald J. Mazukelli
Loyola University Chicago

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A STUDY OF HOST RESISTANCE OF THE PERIAPICAL TISSUES OF DOGS WHEN EXPOSED TO STREPTOCOCCUS FAECALIS

BY

RONALD J. MAZURELLI, D.D.S.

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

MAY 1975
DEDICATION

To my loving wife, Judith, and my four sons, Joseph, Anthony, Michael, and Ronald, Jr., whose devotion, love, and sacrifices have made it possible for me to pursue further my dental interest, I dedicate this thesis.
ACKNOWLEDGEMENTS

I would like to express my deep appreciation to the following people:

To Dr. Franklin S. Weine, a man who has been genuinely interested and actively involved in bringing his graduate students an excellent, well balanced education.

To Dr. Marshall H. Smulson, whose inexhaustible dedication to the education of his students stands as a monument to higher educational standards.

To Dr. John V. Mandonia and Dr. Alicia Rubenstein for their unselfish assistance as my advisors.

To Dr. Henry Kahn, Dr. Charles Larsen, veterinarian, and Mr. Ornce Payne, laboratory technician, for their technical assistance.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. REVIEW OF THE LITERATURE</td>
<td>4</td>
</tr>
<tr>
<td>A. HOST RESISTANCE</td>
<td>4</td>
</tr>
<tr>
<td>B. INTRACANAL TREATMENT PROCEDURES</td>
<td>7</td>
</tr>
<tr>
<td>C. INCIDENCE OF BACTERIA IN THE ROOT CANAL</td>
<td>13</td>
</tr>
<tr>
<td>D. ETIOLOGY OF PERIAPICAL PATHOLOGY</td>
<td>17</td>
</tr>
<tr>
<td>E. ROENTGENOGRAPHIC AND HISTOLOGIC INTERPRETATION OF PERIAPICAL LESIONS</td>
<td>22</td>
</tr>
<tr>
<td>F. INTRACANAL SAMPLING METHODS</td>
<td>27</td>
</tr>
<tr>
<td>G. ENDODONTIC SURGICAL TREATMENT</td>
<td>33</td>
</tr>
<tr>
<td>H. HEALING AND REPAIR</td>
<td>35</td>
</tr>
<tr>
<td>I. ANIMAL EXPERIMENTATION</td>
<td>38</td>
</tr>
<tr>
<td>III. METHODS AND MATERIALS</td>
<td>41</td>
</tr>
<tr>
<td>A. LABELING AN ORGANISM</td>
<td>41</td>
</tr>
<tr>
<td>B. PILOT STUDY</td>
<td>42</td>
</tr>
<tr>
<td>C. EXPERIMENT</td>
<td>44</td>
</tr>
<tr>
<td>IV. RESULTS</td>
<td>51</td>
</tr>
<tr>
<td>A. LOSS OF SPECIMENS</td>
<td>51</td>
</tr>
<tr>
<td>B. RADIOGRAPHIC FINDINGS</td>
<td>52</td>
</tr>
<tr>
<td>C. HISTOLOGIC FINDINGS</td>
<td>54</td>
</tr>
<tr>
<td>D. CORRELATION</td>
<td>57</td>
</tr>
<tr>
<td>V. DISCUSSION</td>
<td>59</td>
</tr>
<tr>
<td>VI. SUMMARY AND CONCLUSIONS</td>
<td>65</td>
</tr>
<tr>
<td>CHAPTER</td>
<td>PAGE</td>
</tr>
<tr>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>VII. BIBLIOGRAPHY</td>
<td>67</td>
</tr>
<tr>
<td>VIII. APPENDIX</td>
<td>75</td>
</tr>
</tbody>
</table>
CHAPTER I
INTRODUCTION

According to Stedmann's Medical Dictionary, disease is an acquired morbid change in any tissue of an organism, or throughout an organism with characteristic symptoms caused by specific microorganismal alterations. We all have natural or acquired immunity to certain diseases, however, there are many to which we are susceptible. Whether or not a person acquires a disease depends on three very important factors: the number of microorganisms, the virulence of the microorganisms, and the host resistance of the person infected. This is best illustrated in the equation:

\[
\text{disease state} = \frac{\text{number of microorganisms} \times \text{virulence}}{\text{host resistance}}
\]

According to the above formula, when an infection of microorganisms occurs, the lower the number of microorganisms, the less the virulence and the greater the host resistance, the less chance that a disease state will exist.

In Endodontics we very often see a tooth with a necrotic pulp exhibiting a periapical radiolucency. In treating this tooth, endodontists attempt to remove the bacteria
and the necrotic debris upon which they thrive by both chemical and mechanical means. Ideally, once this is done throughout the length of the canal and the apex is sealed, the lesion or disease state produced in the periapical area will heal.

However, in normal endodontic procedures ideal root canal preparation is not always achieved. At times the periapical regions are violated by overextension of an instrument through the apex of the tooth or removal of the apical segment of a tooth surgically. Sometimes a disease state will be brought about after this treatment, as evidenced by a radiolucient area in the apical region of the tooth involved. Very infrequently, however, will a tooth which appears to be cleansed, disinfected and filled adequately exhibit a periapical lesion (granuloma or cyst usually) after a few months observation. It is generally thought that instrumentation of a root canal should be confined to the root canal and not violate the periapical area. The most radical treatment which can be performed in endodontics is surgery. Therefore, in order of severity, surgical procedures are greatest, followed by overinstrumentation and then instrumentation confined to the root canal. It seems, then, that altering the host resistance of the periapical tissues plays an extremely important part in the success or failure of endodontic treatment.
In order to determine the influence which varying amounts of periapical irritation exhibits on endodontic treatment, it is necessary, according to the equation previously stated, to keep the number of microorganisms and their virulence constant while varying the host resistance. The resultant periapical reactions will give an insight into the effects of various endodontic treatment procedures on the periapical tissues and hopefully verify our assumptions of the severity of the reaction of these different procedures.
CHAPTER II

REVIEW OF THE LITERATURE

A. HOST RESISTANCE

In almost every dental article written concerning the reaction of the perirapical tissues to an irritant in the pulp canal, the role of the irritant, microorganisms and their products or necrotic tissue in the canal, was stressed as the most important factor in the production of the periapical response. Very seldom was the host resistance of the animal or the tissues in the area even considered. Many of the authors failed to recognize the fact that, although the presence of bacteria and necrotic debris in the root canals of teeth they were studying was evident, some of these teeth showed no periapical reaction to these irritants while others did. Although it cannot be stated unequivocally that this lack of reaction was due to an increased host resistance or immunity, it seems reasonable to assume so. However, in the light of the recent advances and interest in immunity and immunologic responses, the role of host resistance is being brought forward.

The importance of host resistance is not entirely a recent concept. In 1905 Metchnikoff expressed the importance of host resistance in the pathogenesis of infectious diseases. He stated that normally Staphylococcus pyogenes,
an organism found in abundance on the skin of healthy individuals, does not induce an inflammatory response in the host by its presence. However, if the host's resistance is altered, such as by the presence of diabetes, this normally non-irritating bacteria can cause the formation of a boil on the skin. He concluded that the diabetes is the cause of the suspension of immunity which exists in the healthy individual. Any factor which alters the normal resistance of the individual can set up an imbalance in immunity and make the individual more susceptible to infection or injury.

Bellanti (1971) gives a more contemporary definition of host resistance or immunity: "all those physiologic mechanisms which endow the animal with the capacity to recognize materials as foreign to itself and to neutralize, eliminate, or metabolize them with or without injury to its own tissues." The major functions of immune responses are defense, homeostasis, and surveillance. The function of defense deals with resistance of the host to infection by microorganisms and this is what we are most concerned with here. He also states that there are modifying factors which affect immune mechanisms which are genetic, age, environment, anatomic, physiologic and microbial factors.

Narrowing down the factors of host resistance to the perianal tissues of teeth, Naidorf (1972) states that an increase in the virulence of a strain of bacteria in a
root canal or a decrease in the host resistance can result in an infection of the periodontal tissues by these bacteria. This will lead to either an acute exacerbation or a chronic situation which he calls asymptomatic granuloma. He emphasizes that we should pay more attention to the immune response because it plays a big part in resistance and defense against the toxic products emanating from the root canal.

Nygaard-Ostby and Schilder (1972) state that periodontal areas of rarefaction are "regions of reaction to what has occurred within the root canal system." They also ask the question as to whether different pathologic conditions could be caused by the same or morphologically related organisms, and, if possible, is it related to the host-parasitic relationship?

Zeldow and Ingle (1963) and Hobson (1965) realize the importance of microorganisms and their toxic products in the root canal but also stress the importance of the host in producing a disease state.

Other indications that the establishment of periodontal infection may be due to host resistance as well as the presence of microorganisms arose from experiments by Kennedy, Hamilton, and Syvertson (1957) and Rosengren (1962). Both researchers experienced difficulty in creating periodontal lesions in laboratory animals following experimental infection of the dental pulp with specific bacteria.
B. INTRACANAL TREATMENT PROCEDURES

Endodontic treatment in the nineteenth century was largely a matter of trial and error and successful treatment was determined by a lack of symptoms. Some of the earliest literature concerning endodontics came out of Europe. In 1872 Adolph Witzel advocated amputating devitalized pulps and mummifying pulp stumps as reported by Julius Levine (1934). Levine also credited Herbst as the first person to protect the exposed pulp by placing thin metal foil over it in 1878. As time progressed the theories concerning treatment of the exposed pulp split the dentists into two different factions: those advocating pulp cannling (Gysi, Meyer, Hess, Lutz, Muller, Hellner, Aisenherr, and Zender and Teuscher); and those advocating root canal therapy. It is the latter with which we are most interested here.

Before 1910 many dentists subscribed to the idea that root canals should be filled beyond the apices of the teeth and, therefore, through the apical foramen.

In 1921 Carl Grove condemned the practice of cannling exposed pulps and advocated removal of the pulp tissue down to the apical foramen but not through it. He said that the apical foramen is formed to accomodate the periodontal ligament and great care should be exercised not to disturb this tissue. Instrumenting beyond the apical construction along with the use of caustic drugs destroys the vital tissues present there, especially cementum which is
necessary for healing.

It was later shown by Coolidge (1928, 1931, 1933) and Blayney (1927, 1928, 1929) that accessory canals and foramina which were inaccessible to cleansing methods and still contained vital tissues showed a transformation of that pulp tissue into fibrous tissue and hard calcifications. They, therefore, concluded that the greatest success in root canal therapy was achieved when the living tissue within the foramina and around the roots of the teeth were preserved and that caustic drugs which penetrated the periodontal tissues, e.g. arsenic, were more destructive than helpful in repair.

Another of the early investigators, E.A. Jasper (1949), set forth what he thought were the essentials in endodontic practice. One of these was minimum trauma to the supporting tissues of the teeth which included keeping all canal instrumentation within the root canal and also judicious use of drugs. He stressed the importance of knowing the exact length of the tooth and he explained a method of doing this which is the same as G.V. Black's method introduced in 1908. He placed a sterile, fine steel wire in the canal and took a radiograph. The length of the tooth was then determined by the length of the wire plus the distance to the end of the root, assuming the wire was short of the end of the root.

Shevelton (1964) studied the presence and distribution of microorganisms within non-vital teeth. He decalcified
teeth and made transverse sections through the root at different levels. These sections were stained to show the presence of both Gram positive and Gram negative microorganisms. He noted that bacteria appeared in smaller numbers in the root canal as the apical foramen was approached. He showed in two pictures that a section cut 3 mm. from the apex contained considerable amounts of bacteria but a section cut 1 mm. from the foramen showed inflammatory cells but no microorganisms. This was the trend he found most common. He also noted little or no infection of the periapical granulomas associated with these teeth. Shovelton, therefore, concludes that the presence of a defense reaction inside the apical foramen and lack of infection is of clinical significance in determining the extent of mechanical preparation of a root canal. The preparation should be at the cemento-dentinal junction. Overpreparation, he says, would disturb the natural defense reaction of the body.

Figg, Hatton, and Hewitt (1944) observed a similar phenomena. "Regions bordering those containing bacteria are heavily infiltrated with leukocytes and the line of separation, in some instances, is quite sharp and the transition sudden. One characteristic location for this sudden transition is at the apical foramen, with the necrotic material heavily loaded with bacteria within the
canal and the region of leukocyte infiltration devoid of bacteria in the adjacent apical space."

Up until now all the investigations studied showed that it was best to instrument to the apical foramen but none showed what occurred when instrumentation was through the apical foramen and into the surrounding tissues. In 1968 Seltzer, Soltanoff, Sinai, Goldenberg and Bender studied 27 human teeth; 15 of which were instrumented beyond the apices and 24 monkey teeth; 12 of which were instrumented beyond the apices. He studied these histologically at nine time intervals ranging from immediately after instrumentation to one year after. He concluded that tissue reactions were much milder in those teeth instrumented short of the apex and that epithelial proliferation occurred frequently in those overinstrumented. Therefore, for optimum results in cases of vital pulp extirpation, instrumentation should be confined to the root canal, thereby retaining a vital pulp stump.

In a more recent article, Seltzer, Soltanoff, and Smith (1973) studied the perianical reaction of human and monkey teeth to overinstrumentation and either over or underfilling of the root canal. Again, as in their previous study they used small numbers of teeth which seems to detract from the credibility of the study. Nineteen human teeth and fifteen roots of monkey teeth were used. It was found that those teeth overinstrumented and
underfilled initially exhibited an acute inflammatory response, followed by a chronic inflammatory response which gradually decreased giving way to repair. In teeth overinstrumented and overfilled, initial reactions were the same as in the latter but chronic inflammation persisted and large periapical lesions were seen in the period after five months. They also discovered a greater tendency toward epithelial proliferation and cyst formation in this group.

A similar study was reported by Davis, Joseph and Bucher (1971). They utilized dogs in their experiments, dividing their teeth into five groups: 1) overfilled with gutta percha and sealer; 2) filled to the apex with sealer extruded; 3) filled to the working distance; 4) instrumented to within 1 mm. of the apex and filled 3 mm. short; and 5) instrumented to or slightly beyond the apex and filled 3 mm. short. They cultured the canals before filling to check bacterial contamination. Histologically, they found that teeth instrumented to or slightly beyond the apex and filled 3 mm. short and teeth filled to the working distance 1 mm. short of the apex exhibited the greatest amount of periapical healing. Those teeth instrumented beyond the apices and overfilled showed no healing at all.

Bhaskar and Rappaport (1971) performed root endodontic therapy on dogs' teeth whose root canals had previously
been exposed to the oral environment to produce necrotic pulps and periradicular lesions. They divided these teeth into six groups: 1) overfilled with gutta percha (1-2 mm.); 2) underfilled with gutta percha (4-10 mm.); 3) overfilled with silver cones (1-2 mm.); 4) underfilled with silver cones (4-10 mm.); 5) furcation perforation followed by occlusal seal; and 6) occlusal seal without root canal filling. The dogs were sacrificed eighteen months after therapy and the teeth here histologically examined. They found that overfilled canals have a greater apical inflammatory reaction than underfilled canals or those with no filling. They also discovered that once the root canals were debrided and sealed, the radiolucencies in posterior teeth were larger than those in the anterior teeth and this was attributed to a larger amount of necrotic material in the posterior canals causing a greater degree of inflammation.

To determine clinically exactly where the ideal root canal instrumentation should end seems to be very difficult. Palmer, Weine, and Healy (1971) demonstrated that radiographically most files which extend to the apex of the tooth, in actuality, protrude at least 1 mm. beyond the tooth substance and that if teeth were instrumented to this point, they would be overinstrumented. This was due to the deviation of the apical foramen from the radiographic apex of the tooth. A similar study was also performed by Levy and Glatt (1959).
At present, most of today's investigators advocate instrumentation to the cemento-dentinal junction keeping all mechanical debridement confined to the root canal and avoiding encroachment on the periradicular tissues as evidenced by their articles: (Hara, 1967, Yuttlér, 1955, Heuer, 1963, Ingle, 1961, Ingle and Zeldow, 1958, Moodnik, 1963, Weine, 1972, and many others).

G. INCIDENCE OF BACTERIA IN THE ROOT CANAL

Bacteria in the oral cavity and more specifically in the root canals has been a subject of bitter controversy since W.D. Miller proposed the focal infection theory in 1894 and advocated the removal of pulpless teeth because of their ability to harbor disease producing microorganisms. Miller demonstrated the presence of various kinds of bacteria in the root canal and adjacent tissues and Onderdonk verified their presence by culture techniques in 1901 (Sommer, Ostrander, and Crowle, 1956, and Anthony and Grossman, 1945).

Gelner and Moody (1914) cultured bacteria from 40 infected root canals both aerobically and anaerobically on blood agar and ascites-dextrose agar slants. They found that the predominant organism was streptococcus, and it was found in varying species. They also cultured Bacillus fusiformis, Staphylococcus aureus, Staphylococcus albus and Micrococcus catarrhalis. They concluded that jaw abscesses were caused by these microorganisms and that removal of them by endodontic procedures through the root or

13
surgically can cure the abscess and that extraction was not essential.

In 1943 Hayes studied 340 teeth for the presence of bacteria. He selectively cultured the root canals and studied the bacteria microscopically. Of 211 teeth which exhibited positive cultures, 95 contained some species of streptococcus (hemolytic, non-hemolytic and viridans), 61 contained Staphylococcus (aureus and albus), and 18 Bacillus acidophilus in pure cultures. The other 37 were combinations of bacteria.

Teeth with root canals which were unexposed to the oral environment were studied for the presence of bacteria by Brown and Rudolph in 1957. They used blood agar plus ascitic fluid anaerobically and aerobically, chocolate agar in 10% CO₂ environment, PPLO agar plus PPLO serum (Difco) aerobically for 10 days, Sabouraud's agar plus dextrose at room temperature for 7 days, fusiform agar anaerobically for 3 days, and thioglycollate broth, with resazurin indicator, plus dextrose aerobically. Where otherwise indicated the cultures were kept at 37°C for 48 hours. Specimens were also studied under phase contrast and dark field microscopes. Streptococci, diphtheroids and micrococci were most frequently isolated and mixed infections were prevalent. Staphylococcus aureus and albus were not isolated proving that bacteria flora of unexposed pulp canals varies from that of exposed canals.

In a similar study by Ernström and Frestell (1961).
bacteria from non-vital pulps with intact pulp canals were isolated and identified. 26 out of 36 teeth studied exhibited positive cultures and of those with positive cultures, sixteen contained streptococci. The results were similar to the previous study and again Staphylococcus aureus and albus were absent.

Blechman (1957) states that the bacteria most frequently isolated from root canals are alpha hemolytic (viridans) streptococci, Staphylococcus albus and the gamma (non-hemolytic) streptococci. In two different studies, one by Blechman and the other by Sommer, Ostrander, and Crowley, over 80% of a series of cultures contained streptococci and totally about 48% were pure cultures.

In yet another study (Leavitt, Naidorf, and Shurakovsky, 1958), this time utilizing trypticase soy broth and agar, streptococci again proved to be the most prevalent microorganism found in the root canal, however, staphylococcus also was very prevalent.

Shovelton and Sidaway (1960) cultured 147 teeth in Robertson's meat medium and tomato juice medium. They were incubated at 37°C for 4 days and then subcultured to determine the type of organisms present. α-hemolytic streptococci were the most prevalent microorganisms, followed by anaerobic streptococci and gamma hemolytic streptococci.

Crawford and Shankle (1961) compared the flora of root
canals which were open and closed to the oral environment. They found that the flora were similar in each but restricted in the closed teeth. Also, non-beta hemolytic streptococci were found to predominate in both environments.

Winkler and van Amerongen (1959) reported on the bacteriologic results from 4,000 root canal cultures. Cultures were taken on teeth prior to and after endodontic treatment. Bacteria were grown in BHI broth at 37°C, until turbidity was evidenced and then subcultured on four solid media for identification. Once classifications were made, they were confirmed by biochemical or serologic tests. They found that of 1,141 positive cultures, 577, or about 50%, were streptococci in pure culture and that in 315 cases streptococci were found in mixed cultures (61% totally). Of those found in pure cultures, Streptococcus faecalis was by far the most predominant organism. All strains presented under the heading of Streptococcus faecalis showed characteristic large α-hemolytic colonies. They withstood heating at 60°C, for 10 minutes and fermented mannitol and esculin. This bacteria was often isolated more than once from one canal which proves its ability to persist longer in the root canal.

In studying the bacteriology of root canals, Cran (1959) states that the most prevalent and the most difficult bacteria to get rid of are streptococci. They find an
anaerobic or micro-aerophillic environment most favor-
able for growth which is one reason for their persistence in the root canal. The enterococci are also very insensitive organisms, e.g. Streptococcus faecalis, and are, therefore, resistant to many antibiotics.

Finally, in considering the incidence of bacteria in the root canals, Matusow (1967) brought to light three factors which influence the isolation of microorganisms. First, systemic infectious disease could have significance in the characterization of microorganisms cultured from root canals and periodontal tissues. The anachoretic effect of an inflamed pulp or periodontal tissues can attract these blood borne bacteria. Second, pulps exposed to the oral flora have a broad spectrum of bacteria present but those with no evidence of pulp exposure generally exhibit a limited microbial spectrum. Finally, the frequency of microbial isolation from root canals can vary with culturing and microscopy procedures. The addition of dextrose, cysteine, ascitic fluid, agar and drug inhibitors to different base media and the use of dark field and phase contrast microscopy can alter the characterization and incidence of microorganisms present.

D. ETIOLOGY OF PERIAPICAL PATHOLOGY

As early as 1894 when W.D. Miller proposed his focal infection theory which stated that pulpless teeth were mausoleums of infection, it was recognized that bacteria in
the root canal were associated with perianical pathology.

In 1932 Thomas J. Hill performed a study on dogs in which he initiated granuloma formation at the apices of their roots by removing pulp tissue aseptically and introducing *Streptococcus hemolyticus* and also a non-hemolytic streptococcus. He also used a control by removing pulp tissue and sealing the chamber without inoculation with streptococcus. He noted a greater number of apical foramina in dogs than in humans and attributed the rapid formation of granulomas in his dogs to this fact. He observed granuloma formation at the apices of all his teeth, experimental and controls; however, the presence of bacteria in mixed strains could be demonstrated in all the teeth indicating a leak in the occlusal seals of these teeth.

In another study Dixon and Rickert (1938) induced granulomas in the perianical region of dogs' teeth by removing the pulp tissues and inoculating the root canals with *Streptococcus viridans*. They then performed routine endodontic treatment on these teeth and observed the reaction of the perianical tissues histologically on a chronological basis.

An interesting and unusual study of bacteria found in the root canal was conducted by Smith, Thomassen, and Sweet. They cultured 90 non-vital teeth with perianical radiolucencies which had not been exposed to the oral cavity. 70% of these teeth were infected. The bacteria were then
isolated and their enzyme activity determined. It was discovered that these bacteria produced enzymes associated with pathogenicity and invasiveness and they concluded that these microorganisms are potential pathogens and are capable of producing perianal lesions.

According to these studies and many others, perianal pathology is directly associated with bacteria found in the root canal. Once all the bacteria found in the root canals were removed, as indicated by a negative culture, the perianal lesion could heal because the cause of it was removed. However, in 1960 Matsimiyia and Kitamura performed normal endodontic therapy on 215 infected root canals of dogs using four different disinfectants in the root canals and filling the canals with calcium hydroxide. They histologically and bacteriologically observed conditions in and around the canals at three time intervals: immediately after filling; 25 days after filling; and 50 days after filling. At 25 days, healing and repair of perianal tissues was evident, however, in some teeth bacterial infection was greater than immediately after filling. At 50 days healing was increased and bacterial infection decreased. Therefore, healing of perianal tissues can and does occur in the presence of bacteria because it is impossible to sterilize the root canal by present cleansing methods and medications.

Since the advent of the previous article most researchers agree that the presence of bacteria alone is not the...
only cause of periapical lesions. In defending the importance of obtaining a negative culture before completing root canal therapy, Zeldow and Ingle state that we can expect a 94-96% success rate if canals are filled after a negative culture as opposed to 83.3% success after a positive culture. They are quick to admit, however, that the mere presence of bacteria is not the determinant of success or failure. Virulence, the number of microorganisms present and the host resistance are the primary antecedents and as long as an equilibrium exists between the host and the parasite, disease will not result.

An interesting retort to these culture conclusions came in 1964 in an article written by Seltzer, et al. The effects of filling root canals after both positive and negative cultures were determined and they concluded that there was no significant difference in the repair of the periapical tissues both histologically and radiographically between the teeth filled with positive or negative cultures. They also concluded that the role of the host is of critical importance in the end result of endodontic treatment.

As far as establishing a disease in the pulp or periapical tissues of exposed teeth is concerned, it is evident that microorganisms play the primary role. Kakehashi, et al. (1965) and Kakehashi, et al. (1969) used conventional laboratory rats and gnotobiotic rats to prove this. They exposed the pulps of these rats, fed them identical diets,
and observed the teeth histologically at intervals from 1 to 42 days postoperatively. The investigators showed that, in the absence of microorganisms, inflammation of the pulp tissue was minimal and healing occurred readily. But, when bacteria were present, necrosis and abscess formation occurred. It was concluded that microorganisms are the major cause of pulp and periapical disease.

A similar study involving monobiotic rats was published by Korzen, Krakow, and Green (1974). These researchers exposed the maxillary first molars of monobiotic rats and conventional rats. One group was mono-infected with Streptococcus mutans and the other contained the normal oral flora. Half from each group were then sealed and the other half left open changing the quantity of bacteria in each tooth. The inflammatory response to mono-infection with Streptococcus mutans was much less severe than that to the mixed infection. Also, the severity of the inflammatory response of the pulpal and periapical tissues is related to the quantity of bacteria in the root canal and the length of time of exposure.

Up to this time, periapical pathology was considered as one entity but we know that it can be divided into two major histologic categories: granulomas; and cysts. Although both are caused by the same factors, cyst formation goes one step further. In the periodontal ligament are found small groups of epithelial cells called cell rests of Malassez.
According to histochemical and ultrastructural studies performed by Ten Cate (1965) and Valderhaus and Nylen (1966) these cells show a low ribonucleic acid content, low glycogen content, scarcity of Golgi complex and mitochondria with smooth cristae indicating that they are in a resting state. However, in 1967 Grupe, Ten Cate, Zander confirmed by in vitro and vivo studies that cysts formed from stimulation (probably a change in oxygen/carbon dioxide tension) of the epithelial cell rests of Malassez and proliferation of these cells due to the ability of these cells to undertake anaerobic glycolysis.

The incidence of cysts as compared to granulomas has been a topic which has been hotly debated for years. Bhaskar (1966) and Lalonde and Luebke (1968) collectively studying more than 3,000 periapical lesion biopsies concluded that granulomas and cysts occurred in almost equal frequency with slightly more being granulomas. But, Sommer, et. al. (1956) and Patterson, et. al. (1964) in their histologic studies of almost 700 teeth reported a much higher incidence of granulomas than cysts (83% to 6.4% and 84% to 14% respectively).

E. ROENTGENOGRAPHIC AND HISTOLOGIC INTERPRETATION OF PERIAPICAL LESIONS

For many years it was assumed that a periapical lesion which appeared on a radiograph was approximately the same
size and shape of the actual lesion in the bone. In recent years, however, this theory has been disproved. In 1961 Bender and Seltzer simulated pathologic conditions in wet and dry cadaver mandibles and maxillae by drilling holes of different depth in the cortical bone and also by removing cancellous bone from inside the mandible. They discovered that removal of a superficial layer of cortical bone showed no radiolucency on an x-ray and that as more and more bone was removed a shadow appeared and became more pronounced. They also found that all the cancellous bone in the mandibles and maxillae could be removed up to the junction of the cortical and cancellous bone without any change in trabecular pattern or radiolucency radiographically. Only when the junction area was affected could a radiolucency be demonstrated. They conclude, therefore, that early stages of bone disease cannot be detected radiographically and also the size of the rarefied area on a radiograph cannot be correlated with the amount of tissue destruction.

In a similar study, Regan and Mitchell (1963) took head plate radiographs of cadaver skulls to find periapical radiolucencies. Once this was accomplished, the areas were dissected to determine the amount of cortical bone destruction. From their results it was determined that radiographically one could not tell if the cortical plates were perforated, there was always destruction of the cortical plate
and junctional trabeculae and the amount of bone destruction could not be determined accurately from radiographs.

Schwartz and Foster also studied dried human adult skulls in a similar manner. They removed cancellous bone from the mandible and found results similar to those above. They also removed cortical bone from the maxillary antrum and septal bone forming a three-walled bony pocket from a second molar. Preoperative and postexperimental radiographs revealed no diagnostically significant changes in the calcified structure. Therefore, unless a lesion in the bone involves the cortical bone or the junctional area where the cancellous and cortical bone meet, radiographically, there will be no evidence of pathology.

There is a very obvious drawback to studying radiolucent periapical areas on radiographs alone. Bauman and Rossman (1956), Forsberg and Hagglund (1960), and Sommer, Ostrander and Crowley have shown that there is no absolute method of making a differential diagnosis of the cyst or granuloma from radiographic evidence alone. Therefore, the histopathologic interpretation of these periapical lesions must also be determined.

A review of the literature concerning the histology of periapical pathology would not be adequate unless the article by Fish (1939) were quoted. He established foci of infection in the jaw bones of guinea pigs by drilling.
holes in their mandibles and inserting cotton pellets saturated with broth cultures of different microorganisms. The animals were sacrificed at varying intervals from 4 to 46 days postoperatively and the tissues were prepared for histologic study. Fish described four distinct but interrelated areas around the foci of infection, each containing a characteristic type of cell. The zone of infection contained the focus itself with microorganisms and polymorphonuclear leukocytes at its periphery. The zone of contamination contained necrotic bone and tissue and the predominant cells were lymphocytes. Around this was the zone of irritation where osteoclasts digested bone and histiocytes dissolved collagen. The outermost zone was called the zone of stimulation. Here the toxic substances produced in the inner zone were diluted to such an extent that they served as a stimulus to repair. Osteoblasts and fibroblasts were evident here.

According to Grossman (1967) the bacteria are essentially found in the root canal and the surrounding tissues are sterile, except for occasional inroads. As bacteria enter the perianical tissues, they can be destroyed by polymorphonuclear leukocytes and a chronic condition results, but if they overcome the PHN's, an acute abscess is formed. Where defense is adequate the perianical bone will be destroyed, but a wall of fibrous tissue develops and the lesion is called a granuloma. In some cases the
epithelial cell rests in the periodontal membrane are stimulated to form a cyst.

Boyle (1955) describes the progression in the formation of a granuloma and a cyst. Initially, the root ends reveal a circumscribed area of inflammatory cell infiltration in the periodontal membrane next to the apical foramen. These are essentially plasma cells. This inflammation continues and extends beyond the periodontal membrane and into the alveolar bone. In the area around the root end granulation tissue forms. At the apical foramen is a small accumulation of inflammatory exudate cells and the affected root surface shows resorption of cementum and dentin. The granulation tissue is surrounded by a fibrous capsule. Fibroblasts and connective tissue fibers are seen here. As the granuloma increases in size, we can get tissue breakdown opposite the apical foramen and a small area of liquefaction and pus formation appears. In the periphery of this lesion new bone formation occurs. These areas described correspond to Fish's four zones.

Boyle describes two ways in which a cyst can form: an abscess cavity may develop in a granuloma and epithelium, because of its inherent tendency to grow over raw surfaces, covers the walls of the abscess cavity or epithelial cells proliferate, hollow out, enlarge, and undergo cystic degeneration. The histopathologic finding is essentially the same as in a granuloma but a large epithelial lined cavity
is present. Cholesterol slits are also often seen. He divides radicular cysts into four categories: growing young cyst; mature cyst (stationary or only very slowly growing); infected cyst; and mature cyst (decreasing in size).

McCall and Wald (1954) also describe cysts and granulomas in a similar fashion.

F. INTRACANAL SAMPLING METHODS

The detection of bacteria in the root canal was first demonstrated by Onderdonk in 1901. He advocated a thorough antiseptic and mechanical cleansing of the root canal, followed by sealing paper points in the canal. At the second appointment he cultured the paper points sealed in the canal previously to insure a thorough disinfection of the canal.

In 1919 Coolidge suggested culturing the root canal and periapical tissues to insure the sterility of the pulpless tooth before filling the root canal.

Coriell (1918) described a method of culturing the periapical tissues by using a dental trocar. After the tissues covering the apical area have been cauterized, the sterile trocar is driven through the bone into the lesion by means of a dental engine. The drill in the center of the trocar is removed and a sterile platinum needle is passed through the trephine and into the periapical tissues. This
is then cultured.

In 1932 Grossman described the same method of culturing apical lesions, calling the trocar an anicostone and the procedure apicostomy. He insured access into the lesion by injecting a radiopaque material into the lesion postoperatively and taking a radiograph.

More recently Hedman described a method of culturing the periapical region of teeth by means of a sterile cannula. These cannulas were introduced into the root canals of teeth which had been prepared for endodontic therapy and pushed to the apex of the teeth. A sterile wire was then threaded through the cannula exiting in the periapical tissues. This wire was then cultured in the conventional manner.

Shindell performed a study similar to Hedman's in that he introduced a cannula into endodontically cleansed teeth and threaded a stylet through it into the periapical tissues. He found that only 3 of 63 teeth had periapical cultures which were positive. He recognized the fact that false negative cultures were possible due to the small quantities of bacteria which might be present but concluded that most periapical lesions were sterile and what few bacteria were present were insignificant.

Again in 1959 Grossman described a method of culturing the periapical area by making a surgical window in the bone and culturing the exposed root tip or soft tissue. Of 109
cases studied 93 were negative indicating the use of an aseptic technique and also the absence of microorganisms in the perianical area.

Another method of intracanal sampling was described by Grossman (1938) although it had been used as far back as the late 1800's. This is the smear technique. The dressing in the root canal is aseptically removed from the canal and smeared over a clean glass slide and allowed to dry. The bacteria are fixed by passing the slide over a flame and stained with methylene blue, gentian violet, and carbol fuchsin. It is washed, dried and examined microscopically for the presence of bacteria.

He also describes his culture technique. The tooth is isolated and disinfected. The temporary seal is removed by means of a sterile bur and the cotton pellet is discarded. A sterile paper point is introduced into the canal to remove any medicament present and it is discarded. A second sterile absorbent point is placed in the canal to the apex and left in place for one minute. It is then removed and dropped in a tube of culture medium if the tip is moist. If not, a drop or two of culture media is placed in the canal to provide moisture.

Serene and McDonald (1969), however, investigated the ability of four successive paper points placed in a canal medicated with camphorated parachlorophenol or Cresatin to produce an accurate culture reading. They discovered that
wiping the root canal with a paper point significantly reduces the inoculum, thereby reducing the validity of the results. When the first paper point is used for a culture, the most accurate results are obtained.

Another more commonly accepted description of culturing technique is described by Weine. After the rubber dam is applied and the area disinfected, the temporary seal is removed with a sterile bur and the dressing with a sterile broach. A sterile paper point, slightly smaller than the width of the last file used in canal enlargement, is picked up with sterile cotton forceps and placed to reach the apical portion of the preparation. Once here, the point is rotated to gain contact with all the walls of the preparation. It is removed and then inserted into the tube of medium. If the point is dry when removed, it is dampened slightly with sterile water and replaced into the canal before placing it in the culture medium. Incubation is at 37°C. for a minimum of 48 hours but preferably for seven days.

Now that the culturing techniques have been described, how effective they are and how important they are must be considered. Grossman (1966) studied four different species of bacteria (Streptococcus salivarius, Streptococcus mitis, Streptococcus faecalis and Staphylococcus aureus) in three different media (BHI with 0.1% Bacto agar, fluid thioglycolate, and cooked meat broth). Serial dilutions of 10⁻¹
to 10-10 of bacterial cultures were made and introduced into the media which were incubated. It was found that certain strains of microorganisms required only one organism to initiate growth in a culture medium. He also concluded that BHI with .1% agar was better for the growth of the test bacteria than the other two media.

In a research project which parallels the previous study, Munoz (1970) investigated several different media (BHI Broth, BHI Broth with .1% agar, glucose-acites media with .1% agar, tripticase soy broth with .1% agar, and fluid thioglycollate) on the basis of their ability to support the growth of two common root canal contaminants (Streptococcus faecalis and Actinomyces israelii). Serial dilutions were made of the bacteria from 10^-1 to 10^-12 and incubated aerobically and anaerobically at 37°C, for 2, 3 and 10 days. He concluded that the glucose-acites with .1% agar and fluid thioglycollate broths were most suited for culturing these two organisms and that they grow best in an anaerobic environment.

In recent years the validity and the importance of culturing during endodontic treatment has been challenged. Buchbinder and Bartels (1951) raised the question as to whether or not antibiotics used as medicaments in root canals could inhibit the growth of microorganisms in the culture media if transferred there by the paper point used in culturing. Bender and Seltzer (1954) proved that
antibiotic activity was transferred to the culture tube, mainly, by the first paper point inserted in the canal and also that inhibition of growth of microorganisms occurred. They also show an incidence of 14% false negative cultures. They conclude that, if antibiotics are used in root canals, inactivators should be placed in the culture media to prevent inhibition of growth.

Bender, Seltzer and Turkenkopf (1964) again challenged culturing pertinence by proving that there was no significant difference in success rate in 6 month and 2 year recalls of teeth which were filled following both positive and negative cultures. They also found culture reversals (negative culture initially and positive culture at the time of fill) in 16.6% of their cases which was blamed on the fallability of the present culture technique, seal leakage, loss of antibiotic activity, and a short period of time between cultures.

Morse (1970) believes that the culture is irrelevant in routine endodontic therapy but it is important to reduce the number of microorganisms in the root canal to as low a level as possible. He believes culturing and antibiotic sensitivity are necessary only in cases of acute apical abscess and cellulitis.

This view is not universally accepted by all, however. Zeldow and Ingle (1963) and Grossman (1938, 1966) as we have already seen advocate culturing root canals and
place much emphasis on obtaining a negative culture before filling a root canal. Madonia also advocates culturing root canals as a means of relatively determining the degree of contamination in the canal before filling.

G. ENDODONTIC SURGICAL TREATMENT

At just about the time when the focal infection theory was in high gear and dentists were extracting almost all non-vital teeth, a few pioneers of endodontic therapy lashed out at the advocates of this theory and eventually proved them wrong. These men demonstrated large lesions over the apices of teeth which were resolved after treatment of the root canal. Levine in 1935 described a method of successfully eliminating the persistent periapical pathosis. His technique consisted of a semi-lunar incision followed by removal of cortical bone overlying the apex of the involved tooth. The area was then curetted mildly and all necrotic and granulomatous tissue removed. Then an occlusal access was made into the root canal, and the canal debrided and filled. Any filling material extruded was removed by curettment and the flap was repositioned but not sutured.

Weaver described an unusual flap design which he uses for surgical treatment of periapical lesions. This is a vertical periapical flap over the root of the tooth which
is then spread mesially and distally. He advocates curettage and does not cut back the apex of the root because it reduces leverage and strength of the tooth and exposes dentinal tubules.

Most surgical techniques from this point in time to the present are very similar. A few changes have been made and some precautions taken which will be outlined.

Sommer (1946) stressed care in reflecting the surgical flap to minimize post-operative swelling and pain. He also advocated probing the cortical plate for defects rather than removing bone promiscuously with a bur upon flap reflection. Beveling the apex with a bur, gentle treatment of the bone, and similarly angled x-rays were also parts of his therapy.

Maxmen (1959) advocated the use of a surgical technique when filling teeth with blunderbus apices, perforated root canals, broken instruments, internal and external resorption, etc. His method consists of raising a flap, enucleating the lesion, debriding the canal and then filling the canal holding a burnisher over the open end to condense gutta percha against. Any excess is removed after complete condensation.

In 1972 Rud, et. al. investigated the healing of perianical areas which had been treated surgically. 1,000 teeth were reexamined radiographically from one to fifteen years after treatment. They found a higher rate of success
in teeth which had been previously filled with gutta percha and then surperized than in those which had only an apical amalgam reverse filling and not root canal filling. This was attributed to the fact that infectious or necrotic materials from the root canals would seep out into the perianical tissue due to insufficient sealing of the amalgam or unfilled lateral canals. They also found that teeth treated surgically showed more inflammation than those filled in the conventional endodontic manner.

Andreasen and Rud (1972) tried to correlate a radiographic view of post-operative changes with their histologic findings. They discovered that although moderate or severe inflammation persisted in almost one-half of their cases, the radiographs showed a decrease in the size of the rarefaction. The most favorable results were seen in those teeth which showed scar tissue histologically and a decreased or absent rarefaction radiographically. It seems obvious from their results that it is impossible to correlate a radiographic image with actual histologic findings.

H. HEALING AND REPAIR

Healing and repair of the perianical tissues usually occurs in a manner similar to any other tissues of the body. First, the cause of the pathology must be eliminated or decreased sufficiently so the normal healing process can occur. In endodontics the removal of the toxic products in the root canal and the sealing of the apical end of the
canal will provide the environment necessary for this to occur.

In 1928 Blayney histologically surveyed the peri-apical tissues after pulps had been removed from teeth and the teeth had been instrumented and filled to different levels in the canal. He found that the dental pulp may be removed without causing irreparable damage to the periapical tissues if normal, conservative endodontic therapy is instituted. After the pulp is removed, resorption occurs causing enlargement of the apical foramina. These heal later with the formation of calcified material resembling cementum being deposited in the foramina but never closing them completely. Healing in the areas containing granulation tissue occurred by replacement of inflammatory cells with fibroblasts which are active in the repair process.

An important histologic observation in healing and repair is the reaction of cementum. Cementum serves as a connecting medium for fibers of the periodontal ligament within the root dentin and insures root surface vitality. It is bone-like in substance but is not physiologically resorbed and rebuilt. It is covered over and increases in thickness. Following vital pulp extirpation inflammation is induced apically and resorption of cementum and dentin takes place. Soon after inflammation subsides, new cementum repairs the area and restores the normal outline
of the root. In teeth with apical granulomas, once the etiology is eliminated, new cementum is laid down and new bone is deposited adjacent to it. The presence of vital cementum stimulates the functional repair of bone and the reestablishment of the periodontal ligament.

After an apicoectomy cementum would be deposited on the cut dentinal surface and a new periodontal ligament will be attached if no inflammation is present. (Coolidge, 1931)

Again in 1932, Coolidge proves perianical repair can occur in both vital and non-vital endodontically treated teeth and in surgically treated cases.

In his text, Kronfeld (1933) describes what occurs perianically when a pathologic lesion begins to heal. This lesion consists of granulation tissue which is a defensive or reparative reaction. If infection is controlled, the proliferation of connective tissue elements and the decrease of inflammatory cells will advance from the periphery toward the center until the entire mass is transformed into scar tissue and ultimately into bone.

Another investigation into the repair of the perianical tissues utilized rats whose root canals were overfilled, partially or totally occluding the apical periodontal space. A lesion appeared almost immediately. At 24 hours postoperatively the apical region showed necrosis of the periodontal ligament, polymorphonuclear leukocytes,
extravasation of blood, and bone and cementum necrosis. Healing occurred at different rates according to the damage inflicted. Repair began with resorption of necrotic bone and removal of the damaged periodontal ligament. Highly vascularized tissue replaced this and was instrumental in resorption of the necrotic cementum. Next, corticoalveolar bone was formed, new cementum was laid down, and the periodontal ligament returned to normal. (Erausquin, et. al., 1966)

Nygaard-Ostby (1961) attempted to demonstrate that perianical bleeding and the formation of a blood clot stimulated healing in this area. Even though healing in his experimental teeth was evident, the amount of time required for inflammation due to overinstrumentation to decrease for healing to occur was prohibitive.

After presenting many cases of endodontically treated teeth which initially revealed perianical pathosis but subsequently resolved, Enlander (1940) concluded that surgical intervention was not required for all teeth with perianical lesions.

I. EXPERIMENTATION WITH ANIMALS

As early as 1932, it was noted that the perianical tissues of dogs' teeth are very sensitive to any kind of injury and are in fact more sensitive than human tissues. They have a tendency to form perianical lesions readily
due to the great branching of the pulp canals at the
noises of these teeth and the difficulty of removing
organic material from these canals. However, other than
the apical branching, the roots and periapical tissues along
with the granulomas which are formed here are comparable
to those found in humans. (Orhan, 1932 and Hill, 1932)

Dixon and Rickert (1938) and Stein (1931) also con-
cur with the above conclusions.

Torneck (1969) says that the basic response of the
periapical tissues to injury is similar for both dogs and
human beings but the pattern of hard tissue resorption
and deposition may be unique to the dog because of lack
of direct correlation with human patterns.

Barker and Lockett (1971) evaluated the dog as a
suitable animal for endodontic research. They concluded
that the premolars are readily accessible for pulp treat-
ment procedures and the root canals were sufficiently
wide to permit instrumentation with standard endodontic
instruments. Apical perforation is readily achieved and the
reaction of the periapical tissues can be easily observed
histologically. They also describe a technique for
experimentally infecting root canals.

In conclusion, therefore, it seems correct to assume
that the reactions exhibited by periapical tissues of
dogs after root canal experimentation are representative of
that which would occur if a similar treatment were performed
on a human.
CHAPTER III

METHODS AND MATERIALS

A. LABELING AN ORGANISM

In evaluating the literature concerning bacteria, it was found that Streptococcus faecalis was the strain of bacteria most often found in root canals in a pure culture. It is also the most common contaminant found in root canals after the canals have been instrumented and disinfected. Because of its ability to exist in a root canal which has already been instrumented mechanically and its ability to be labeled so it can be identified by conventional culturing methods, Streptococcus faecalis was chosen as the organism to be used in this study.

A lyophilized sample of Streptococcus faecalis was obtained from the Microbiology department and introduced into a test tube containing tripticase soy broth. It was incubated at 37°C for 48 hours.

Meanwhile, a mixture of tripticase soy agar was prepared, sterilized, and cooled to 54°C. At that time, streptomycin was added to the agar to create a concentration of 2 milligrams of streptomycin per milliliter of agar. This was poured into sterile petri dishes which were slanted to create a higher concentration of streptomycin at one end of the dish. When this was cooled, the dishes were laid flat and topped with plain tripticase soy agar. After

41
solidification, one petri dish was inoculated with 0.5 ml. of the 48 hour culture of *Streptococcus faecalis* and incubated at 37°C. for 48 hours. Growth occurred more densely at one edge of the dish and became more sparse as it approached the area of greater streptomycin concentration. Colonies closest to this area were picked up on a sterile wire and inoculated into a tube of tricoptase soy broth and the latter process repeated. When growth covered much of the streptomycin slant plate, a stock growth of the culture was made and two Gram stains were made to insure purity.

Six test tubes containing varying concentrations of streptomycin in tricoptase soy broth were made. These were inoculated with the streptomycin-resistant *Streptococcus faecalis* to determine at what concentration of streptomycin they would grow. Incubation was 37°C. for 48 hours. It was determined that the bacteria would grow in broth containing 1 mg. streptomycin/ml. broth. The stock culture was kept refrigerated and at 7 day intervals was re-inoculated and Gram stained to insure viability and purity.

**B. PILOT STUDY**

In order to fulfill the requirements for this study, the number of bacteria and the virulence must be kept constant. The virulence is kept constant by the introduction and maintainence of only one strain of bacteria, but
the number of microorganisms necessary to produce a lesion had to be determined and this necessitated a pilot study.

A 48 hour culture of streptomycin-resistant Streptococcus faecalis in tripticase soy broth was diluted in test tubes containing sterile pentone water in the concentration of 10 grams/100 ml. The tubes contained $10^{-2}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$ dilutions of the 48 hour culture.

A 20 kilogram dog was administered 5% sodium pentobarbital intravenously in the amount of 0.5 cc/kg. as a general anesthetic. Radiographs were taken of all teeth to be worked on to insure the potency of the periapical region. The maxillary and mandibular bicusps were then disinfected with alcohol-iodine solution and the cusps reduced out of occlusion with a heatless stone. Access cavity preparations were made into the root canals with a sterile bur. The pulps were removed from the entire length of the canal with various sized barbed broaches. Next, the different concentrations of the streptomycin resistant bacteria were introduced into different root canals and they were covered with a cotton pellet and sealed with zinc oxide and eugenol. In two canals the pulps were removed and no bacteria were introduced. These were used as controls. Visual examinations were given and radiographs were taken at two and four months after treatment to determine at which concentration lesions began to
It was discovered after four months that a dilution of $10^{-4}$ was the point at which lesions occurred in half of the canals studied. No lesions formed apically to teeth with greater dilutions or in the control teeth and lesions occurred 100% of the time on teeth with less dilution (greater concentrations of bacteria).

C. EXPERIMENT

Five adult mongrel dogs, four females and one male, ranging in weight from 18.5 to 25 kilograms were chosen as experimental animals for the study. They were housed in individual cages at the Animal Research Facility of Loyola Hospital and were fed the normal diet. All were given the normal vaccinations, deworming procedures and isolation.

The usual endodontic armamentarium was used in this study: a portable dental engine, a vacuum system, syringes, anesthetic, needles, alcohol, assorted burs, endodontic explorers, excavators, mirrors, rulers, cotton pliers, lock pliers, temporary filling instruments, glass slabs, mixing spatulas, root canal reamers and files with silicone rubber stops, barbed broaches, sterile paper points, sterile cotton pellets, glass bead sterilizer, temporary filling material (I.R.M.), assorted sterile disposable syringes for irrigation and inoculation, sodium hypochlorite, amalgam and mercury capsules (Spheralloy capsules), alcohol lamp, amalgam squeeze cloths, amalgam condensors, x-rays, fixer.
and developer in a quick developing box, x-ray machine and sterile towels. Other materials used which are not part of a normal endodontic armamentarium were 5% sodium pentobarbital, electric sheers, indwelling catheters, adhesive tape, mouth props, surgical instruments (scalpels, scalpel blades #15, surgical burs, periodontal elevators, retractors, sterile saline, irrigation syringes, needles and suture material), formalin, Striker saw, decalcifying solution, hematoxylin and eosin stains, slides and light microscopes.

All media used in this study were freshly prepared to avoid contamination or chemical change. Fresh bacterial cultures of the *Streptococcus faecalis* were made every seven to ten days to insure a viable culture of the same bacteria and inoculations of the root canals were only made with bacteria from a new 48 hour broth culture to insure bacterial freshness.

All autoclavable equipment and media were sterilized before use in a steam autoclave for at least 20 minutes at 121°C and 15 lbs./sq. in. Autoclave tape was used to insure sterility.

Each dog was weighed and then administered 5% sodium pentobarbital intravenously (0.5 cc/kg. body weight) as a general anesthetic. The dog's mouth was then kept propped open with a device which fit over the maxillary and mandibular cuspids and separated the jaws. The maxillary and mandibular pre-molars were inspected visually for caries,
fractures, or other abnormalities. Radiographs were then taken of all the teeth clearly showing the perianical areas of these teeth.

Next attempts were made to isolate these teeth with a rubber dam. However, due to the conical shape of these teeth and the periodontal involvement of the tissues, it was found that blood and saliva continually flowed into the area. With careful isolation of the teeth with sterile cotton rolls and gauze, little or no contamination occurred. Once the quadrant was isolated, the teeth were disinfected with an alcohol and iodine solution. A sterile heatless stone was used to reduce the cusps of the teeth to be studied to keep them out of occlusion. The teeth were again disinfected with the alcohol and iodine solution. A sterile 558 bur was placed in the handpiece and an access cavity preparation was cut in one tooth. The pulp was removed by means of a sterile barbed broach and the canal was measured in the normal endodontic manner and then cleansed.

Sterility of the files was insured by use of the glass bead sterilizer and the canals were shaped using endodontic files only. A 5% sodium hypochlorite solution was used for irrigation and disinfection. The canals were instrumented until clean dentin shavings were seen at the tip of the last file used. In this experiment three different apical procedures are going to be studied: filing to the histologic apex; filing through the histologic apex and into the
periapical tissues about 2-3 millimeters and surgically removing the apical 1/4 of the root after filling the canal. Each of these procedures was performed on different teeth in different quadrants of the mouth except for the surgical procedure which was performed only on the first and second bicuspids because of accessibility of the apices. A semi-lunar flap over the apices of the teeth was made with a #15 Bard-Parker blade and reflected with a periosteal elevator. Hemorrhage was controlled by use of a combined irrigation-suction device. The overlying cortical bone was removed with a sterile 558 bur and the apices of the teeth located by using a file, premeasured to the length of the root canal. About 1/4 of the root was resected and when possible the apical segment removed. The area was irrigated with water, dried, and the flap repositioned and sutured with 3-0 silk sutures. These sutures were removed seven days postoperatively. The canal was then dried with sterile paper points, covered by a cotton pellet, and sealed with zinc oxide and eugenol. This procedure was repeated for each tooth in the quadrant and each quadrant in the mouth.

Seven days later the teeth were again isolated and disinfected with alcohol and iodine. The zinc oxide and eugenol temporary fillings were removed with a sterile 558 bur. The cotton pellets covering the orifice of the canals were removed and a sterile paper point was introduced into the canal and left for 30 seconds. The point was then
removed and immediately placed in a sterile test tube containing thioglycolate broth. Some of the canals which had been over-instrumented or surgirized exhibited a hemorrhagic exudate upon opening and the paper points were saturated with this exudate before placing in the culture tube. All precautions to maintain sterility were followed. Again, sterile cotton pellets were placed over the orifice of the canals and the access cavity preparations sealed with zinc oxide and eugenol cement. The culture tubes were then placed in an incubator at 37°C. for seven days. After this period of time the tubes were inspected for turbidity. Those teeth with cultures exhibiting bacterial growth were re-instrumented, re-irrigated and re-cultured. If the culture was again positive, the teeth were eliminated from this study.

After the cultures had been determined, the dogs were again anesthetized, the quadrants isolated and the temporary fillings removed. Very few canals showed a hemorrhagic exudate when opened and these were dried with sterile paper points until hemorrhage ceased. Each canal which showed a negative culture was then innoculated with 0.1 milliliter of a 48 hour culture of the streptomycin-resistant Streptococcus faecalis diluted to $10^{-4}$ in pentone water by means of a syringe and sterile 25 gauge needle. Any excess solution in the chambers was dried with sterile cotton pellets. Sterile paper points which were cut into 2 millimeter sections were placed in the orifice of the canals and
a thin layer of zinc oxide and eugenol was placed over the floor of the chamber and the orifice. Then, amalgam (Spher-alloy pre-mixed capsules, Kerr) was triturated and condensed into the access cavity preparations. Until corrosion of the amalgam occurs, the layer of zinc oxide and eugenol provided a seal against salivary contamination.

Radiographs were taken at eight weeks and again at sixteen weeks. At the end of sixteen weeks the dogs were sacrificed by intravenous injection of Euthanol. An electric bone saw was then used to remove sections of the mandible and maxilla containing the bicuspid teeth. The access cavity fillings were then removed and cultures taken in a similar manner as described above. Holes were then drilled through the cortical bone and the cortical bone along the inferior border of the mandible was removed to insure penetration by the fixative. The sections were then placed in 10% neutral buffered formalin for 10 days and decalcified in 5% formic acid for 4-6 weeks. They were then prepared for histologic study and stained with hematoxylin and eosin. Cultures were placed in an incubator at 37°C. for seven days.

Gram stains were made on these cultures and those not showing only Gram positive cocci were eliminated from this study. 0.5 milliliter of this broth culture was then transferred to sterile test tubes containing triticate soy broth and 1 mg. streptomycin/ml. of broth and incubated at 37°C. for seven days. Those cultures not exhibiting turbidity
were eliminated from this study.
CHAPTER IV
RESULTS

A. LOSS OF SPECIMENS

Initially, 102 root canals were prepared for study: 40 were instrumented to within 1 mm. of the radiographic apex, 37 were instrumented through the apex and into the periapical tissues, and 25 were treated surgically. However, some of these specimens had to be eliminated from this study for various reasons. Seven root canals were eliminated because of fracture of the tooth during the post-operative time period. These teeth lost their temporary amalgam restorations and the canals became contaminated (Table 1). Seven specimens were eliminated from this study due to the presence of contamination of the root canals or no bacteria present when Gram stains were made on the four month post-operative cultures. (Table 2). Finally, four root canals were eliminated on the basis of failure to show turbidity in the streptomycin broth after seven days incubation at $37^\circ$C. (Table 3). Therefore, a total of 84 root canals were used in this study: 34 instrumented to within 1 mm. of the radiographic apex (6 controls and 28 inoculations); 29 instrumented through the apex (4 controls and 25 inoculations); and 20 treated surgically (3 controls and 17
innoculations).

B. RADIOGRAPHIC FINDINGS

Pre-operative radiographs taken on all teeth to be prepared in each dog revealed no periapical pathology. All teeth exhibited normal root canal and pulp chamber morphology. Periodontally, slight crestal bone loss was evident on a few radiographs. Furcation areas were normal and a lamina dura could be traced around each root. The floor of the maxillary sinus closely approximates the apices of the maxillary teeth. Maxillary first pre-molars routinely exhibit a single root with one canal but all other pre-molars show two roots with a single canal in each. In one dog (505) a supernumerary tooth had formed and erupted next to a first pre-molar and this was included in the investigation.

Upon examination of the four month post-operative radiographs, the root canals which had been instrumented to within one millimeter of the radiographic apex revealed relatively little periapical pathology. None of the control teeth showed any radiolucencies and only 7 of 28 (25%) exhibited roentgenolucent areas. Of these, five were seen in one dog indicating that this dog may have been more susceptible to the bacteria or more prone to the formation of granulomas or cysts. The radiolucencies seen here were usually small with diffuse borders and limited to the apical 1/4 of the root. These teeth normally exhibited
an intact bifurcation and no root resorption.

Of those teeth which were inoculated with bacteria and were over-instrumented, 13 exhibited radiolucencies and 4 were obscured from view due to superimposition of the floor of the maxillary sinus over the apex of the root or thickness of the bone in the area. All control teeth showed no radiolucencies. Therefore, 59% of these teeth showed periapical radiolucencies. These lesions were usually large with diffuse borders. They usually formed at the apex of the tooth, but some were observed laterally on the root. Most of the teeth with roots that were perforated showed much root resorption both apically and laterally (somewhat of a moth-eaten appearance). One of the control teeth also showed root resorption but complete bone fill-in. Many also exhibited radiolucencies in the bifurcation areas.

Those teeth which were treated surgically and inoculated showed only 7 radiolucent areas at the root ends (41%) and most of these were much smaller than the size of the area made when surgical treatment was performed. One control root showed a small radiolucency around its apex, but bone regeneration was apparent. Most of these teeth treated surgically showed no resorption of bifurcation bone or dentin. Most lesions showed diffuse borders radiographically but two were well circumscribed.
C. HISTOLOGIC FINDINGS

The specimens were sectioned so as to include the entire root and root canal space down to the apex as well as the periapical tissues. They were then stained and observed under high and low power with a light microscope. Each section examined was graded on a scale ranging from 0 to 3+, with 0 showing no inflammatory cells present periapically and a normal histologic appearance, and 3+ showing a tremendous influx of inflammatory cells, much hard tissue resorption, necrosis, etc. indicating absence of healing. A general histologic description of each group is given.

Those teeth which were instrumented to the histologic apex and inoculated showed normal dentin and cementum with no resorption. Many pulp canals had some dentin filings packed apically but the walls were free of debris. In all but seven roots, the periodontal ligament was intact around the tooth. Few, if any, inflammatory cells were present and those there were mostly lymphocytes. Bone was viable in most sections and little resorption was present. Most of the trabeculae were surrounded by osteoblasts and the bone marrow had a fibro-fatty appearance. In those seven roots (five from one dog alone) where the periodontal ligament was not intact, granulation tissue was in evidence. There was some fibrous tissue surrounding the lesions and varying amounts of bone loss. No necrotic areas were seen but the tissue was, in a few areas,
heavily infiltrated with chronic inflammatory cells. The tissue was also highly vascular. Control teeth, for the most part, appeared normal histologically, with only two showing some signs of slight inflammation.

Teeth instrumented through the apex generally exhibited much dentin and cementum resorption both apically and laterally. A few showed some necrotic cementum along the root. The root canals were free of dentin fillings but inflammatory cells and necrotic tissue were often found here. The periodontal ligament was usually destroyed in the apical half of the root and replaced by granulation tissue. At times the inflammation extended all the way up the root surface and into the bifurcation areas or periodontal sulcus. The apices were surrounded by granulation tissue heavily infiltrated with chronic inflammatory cells (mostly lymphocytes). These lesions ranged from a few millimeters to twelve millimeters in diameter with the majority showing a central area of necrosis. Very often a fibrous capsule could be seen surrounding the lesion. The bone outside the capsule exhibited both resorption and formation. In a few sections small amounts of bone formation could be seen within the circumference of the lesion indicating some healing taking place. The bone marrow was infiltrated with chronic inflammatory cells to varying degrees depending on the amount of inflammation present. Healing seemed non-existent in
many specimens. Controls, for the most part, displayed periapical inflammation, bone loss, inflammatory cells, etc., but to a lesser degree, and healing was obvious. A few specimens in this category were eliminated from this study due to failure to observe a section through the perforated apex.

Finally, those roots which were treated surgically demonstrated little or no dentin or cementum loss on the surface where the root had been cut. The lateral aspects of the roots showed no resorption. Occasionally, new cementum was observed on the cut dentin surface of the root. The canal was normally free of dentin shavings but contained inflammatory cells and vascular granulation tissue. In many cases the area of inflammation took the shape of the apex of the tooth which was removed surgically. This tissue was granulation tissue which was moderately infiltrated with chronic inflammatory cells. Two specimens showed cyst formation around the surgically treated apex. The bone here was viable and in a few cases spicules were seen extending into the defect area. Most trabeculae were surrounded by osteoblasts but resorption and osteoclasts were seen in some sections. Generally, the bone marrow was sparsely infiltrated with chronic inflammatory cells. Again, the control teeth showed some signs of inflammation, bone loss, etc., but to some degree less than the inoculated specimens. Some of these surgically treated teeth
had small apical segments which remained in the bone after surgery. They were not visualized due to the intense hemorrhage in the area during the surgical procedure and subsequently not removed.

The control teeth which had not been treated in any manner showed normal pulp tissue in the canal and normal periapical tissues, histologically.

D. CORRELATION

Table 4 shows a compilation of the data obtained after viewing each slide histologically based on the grading system (0-3+) explained earlier. It is seen that those roots treated in an ideal endodontic manner and inoculated with Streptococcus faecalis, group N, had an average degree of periapical inflammation of 0.76, whereas the controls had an average of 0.30. The difference between the two being 0.46. Group P, those roots which were perforated, showed an average degree of inflammatory response of 2.63, whereas the control average was 1.75. The difference is 0.88.

Finally, group S, those roots treated surgically, had an average of 1.50. The control average was 1.33 and the difference is 0.17.
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* some revealed slight widening of the periodontal ligament space
CHAPTER V

DISCUSSION

The most difficult task an examiner can confront is that of subjectively evaluating his results. If observations can be assigned numbers which correspond directly to what is visualized, such as using weights or measures, it becomes very easy to evaluate data. However, in this case radiographic and histologic findings do not reveal measurable results and therefore, observations must be weighed and evaluated before assigning them arbitrary figures. The drawback confronted in this situation is the fact that a bias viewpoint may enter into these evaluations, thus negating the results of the experiment.

To avoid such a pitfall in this experiment, observations of radiographs were made and recorded before mounting in their proper position. Histologic sections were coded with no indication as to controls, dog number, tooth number, or root. Another unintended advantage in producing non-bias results was the fact that five months had elapsed between the time the root canals were treated and the radiographs were observed and six months between treatment and histologic observation.

In actuality, eight different grades of histologically observed inflammatory responses were recorded. At first
it was determined to use four categories, however, as the slides were reviewed it became evident that many fell into a so-called twilight zone between two divisions and adjustments were necessary. Grading was kept on a scale of 0 to 3+, however, new categories of 1½, 1¾, and 2½ were added.

Grading of the specimens was based on a comparison of a section of the treated tooth with a similar section of a control tooth which had not been instrumented. The control tooth had a grade of 0. This section exhibited normal dentin and cementum, a root canal containing vital pulp tissue, an apex showing many apical foramina leading to the periodontal tissues, a periodontal membrane of equal thickness on the lateral aspects of the root and slightly thicker apically, a layer of cortical bone on the lateral aspects of the root with heavy trabecular bone apically, bone trabeculae surrounded by osteoblasts, and a fibro-fatty bone marrow. There was no evidence of resorption, osteoclastic activity, or inflammation present. In grading the slides the presence of the following was deemed important: cementum; dentin; bone resorption and apposition; inflammatory cell density and extension; size of the lesion; fibrous capsule and thickness; types of inflammatory cells; areas of necrosis; periodontal ligament destruction and bifurcation involvement.

After all the above factors were considered a
judgment was made as to whether the area was healing or the destructive process was continuing. Cementum and bone apposition, well encapsulated lesions with a sparcity of chronic inflammatory cells and no invasion outside the lesion, lack of necrosis, minimal periodontal ligament involvement and no bifurcation involvement was generally considered a lesion which was healing. It seems relevant to mention here that classification of inflammatory cells was difficult because the decalcification process necessary for sectioning affects the cellular structure.

According to the results in table 5, those specimens which were instrumented to the cemento-dentinal junction and inoculated with *Streptococcus faecalis* showed a very mild degree of inflammation if any at all four months after being exposed to the bacteria. Teeth whose ances were perforated 2-3 mm. into the periodontal tissues and inoculated revealed a tremendous degree of bone loss, root resorption and inflammation. Those teeth treated surgically and inoculated exhibited a moderate degree of bone loss, some inflammation but little root resorption. It was also evident that the presence of *Streptococcus faecalis* in the root canals added to the inflammatory effect on the tissues as evidenced by the fact that the controls showed much less degree of inflammation than those teeth which were inoculated.
The figures indicate that the periodontal tissues more readily resisted the inflammation producing products of the bacteria in those teeth which were filed to the radiographic apex than those of the other two categories. One very evident reason is that the tissues in the periodontal region were not violated as in the other two instances, thus allowing for a better defense against the onslaught of the bacteria and their products.

Another, but more minor, reason could have been that the canals in the other two groups were wide open apically, whereas those in this group remained patent. This, however, does not seem to be important because of the numerous lateral canals in the apical region giving the bacteria the pathways to the apical tissues. An enigma arises, however, when the surgical and perforated categories are compared. One would expect to observe a greater inflammatory response to surgically treated teeth than to those which were perforated but the converse was true. An explanation which seems reasonable is that the periphery of the surgical defect was too far removed from the toxic products of the bacteria emanating from the root canal to be affected by them. Therefore, healing proceeded in a normal manner toward the severed root end. Because the healing process was in progress, the effect of the bacteria was lessened. However, no healing was occurring in the perforated areas because the affected tissues were too close.
to the bacterial toxins and, therefore, the full effect was produced.

From the results obtained in this investigation, it seems reasonable to conclude that the host resistance of the perianical tissues is greatest if they have not been violated by root canal instruments. Also, surgical treatment, by the fact that the effects of toxic products are reduced peripherally and healing is initiated in this area, shows greater host resistance than those teeth in which the canals are severely overinstrumented.

Now that these facts have been established, another question arises: How do these results relate to endodontic therapy?

It is generally uncontested that, of the three objectives of root canal therapy (preparation of the canal to receive a filling, elimination of canal contaminants, and a root canal filling which obliterates the canal in three dimensions), root canal preparation is the most important aspect. This part of total root canal therapy encompasses the other two. Without an adequate preparation the debris and microorganisms present in the canal will not be completely eliminated lending to a greater chance of failure. Again an adequate mechanical shaping of the canal is necessary to insure adequate room for condensing instruments when filling the canal. If the canal is not widened sufficiently, the condensers will not penetrate the apical
region thereby not producing enough space for adequate condensation of the filling material. This leads to an improperly sealed apical foramen which can allow the passage of fluids into the canal and act as a harbor for existing microorganisms.

In his book, Weine suggests that in preparing teeth for endodontic therapy the instrumentation should be limited to the confines of the root canal and should never be extended into the periodontal tissues. In this way the operator can prepare a "dentin matrix" at the cemento-dentinal junction against which he can condense a number of gutta percha cones without fear of overextending the filling material into the periodontal tissues and having a poor apical seal. The results here indicate that filing to the cemento-dentinal junction in these dog teeth produced a much more favorable periodontal tissue reaction than filing 2 to 3 millimeters through the apex both in the presence of Streptococcus faecalis and in a culturally "bacteria-free" environment. Therefore, it seems safe to assume that filing a root canal to the cemento-dentinal junction is ideal from both a mechanical and tissue preservation point of view.
CHAPTER VI
SUMMARY AND CONCLUSIONS

The host resistance of the periapical tissues of dogs when exposed to *Streptococcus faecalis* was studied in this experiment. Twenty-eight root canals were instrumented to the cemento-dentinal junction, twenty-five were instrumented two or three millimeters through the apex and seventeen were treated surgically. After two weeks the canals were inoculated with labeled *Streptococcus faecalis* in a dilution of $10^7$ and sealed with zinc-oxide and eugenol and amalgam.

After four months the teeth were examined and radiographed. The animals were then sacrificed and the teeth and associated structures were removed in block sections. Cultures were taken on all root canals to insure the presence of the labeled microorganisms. The sections were then prepared for histologic evaluation.

Radiographically, those teeth filed to the cemento-dentinal junction showed radiolucencies in 25% of the cases, those perforated showed radiolucencies in 59% of the cases, and those treated surgically showed radiolucencies in 41% of the cases. Histologically, similar results were observed. Those teeth filed to the cemento-dentinal junction showed the least amount of inflammation,
followed by those treated surgically and then those perforated.

From these results it can be concluded that the host resistance of the periodontal tissue is greatest if they have not been violated either surgically or by overinstrumentation. Also, the periodontal tissues treated surgically are more resistant to inflammation caused by bacterial products than the periodontal tissues which were violated by overinstrumentation. This is probably due to the fact that peripherally the effects of the toxic products are reduced and healing is initiated here. This healing process, in turn, offers a greater resistance to the spreading inflammation. It can also be concluded, therefore, that filing a root canal to the cemento-dentinal junction is ideal for periodontal tissue preservation.
CHAPTER VII

BIBLIOGRAPHY


CHAPTER VIII
APPENDIX
TABLE 1

PROCEDURE PERFORMED AND INOCULATIONS

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N - Normal Endodontic Therapy
P - Perforated
S - Surgical
C - Histologic Control

*Bacterial Control
**Fractured Tooth

76
**TABLE 2**

**GRAM STAINS**

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OK - Gram positive streptococci only

NB - No bacteria

C - Contaminated
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**CULTURES**

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+ Positive culture
- Negative culture
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<td>1\textsuperscript{1/2}</td>
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<tr>
<td>2\textsuperscript{1/2}</td>
<td>2\textsuperscript{1/2}</td>
<td>2\textsuperscript{1/2}</td>
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</table>

22 \quad 50 \quad 25\textsuperscript{1/2} \quad 0

Ave. 0.76 \quad Ave. 2.63 \quad Ave. 1.50

Ave.*0.30 \quad Ave.*1.75 \quad Ave.*1.33

* Controls which were instrumented but not inoculated
APPROVAL SHEET

The thesis submitted by Dr. Ronald J. Mazukelli has been read and approved by three members of the Graduate School faculty.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with references to content, form and mechanical accuracy.

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

May 15, 1975

Date

Signature of Advisor