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The Anachoretic Effect of Periapical Tissues Following Overinstrumentation of the Radicular Foramen

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THE ANACHORETIC EFFECT OF PERiapICAL
TISSUES FOLLOWING OVERINSTRUMENTATION
OF THE RADICULAR FORAMEN

BY

PETER J. LIO, B.S., 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
1974
DEDICATION

To my loving parents, Carmelo and Rose, whose devotion, loyalty, and personal sacrifice are unending, I dedicate this thesis.
ACKNOWLEDGEMENTS

To Dr. Franklin S. Weine for his genuine interest, professional assistance, and warm, personal friendship throughout my entire graduate education.

To Dr. Marshall H. Smulson, an everlasting flame of high educational standards.

To Dr. John V. Madonia and Dr. Robert Pollock for their unselfish assistance as advisors.
AUTOBIOGRAPHY

Peter Joseph Lio was born in Chicago, Illinois, on November 14, 1944, to Carmelo M. and Rose C. Lio.

He received his elementary education at Ryerson Public School on Chicago's west side and secondary education at St. Mel High School where he was graduated at the top of his class in 1962.

In September 1962, he entered Loyola University Dental School (Chicago College of Dental Surgery). Throughout his four years of dental education he was active as a student leader. He served as president of his class for four years and, upon graduation in 1970, received numerous academic and leadership awards including Blue Key, Omicron Kappa Upsilon, and the Alpha Omega scholastic award.


In 1972, he again returned to Loyola University Dental School to pursue a Masters Degree in Oral Biology in the Department of Endodontics.
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CHAPTER I

INTRODUCTION

The phenomenon of anachoresis, the attraction and fixation of various circulating foreign materials, has been investigated by a host of research workers. Although studies have shown this phenomenon to exist in many physiologic systems, the pertinence in this investigation is to observe its actions in the region of the periapex. Therefore, the scope of this study must not only encompass a bacteriologic-histologic analysis of the periapical tissues but an observation of transient systemic infection and its effect, if any, on periapical cellular components.

Studies of the periapical region cannot be exclusive in nature because they must involve techniques and/or anatomical structures relating to adjacent structures as well. This study necessarily requires root canal preparation methods along with the examination of the structure and position of the radicular foramen. More precisely, the termination of the root canal system and techniques utilized to closely correlate the clinical and anatomical position of this point, along with the systemic microbiologic condition might alter the bacteriologic and histologic condition of the periapex.

Classically, bacterial analysis of the periapical tissues and root canals usually involved the examination of extracted teeth or in situ methods employing intracanal culturing, trocar and cannula or
mucoperiosteal periapical surgery. All of these are subject to considerable potential contamination.

Most microorganisms isolated from any naturally infected root canal and periapical tissue in both the human and the dog are gram positive cocci. Anatomical studies of root structure, both internally and externally, give evidence that in many cases the terminal portion of this system is interpreted erroneously resulting in an insult to periapical tissues. The consequences of this insult, namely inflammation, coupled with the presence of bacterial components should render it possible to conduct a bacteriologic-histologic study of these inflamed periapical tissues. By using culture techniques and tissue bacterial stain, such as John Hopkins Modified gram stain or Brown and Brenn bacterial staining technique on block sections of experimentally-induced periapical areas of inflammation, attraction or fixation of bacterial components could be investigated.

The purpose of this research exercise was to attempt to detect the presence of bacteria in experimentally-induced areas of periapical inflammation.
CHAPTER II

REVIEW OF THE LITERATURE

The localization of circulating bacteria in areas of injury has been recognized from the early days of medicine and is often expressed as LOCUS MINORIS RESISTENTIAE.

Schüller (1880) introduced tuberculous material into animal lungs through tracheotomy wounds and simultaneously injured knee joints by twisting. The infection was found to be present not only as a general tuberculous process, but also and especially, localized about the traumatized joint.

In 1901, Calmette and Guerin injected vaccinia virus into the veins and found it localized in previously shaved areas on the backs of rabbits. Pawlowsky (1909) found that streptococci were uninhibited from dissemination when injected into inflamed knee joints, although they spread profusely from those joints which were uninjured. In 1916, Lewis, working with trypon red, observed that when the dye was given intravenously, it appeared in abnormal amounts and with unusual rapidity in the aqueous humor of slightly congested eyes of rabbits.

Bullock and Cramer (1919) demonstrated the production of lesions by anaerobes; not at the points of inoculation, but in distant areas initiated by the injection of ionizable calcium salts. Treatment of pneumococcal meningitis was accomplished by Netter and Cesari (1923) by the production of fixation abscesses in the thigh. Opie (1924) noted fixation of antigen at the site of injection in immunized animals
and (1929) found that aleuronat-induced peritonitis retarded dissemination of hemolytic organisms subsequently introduced intraperitoneally. Opie also called attention to the importance of the antigen-antibody reaction at the site of injury as a factor in production of local inflammation.

Kettle (1924), using silica, localized tubercle bacilli in lesions and to a lesser degree with calcium chloride injections. In 1926, Land used the anachoretic phenomenon to study the fate of circulating particles in the defense cells. Halley, Turner, and Chesney (1928) injected treponemata into the testicle and veins of rabbits and invariably produced syphilitic lesions in previously prepared wounds. Findlay (1928), found that in animals prepared by intradermal inoculation of histamine, intravenously injected *Staphylococcus aureus*, *Streptococcus hemolyticus*, and pneumococci localized in the histamine-treated areas. He believed localization to be due to increased capillary dilation and permeability produced by histamine or histamine-like substances. A notation was made by Spagnol (1928) that electronegative dyes localize much more readily than electropositive ones.

It should be recalled, in this respect, that bacteria carry negative charges. Menkin (1929, 1930a, 1930b, 1931, 1935, and 1940) conducted an extended series of experiments demonstrating the fixation of dyes, metals, foreign proteins, and bacteria in areas of induced inflammation. In further studies, it was shown that blood-borne materials are attracted to areas of inflammation and that fixation can occur within thirty minutes after application of the irritant. A report of two cases by Quednau (1929) called attention to the tendency for microorganisms, particularly pneumococci, to localize in areas of cerebral
Sager and Nickel (1929) produced sterile abscesses subcutaneously in rabbits and later recovered intravenously innoculated streptococci from the abscesses in some of the animals. It was their belief that a higher percentage of localization would have been evident if the bacteria had been introduced at earlier stages of inflammation.

Menkin and Menkin (1931) demonstrated the accumulation of intravenously injected colloidal iron in induced tumor nodules. Citing clinical cases, Rickert (1931) directed attention to the localization of bacteria from extraoral foci in the pulps of restored teeth. Burrows (1932), in addition to reviewing the literature at some length, illustrated the localization of dyes in various areas of inflammation, such as induction of a palatal ulcer with the accumulation of blood-borne dye. He recorded a number of cases from the literature in which *Treponema pallidum* localized in old tattoo marks. In one case, an obliterated tattoo appeared anew as an Indian, resplendent in red popule outline!

Experiments by Fox (1936) showed that younger lesions attracted dye more rapidly than older ones and that the power to collect blood-borne matter persisted longer where the inflammation was induced by stronger irritants. Bareggi (1937) and Ascoli and Nai (1937, 1938) not only observed attraction of bacteria to areas of inflammation but also noted their retention for long periods of time. It was Csernyei (1936) who credited Ascoli with the introduction of the term anachoresis from the Greek meaning, convocation and refuge.

In 1939, Rigdon confirmed the observation of Fox that younger lesions attract dyes more readily finding that xylene-induced areas of
inflammation failed to attract dyes and foreign proteins one to five days following application of the irritant. Csernyei (1939) provoked chronic periapical inflammation in dogs, and after three weeks, injected Bong bacilli (Brucella abortus) in the saphenous vein. Three weeks later the pulpless teeth and the controls were extracted and suspensions prepared from them for inoculation in guinea pigs. After from three to four weeks, the guinea pig sera were tested for agglutinins against the bong bacillus. The results of this investigation clearly indicated that areas of chronic periapical inflammation can attract bacteria from the blood stream and that the fixed bacteria can remain vital.

LOCALIZATION AND FIXATION OF BLOOD-BORNE BACTERIA

It has been shown that positive blood cultures are sometimes obtained from apparently healthy normal individuals. In studies done by Cameron, Rae, and Murphy (1931) it was found that of 100 blood cultures from supposedly healthy subjects, eighteen were positive although twelve of these were considered contaminated.

In a carefully controlled study, Butler (1937) observed growth in blood cultures in from two to fifty healthy adults, and he believed that with present methods, less than five percent of normal individuals will yield positive blood cultures. Among persons with infected sinuses, tonsils, teeth, and other foci, the incidence of bacteremia observable from time to time will presumably be somewhat higher. Okell and Elliott (1935) found micro-organisms from the blood of ten of 110 patients with periodontal disease.

Some investigators have studied the incidence of bacteremia after
operations or other systemically disruptive procedures. Seifert (1925) found not only that there were seven positive blood cultures in twenty-two goiter operations, but also that operating in re-examining or changing dressings in infected areas was followed by bacteremia in 82 of 195 instances. In a study of bacteremia and its association with urologic disease, Scott (1925) observed 82 cases. Fifty one of the cases were found following operative procedures. That a high incidence of urethral operations exhibit post-operative blood infections was observed by Barrington and Wright (1930). Four out of 64 tonsillectomies were followed by bacteremia, as noted by Bartlett and Pratt (1931). In addition, evidence of bacteremia in sixteen of 51 cases was shown by Fischer and Gottdenker (1936).

The importance of blood clearing time has been emphasized by many workers. The speed with which the blood can be cleared of bacteria has been emphasized by most workers in directing attention to the importance of taking blood specimens immediately or shortly after an operation. The speed of blood clearing was shown by Reichel (1939) in an experimental study expressing the belief that positive post-operative cultures will not persist unless (1) there is a focus of infection in the vascular system itself, (2) an abscess is draining into the blood, or (3) the invading organism is not affected by the defense system of the body.

In more direct relation to the role of dentistry, attention has been directed to the possible role of post-extraction bacteremia in vascular diseases. Richard (1932) initiated various infected foci and produced bacteremia. Massaging the gingiva and rocking the teeth for
periapical tissues for the presence or absence of bacteria.

The popularization of focal infection was given impetus by Hunter (1911), Billings (1912), and Rosenaw (1917), to prove that all infected teeth and radiographic lesions were a source of systemic ailments from tonsillitis to rheumatism. In a study of periapical granulomas, Cook reported a more than 90% incidence of bacterial growth and thought that teeth with granulomas should be removed, particularly in patients with systemic or organic diseases. Such teeth were thought to be "a menace to health and happiness" by Lucas. Rhoades and Dick got similar results for bacterial presence in periapical pathosis. The assumption that all periapical radiolucencies are infected was made by Appleton (1924). He described in detail most of the available techniques for sampling periapical tissues: extraction of tooth with culturing of socket or root apex with and without attached apical resins, in situ methods employing intracanal culturing, intracanal aspiration techniques, and burrowing through mucoperiosteum or mucoperiosteal flaps. He also advocated use of bacterial smears and culturing of periapical contents, regardless of the technique employed. Grossman also advocated three ways of determining the condition of the periapical tissues: histology, radiology, and bacteriology. These appear to be awesome in their scope to study; but bacteriology, extraction, or in situ methods were once again employed.

EXTRACTION

The most popular as well as the most primitive technique for periapical bacterial sampling was the culturing of extracted teeth or their resident sockets. The culturing ranged from the entire surface of the root severed apical third or pulpal contents to determine the
pulpal and/or periapical bacteriology.

In the culturing of 115 extracted teeth, Henrici and Hartzell found positive bacterial cultures in 42% of the teeth with periodontal disease only, 43% of teeth with carious lesions only, and 46% of teeth with periodontal disease and carious lesions together. The total number of control teeth, which were free of all obvious clinical pathology, yielded negative bacterial cultures. They concluded that all periapical granulomas were infected.

Duttem, et al., conducted a similar study in which pulpal contents of normal uninjured extracted human teeth were cultured. They obtained only 2.7% positive cultures from this sample. Pulps with incipient caries only, with gingival inflammation only, and those with both yielded 43.7%, 48.7% and 64.4% positive bacterial cultures respectively. They decided that pulpal and periapical infection are common in even slightly diseased teeth and uncommon in healthy teeth.

A study by Bulleid, using the apical root tips of 200 pulpless extracted teeth, concluded that 1) teeth with radiographic periapical lesions are probably infected, 2) teeth with no periapical lesions radiographically are only occasionally infected, 3) teeth with only slight periapical radiographic change are usually infected to a lesser extent, and 4) granulomas are invariably infected.

The presence of anaerobic and aerobic micro-organisms in periapical tissues was surfaced by Gilmer in 1924. A histological investigation of periapical infection gave evidence that the distribution of bacteria in periapical lesions, when found, is in islands of infiltrating cells near the apical opening or even deeper of the fibrous structure is scant and the cellular collections of polymorphonuclear
leucocytes and plasma cells are abundant. He felt that periapical granulomas act to prevent systemic dissemination of the bacteria.

The use of severed apices of extracted human teeth for bacterial culture of periapical tissues was also employed by Haden, Head and Ross. In both investigations, more than 90% of all radiographic periapical pathoses were positive for bacterial growth. Haden employed glucose brain agar as a medium for his 1,307 samples. Using subcultures and stained smears, Head and Ross isolated an unknown organism, a coccobacillus in 90 out of 130 teeth.

Studies by Figg, et. al., and Shovelton utilized a bacterial tissue stain to determine the presence of bacteria in the canal and periapical tissues. Figg found bacteria in pulps only in areas of partial or complete necrosis and even though the area of necrosis was heavily laden with bacteria within the canal, none were seen in adjacent periapical tissues. Shovelton's observation of organisms in periapical granulomas were rare.

The technique of extracting teeth with the periapical lesion attached was used by many investigators. Hordt concluded that all solid granulomas with no tears in the fibrous wall were sterile. Bacteria were never found within the granuloma and only on the surface of the lesion as contaminants. However, Hordt always found bacteria in granulomas and cysts that were acutely inflamed and had caused pain.

In his studies with numerous extracted teeth, Kanner concluded: 1) that during extraction, bacteria may enter the pulp by capillary action; 2) that even an ideal method of sterilizing the surface of the extracted teeth could not prevent contamination, and 3) that attempts
to sterilize the operative field tend to decrease the incidence of contamination of pulp and periapical tissue but do not eliminate it.

In 1931, Bulleid extracted 400 teeth and found only 15% with attached periapical lesions. It was his feeling that these small "growths" always appeared to come away with the extracted tooth and that it was simple to sterilize the external surface of the lesion and culture the internal contents.

Boyle used 63 extracted human teeth with and without attached periapical granulomas. He demonstrated a large number of gram-positive bacilli within phagocytic cells in the central portion of the solid granulomas using Giemsa, gram and eosin methylene blue stain. Since they were intracellular, he felt that this proved these microorganisms were not contaminants.

Ellingham (1935) examined 42 teeth with radiographically normal periapical tissues and obtained growth in 57% of the periapical areas. He felt that bacteria in the pulp were a result of sucking bacteria back into the vascular system from the periodontal and periapical tissues during extraction.

The fact that bacteria in the periapical and pulpal tissues did not cause an inflammatory response was unexplainable to Appleton and Holton. They found no inflammatory response at all in their extracted specimens and concluded that these bacteria must be contaminants. In two separate studies, Tunnicliff and Hammond come to a similar conclusion. Their observation was that of a tooth creating a positive and negative pressure during extraction which forces bacteria from the gingival sulcus up onto the root surface.
In 1936, Fish and MacLean cauterized the region of the gingival sulcus around the tooth to be extracted and obtained no post-extraction bacteremias from these specimens. Tests for bacterial growth in vital and pulpless teeth were negative. They felt that it was impossible to sterilize the sulcus by chemical solutions. They stated that "a pool of blood wells up around the edge of the gum before the tooth gives way and the blood becomes contaminated from the crowns of the surrounding teeth and finally is smeared over the root as it leaves the socket". They concluded that 1) bacteria never live in harmony with living tissue; 2) for bacteria to live among living cells they must be associated with a necrotic nidus except in the very early acute inflammatory reaction of an infectious nature, and 3) the round cell infiltrates of lymphocytes and plasma cells indicate the presence of bacterial and pulpal toxic products and polymorphonuclear leucocytes indicate actual bacterial presence.

**INTRACANAL SAMPLING METHODS**

This method of determining the periapical tissues for bacterial content was probably first suggested by Coolidge in 1919, and as of this date, still the most frequently used technique. It was again Coolidge who first suggested biologic examination of the root canal to determine the sterility of a pulpless tooth before filling the root canal.

Smith, et. al., conducted an interesting study of the pulpal and periapical bacteria employing intracanal sampling. They cultured necrotic root canals of 90 teeth showing pulp disease with and without periapical lesions. None had been open to the oral cavity and 70% of
the specimens were infected. All of the isolated organisms were tested for enzyme activity as a means of identification. These enzymes were normally associated with the pathogenicity of the microorganisms and were known to play a part in the development of various lesions. The authors concluded that the various enzymes that were found indicate that many of the microorganisms present in root canals are potential pathogens and can produce periapical lesions.

Eight hundred fifty nine intrapulpal cultures were analyzed by Ostrander and Crowley. They found that 40% of the root canals were not infected prior to root canal therapy. Of the 119 teeth that exhibited periapical pathology, 60% were infected, with the organisms mostly cocci.

Hayes employed intracanal culturing and bacterial smears in his endodontic practice and concluded, 1) mostly cocci are found in periapical tissues; 2) 69% of 340 periapical samples were infected in specimens with carious pulp exposures; 3) 43.4% of the vital pulps were not infected and 76.2% of the non-vital pulps were infected, and 4) 20.5% of the vital teeth showed periapical pathology radiographically.

It was Grossman who was responsible for much discussion of intracanal sampling techniques. He has brought to surface various ramifications of sampling from within the canal and has discussed the use of paper points and the proper composition of growth media. He felt that beef heart infusion broth with 0.1% agar and tripticase soy broth with 0.1% agar were the best media. Grossman also touched upon the possibility of obtaining false negative cultures due to dry canals or insufficient amount of bacterial sample. He further stated that a false positive intracanal culture might be obtained because of unsterile paper points, cotton pliers, cavity surface, or cotton plug for the
test tube or because of a delayed transfer of the point from canal to
test tube.

Seltzer and Bender (1963) have done extensive work with both human
and dog specimens. They concluded that due to the myriad fallibilities
of intracanal culturing procedures, it is without reservation an ineffect­
tive "tool" in the bacteriologic control of endodontically-treated teeth.

There have been several unique intracanal studies conducted employ­
ing cannulas and stylets to sample the canal and periapical tissues. In
1918, Corriel introduced a completely new instrument for sampling the bac­
terial content of periapical tissues - the dental trocar and cannula.
It provided a sterile pathway by which a sample may be obtained. Several
other investigators have since used it or a modification.

Shindell attempted to determine if bacteria were present in peri­
apical areas of pathology just prior to completion of root canal therapy.
After mechanically and chemically cleansing the root canal, he used the
cannula and stylet so that he could study the bacteriology of the asso­
ciated periapical pathosis. The results of 63 specimens were: 1) 5%
of periapical tissues were initially infected; 2) 30% of the specimens
had positive pre- and post-reaming cultures and negative periapical cul­
tures, and 3) 65% had positive pre-reaming, negative post-reaming, and
negative periapical cultures. He found only staphylococci and strepto­
cocci when organisms were present. He did recognize that false negative
cultures might have been obtained due to the small quantity of bacteria
picked up by his stylets. On the basis of his study, he felt that granu­
lomas were essentially part of the body's defense and that most of the
periapical radiolucent areas were sterile; what few were positive, had
no practical significance. He also mentioned that the sodium hypochlorite solution used in root canal irrigation could have had a bactericidal effect on the periapical tissue bacteria.

The use of the trocar and cannula was made significant in a report by Hedman on the presence or absence of bacteria in the periapical tissues after conservative endodontic therapy. He used an intracanal cannula and stylet culture wire method on preoperative and prefilling cultures and employed periapical curettage for postfilling cultures. His preoperative cultures in 82 cases of pulp-involved anterior teeth which had periapical radiolucent areas, yielded 56 cases (68.5%) with viable bacteria in both canal and periapical tissues, 7 cases (8.5%) with bacteria in canal only, and 19 cases (23%) with no growth in either area. Therefore, 31.5% of the periapical lesions were not infected. It should be noted, however, that he did use chlorinated soda prior to his preoperative cultures for the removal of the pulp debris and intracanal sterilization of the periapex. After using camphorated monochlorophenol as the intracanal medicament and obtaining two successive negative prefilling cultures, he obtained 100% sterile periapical lesions employing periapical curettage for these postfilling cultures. By using chlorinated soda and an intracanal medicament, he did create some doubt as to the reliability of his bacteriologic conclusions. Wais believed that Hedman's study proved residual periapical infection did not exist after successful conservative endodontic therapy.

A modification of the trocar and cannula technique was used by Grossman, and called the new method an apiostomy. He defined an apiostomy as an operation whereby a sterile channel is created to obtain
cultures from the apical end of a tooth by cutting through the overlying soft tissue and bone using an apicostome, a trocar and cannula, in a dental handpiece. He modified Coriell's device by using a straight handpiece rather than a contra-angle. The assembled apicostome could be autoclaved and a radiopaque bone paste injected into the area penetrated by the trocar so that a radiograph would reveal when the lesion had been reached. In a study sampling 20 teeth with various periapical lesions from a few months to three years after treatment, Grossman used the apicostomy method. He obtained 19 negative cultures but five known untreated teeth with proven positive bacterial periapical infection yielded only three positive cultures even sampled with apicostomy. In another study using 49 human cadavers, Grossman drilled through the alveolar plate after sterilizing the mucosa. The trocar was then removed and the cannula left in place. Next, a sterile nichrome steel wire was injected through the cannula into the periapical lesion. In 49 attempts, Grossman reached the apical lesion only 80% of the time. Therefore, he obtained false cultures in 20% of the cases. It would seem that in the use of the trocar and cannula or the apicostomy method, a negative culture is of little significance since these methods are dependent upon reaching the root apex and it is difficult to be certain about this unless you fall into the lesion.

SURGICAL ROOT RESECTION

The use of the mucoperiosteal flap with a technique of resecting the apex of the root is another popular *in situ* method for sampling the bacterial periapical tissues. This method was first utilized by Garvin in 1919.
Grossman has discussed various methods of sampling periapical lesions. He used the root resection technique on 109 pulpless teeth with periapical radiolucencies and reported a 14.7% incidence of infection.

Genvert and Miller used healthy, mature monkeys in their study employing root resection. Of 93 roots with pulps exposed to the oral cavity, 58% of the apical areas were infected and 7% of these had no radiographic or histologic lesions. A known organism, *Streptococcus viridans* was introduced and sealed into 22 specimens which resulted in 53% recovery of microorganisms in periapical tissues.

A study employing anterior human teeth was conducted by Melville and Birch. They found a 35.3% incidence of infection in periapical tissues. The periapical and root canal bacterial flora were usually found to be identical. However, the authors noted that the periapical area and labial bone plate overlying the periapical lesion were occasionally contaminated by microorganisms carried down by the surgical sampling technique.

Cameron reports of his special surgical aseptic technique for obtaining apical scrapings of periapical tissue. In 19 human specimens, he concluded that in the absence of suppuration or sinus tract, no more than 17 to 34% of the non-vital teeth tested had bacteria in the periodontal membrane space.

Burn, in 1934, established the necropsy periapical specimens as a possible source of new information in the study of periapical bacteriology. He was the first to show significant value of post-mortem bacteriology. In analyzing the bacteria found in blood, viscera, and peri-
apical tissues of 93 human teeth, Burn found practically identical percentage of bacteria at necropsy except that there were different types of organisms. By a mucoperiosteal flap technique, he showed that bacteria in the apical lesions were those of normal flora. From the 195 apical specimens studied, he obtained 47% that were positive for bacteria.

It was Moller who decided to make a controlled bacterial examination of the apical tissues so that future studies could apply this knowledge to the role of infection in this region. He studied 1) the avoidance of contamination from the oral cavity; 2) the demonstration and avoidance of antimicrobial activity connected with endodontic sampling; 3) methods of taking microbial samples from apical regions; 4) the culture media most suitable for maintaining the bacterial reproductive capacity and for cultivating microorganisms in the sample, and 5) improved methods of microbial examination. Among his more pertinent findings was the development of a shielding method to maintain the sterility of the operating field when sampling from the apical region by periapical surgery.

Using this acrylic shield method on 15 cases with initially known infected apical regions, Moller found sterile results in all instances. Since the antiseptics used in intracanal endodontic medication remain active longer than had been previously reported, he recommended specific inactivators in the culture media to help prevent false positive cultures. When the apex is penetrated and only slight pathologic changes in the periapical area exist, he reported that one negative intracanal sample is sufficient to validate root canal sterility if an adequate sampling method is used. In all other instances, at least two negative samples are required. Microorganisms most frequently found in the
apical region were streptococci, lactobacilli, and gram-positive anaerobic bacteria.

In his earliest research, Burket thoroughly reviewed the literature on the methodology of bacterial apical sampling. He employed a resection technique to obtain periapical post-mortem material. He correlated the periapical bacteriology, clinical conditions, and radiographs. In a study of 445 teeth obtained from 138 necropsies, he correlated the gross and microscopic examinations of the blood cultures and the viscera with the medical records. He obtained a 49% incidence of bacteria in 419 periapical lesions. They were mostly gram positive organisms, particularly *Streptococcus viridans*. There were several other facts of interest: granulomas were infected 49% of the time; the larger the lesion, the greater was the tendency to mixed cultures; and the cultures made of the alveolar bone just before resection were negative in 34 out of 35 cases.

In another study in 1934, he employed a resection technique to gain access to the periapical tissues. Again he found mostly gram positive cocci and approximately the same percentage of organisms in the apical pulp and periapex. His results were 46% positive in the apical pulp, 22% positive in the periapex, and 6% positive in the coronal pulp.

**ANIMAL STUDIES**

Rickert and Dixon in 1931 were the first to use this somewhat unusual approach to the investigation of periapical bacteria. The initial studies involved implantation of hollow tubes of silver, platinum, and steel in rabbits. They described a hollow effect around open ends of hollow tubes and a reaction of tolerance and non-irritation around a solid glass rod. In 1966, Tornecch attempted to correlate the results
of these studies with the periapical tissues. He implanted polyethylene tubes filled with sterile and contaminated cellular debris into subcutaneous tissue of rats. Using Giemsa and gram stains, he found that in none of the specimens could microorganisms be identified in the tissue at or beyond the region of the tube orifice. The absence of microorganisms, which were gram negative cocci, demonstrated to Torneck the ability of the surrounding tissues, analogous to periapical tissues, to localize and eliminate these microorganisms.

BLOCK SECTIONS

The block section technique is not very popular due to its traumatic nature. It is most frequently employed in animal studies. Kakehashi, Stanley and Fitzgerald used block sections in a study of the pathologic change resulting from untreated experimental pulpal exposures in germ-free rats as compared to conventional rats. Using Brown and Brenn as the bacterial tissue stain, they noted that a few specimens of the conventional rats demonstrated microorganisms in the periapical tissues by the radicular foramen. It was their conclusion that the presence or absence of bacteria is the major determinant in the healing of exposed pulps.

BACTERIAL FLORA

Many investigators have studied the types and percentages of various bacteria of the oral cavity, root canal and periapical areas, of both humans and research animals. The most common microorganisms isolated from pulp canals, periapical tissues, and the oral cavity appear to be the same even though the variation in techniques of ob-
taining cultures, time of culturing, type of media, and method and criteria used have caused serious disagreement among investigators. Those which are most commonly isolated are the alpha hemolytic streptococci, Staphylococcus albus, and gamma non-hemolytic streptococcus. There have been reports of more than 30 other species. A sound statistical figure is that more than 80% of microorganisms isolated from non-vital root canals, periapical areas of pathology, and the oral cavity of humans and dogs are gram positive cocci, and 20% are gram negative organisms.

EXPERIMENTATION WITH ANIMALS

The validity of sound animal experimentation is dependent upon the similarity of response to the test animal and the human. The use of dogs in pulpal and periapical studies is quite common even though the anatomical configuration of the root apices are somewhat different. Dixon, Rickert, Orban and Torneck all concluded that in the dog the periapical and pulpal reactions are histologically similar to those of humans when subjected to similar irritation, whether it be chemical, mechanical, or bacterial. This supports Coolidge's contention that the reaction of all living tissue to injury and infection has a definite relation.

STAINING BACTERIA

The origin of most bacterial tissue stains can be traced back to 1884 when Gram devised the first tissue bacterial staining method. It was Conn, however, who introduced a counterstain to differentiate between gram positive and gram negative microorganisms.

Bacterial tissue stains make it possible to locate bacteria and study their distribution in root canals and surrounding periapical tissues. Bacteria can be identified by their morphology, motility,
grouping, growth behavior on culture media and staining characteristics. Bacterial tissue staining makes use of only two of these characteristics: morphology and staining characteristic.

There are many bacterial tissue stains other than gram stain or a direct modification of the gram stain. Some of these are Brown and Brenn stain, Nicholle's Carbol-Thiomeine stain, McCallum-Good posture stain, Brown-Hopp's stain and Weigert's stain. That stain which has been most frequently used to investigate bacteria in periapical and pulpal tissue studies is Brown and Brenn. Reeves and Stanley and Gier and Mitchell are two of the more recent studies that have employed this staining technique. Pulpal anachoresis was studied by Gier and Mitchell employing the Brown and Brenn stain and fluorescent antibodies to determine morphology and gram reaction and to furnish a guide for the identification of bacteria in tissue sections found in extracted and surgically removed teeth in dogs.

In 1931, Bulleid made use of Weigert's stain or the Murray-Drew method on 80 extracted teeth with attached periapical lesions to study the histology and bacterial incidence. He consistently found streptococci in the periapical lesions that could not correlate any particular organism with the type of periapical pathosis.

Fish undertook a classic study of bone infection by drilling holes into the jaws of guinea pigs and introducing alpha and beta streptococcus and Staphylococcus aureus. The results of his study were three zones of reaction around one infected central zone. From this study we have been able to develop a classic analogy to the infected dental pulp: the periapical tissue is not an infected area, but contains bacterial toxins liberated from within infected canals. Therefore, if irritant bacterial
toxins and bacteria within the root canal are removed, the periapical lesion will disappear.

An examination of the literature concerning the gram staining method of staining bacteria in tissues and other bacterial tissue stains demonstrates that successful stain, one giving sharp differentiation between gram positive and gram negative organisms, can be obtained by many variations of the above mentioned stains. Since results are of fairly uniform quality, selecting a stain becomes more of a question of technical skill, time necessary to prepare solutions, and actual technique involved in staining specimens.
CHAPTER III

METHODS AND MATERIALS

DEVELOPING A LABELED ORGANISM

In selecting the proper organism to be used in creating a transient bacteremia, certain specifications were sought. First, it must be capable of creating a full-scale effective bacteremia in the dog, such that the number of microorganisms in the blood would be of sufficient duration to be fixed by the inflammatory cells of the periapical region. Secondly, it must not be fatal to the experimental animal. Thirdly, it must be labeled in such a manner as to not only be seen in histologic sections but retrieved by conventional culturing techniques.

The organism selected was *Streptococcus fecalis*. A lyophilized sample was obtained from the microbiology department and grown in BHI broth for 48 hours. The labeling procedure was done by creating a mutant strain resistant to a specific dosage of streptomycin.

A mixture of sterile BHI agar was sufficiently cooled (54°C) before pouring and a prepared solution of streptomycin was added to create a concentration of 2 mg/cc. Slanted petri dishes were ½ poured with this agar mixture and topped with sterile BHI. After cooling, they were streaked with the *Streptococcus fecalis* (.5 ml per petri dish) and spread evenly with a sterile glass rod. As growth occurred more sparsely in the high-concentrated region, those mutant colonies were again grown in sterile BHI broth and the process repeated. When sufficient growth was obtained, a stock growth of the culture was maintained and a serial
dilution done to determine its exact growth limitations. The stock culture was kept refrigerated and re-innoculated into fresh sterile BHI broth at 7-10 day intervals. At each change of culture media, gram stains were done to insure purity of growth.

PILOT STUDY

Bacteremia is a condition that is easily induced but often times difficult to control. Since our intention in this experiment was to create a transient bacteremia, it was of paramount importance that we know the limitations of its process in the experimental animal. For this reason, a study was conducted to determine its duration in a 20 Kg dog.

Initially, it was thought that the vehicle for inducement of the organism by intravenous administration could remain its culture media, namely the BHI growth mixture. In a review of the literature, many experiments dealing with bacteremia take no special precautions and administer the original growth media directly. Since we were, however, greatly concerned with foreign contaminants in this experiment, it seemed less than wise not to re-suspend the organism in a pure vehicle for administration to the experimental animal and sterile saline was selected. A sealed stock laboratory-prepared bottle of sterile saline was obtained from the oral surgery department.

A fresh growth of Streptococcus fecalis was grown in 100 ml of sterile BHI broth. The growth was less than 24 hours old. Four centrifuge vials with 10 mls of broth in each were centrifuged at 10,000 RPM for ten minutes. The supernatent was discarded and the remainder of all four samples was resuspended in 20 ml of normal sterile saline. The result was a loose, milky mixture that appeared conducive to parental administration.
When obtaining and culturing multiple blood samples from a single experimental animal at short intervals, there are precautions that cannot be overlooked. The intention was to obtain 2cc samples of venous whole blood for the first hour after administration of the labeled organism at a time schedule of one minute, three minutes, 5, 7, 9, 12, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 minutes. Sixteen total samples were to be obtained. Initial attempts to obtain individual samples by repeated venous puncture resulted in confusion and collapse of vessels. Even strong, healthy vessels cannot maintain patency for that period of time, nor withstand the insult of repeated short-interval sampling.

A successful method was derived by using a venous catheter.* The device used was obtained from a hospital supply source and is commonly used on hospital patients for various laboratory purposes. When inserted far into the lumen of the vessel, both patency and a contamination-free pathway are insured throughout the procedure.

After intravenous injection into the front paw of the mongrel dog, the catheter was placed and whole blood samples taken at the specified time intervals. The samples were taken into heparinized vacuum tubes and then transferred to BHI agar plates and incubated for growth.

Results of 24-hour incubation revealed diminished growth at 35 minutes and no visible growth at 40 minutes. The clearing time for a 20 Kg healthy mongrel dog could be determined between 35 and 40 minutes.

*Abbot Laboratories, North Chicago, Illinois
EXPERIMENT

Four adult mongrel dogs, ranging in weight from 20-30 pounds, were given distemper vaccinations, dewormed, and were isolated for two weeks. All procedures were performed under general anesthesia induced by intravenous sodium pentobarbital in a dosage of 1cc/5lb body weight.

A radiographic evaluation was made of the lower quadrant of all four animals utilizing the Kasle radiographic technique for dogs. Teeth were selected on the basis of well-defined apices that were apparent radiographically. Whenever possible, the corresponding teeth in the opposing arch were used as controls. Whenever possible, the first lower premolar and the first and second lower molar were used. This gave a possible sampling of 12 apices per dog or a total of 24 test teeth with an equal number of control teeth in the opposing arch.

The materials used were as follows: 5" X 5" rubber dam (heavy gauge), sterile rubber gloves, sterile cotton applicators, sterile towels, matches, BHI broth in stoppered sterile glass tubes, blood culture equipment, bead sterilizer, rubber dam punch and forceps, assorted rubber dam clamps, rubber dam frame, dental floss, plastic instrument incubator, phenol, alcohol sponges, 80% alcohol, fresh zinc-oxide powder and eugenol, disinfecting metaphen, ultra-speed occlusal radiograph of film.

For each dog, a separate sterile tray set-up with the following items was used: mouth mirror, cotton pellets, cotton rolls, 2" X 2"

a) Pitman-Moore, Indianapolis, Indiana
b) Eastman Kodak Company, Rochester, New York
gauze, explorer, excavator, scissors, spatula, glass slab, cotton forceps, #557 and #8 carbide burs for straight handpiece, endodontic files assorted sizes, rubber stops, medicine dropper, medium sterile paper points contra-angle handpiece. All tray set-ups were dry-heat sterilized at 320 F for two hours.

Immediately before the experimental procedure was commenced, a sterile 21 gauge needle and a sterile 10 cc plastic syringe were used to draw a 5 cc sample of venous blood from the front paw of each dog after the area was shoved and wiped with disinfecting metaphen and 80% alcohol each for three minutes. The sample was placed in a sterile tube containing 3 cc of sodium citrate solution and gently agitated. For culturing purposes, 2 cc of this blood sample were transferred to a sterile tube containing 10 cc of sterile BHI broth and 15 cc of the sample were streaked on a plate of sterile BHI agar. These cultures were incubated at 37° C and observed.

The dog's mouth was kept open with a spring-type metal wedge apparatus over the cuspids opposite the quadrant where we were working. The rubber dam was applied to the teeth being worked on with the most anterior and posterior tooth being clamped and the rubber dam being carefully inverted with dental floss and plastic instruments. The rubber dam and clamps were then swabbed with disinfecting metaphen and 80% alcohol, each for three minutes. The bead sterilizer was turned on, the light adjusted, and the unsterile straight handpiece sheath removed. The sterile tray set-up was placed on a Mayo stand over the working area. The operator then put on sterile rubber gloves and four sterile towels were draped around the dog's head isolating the working area.

a) Star Dental Manufacturing Company, Conshohocken, Pennsylvania
b) Johnson & Johnson, New Brunswick, New Jersey
The tray set-up was opened and with a #557 carbide bur an occlusal opening was cut in the tooth through the dentin and the roof of the pulp chamber was removed with a #8 carbide bur. There was a conscious attempt at all times to adhere as close as possible to those procedures followed in a normal endodontic preparatory and instrumentation appointment. The contents of the pulp chamber were removed with a small excavator. Hemorrhage was suppressed by sterile cotton pellets and pressure. The orifice of each canal was located and initial endodontic files were placed within each canal to the point where a constriction was felt. At this point, radiographs were taken to determine the position of the instrument and the length recorded in relation to a non-variable point of reference. Due to the apical structure of dog teeth, a penetration of the apex into periapical tissue is not facilitated without force and rotary action combined. For this reason, the penetration was done by those endodontic a) reamers that can be utilized in a contra-angle handpiece. The rubber stops were set on these instruments to penetrate no more than 2 mm past the radiographic apex. Once this was accomplished with an instrument of sufficient size (#40) the remainder of the canal was prepared utilizing a flare* preparation and irrigation with sodium hypochlorite. At this time, the canals were dried with sterile paper points and a sterile cotton pellet placed within the chamber. The access was sealed with a medium-heavy mix of zinc oxide eugenol cement.

As mentioned previously, the opposing arch of each dog was utilized as a control. Again, the areas were similarly prepared for rubber dam

a) Union Broach, Long Island, New York
placement. In these teeth the access and instrumentation was the same except for violation of the apex. Upon initial instrument placement, a measure of the radiographic apex was taken and the endodontic files were set to at least 3 mm short of the radiographic apex. Again, the teeth were prepared by flare method, then dried and sealed with zinc oxide eugenol cement. At this point, all rubber dam apparatus and mouth props were removed and the animal was allowed to rest undisturbed under anesthesia.

At approximately one hour after preparation of the teeth, a suspended solution of the labeled *Streptococcus fecalis* organism in saline was injected intravenously into the front paw of the animal after it had been shaved and surface disinfected with metaphen and 80% alcohol for three minutes. A quantity of 10 cc was injected via a sterile plastic syringe. Again the animal was allowed to rest undisturbed while maintaining a sufficient level of anesthesia.

After a period of at least 45 minutes duration, all teeth were again placed under rubber dam and surface disinfected for culturing purposes. A fresh sterile tray set-up was used for each quadrant and the culturing technique utilized was that most commonly used for clinical endodontic cultures. The cement seal was disrupted and the sterile cotton pellet removed from the chamber. In the test teeth, a pre-measured sterile paper point was placed to a point beyond the apex to insure a sample of the periapical area. Great care was taken to avoid contact with the remainder of the root canal system. The paper point was placed in a solution of sterile BHI broth and incubated at 37°C. The control teeth were cultured to the point of created stop which again by pre-measured paper points was at least 3 mm short of the radiographic apex.
The media and incubation procedures for the control teeth were identical.

Upon completion of periapical and intracanal culturing procedures, a blood culture sample was again taken utilizing the same technique as the pre-operative blood culture. An overdose of Sodium Pentobarbital \textsuperscript{a)} was used to sacrifice the dogs.

An electric bone saw was used to remove the mandible at the angles and a bone saw was used to cut block sections after the muco-periosteum was dissected away. A minimum of vibration, trauma, and dessication was sought.

The specimens were placed in formalin for five to ten days, then decalcified in 5\% formic acid.

\textsuperscript{a)} Barb-Euthol, Haver-Lockhart Laboratories, Shawnee, Kansas
CHAPTER IV

RESULTS

CULTURE RESULTS

The results of the blood cultures, both pre-operative and post-operative, were consistent with those results obtained from the pilot study. The level of labeled organisms was sufficient to produce a culturable level of microorganisms due to the state of bacteremia. Those cultures taken after sufficient blood clearing time gave no evidence of culturable microorganisms indicating a reproducible blood clearing time.

The results of periapical cultures were overwhelmingly positive in all teeth that had undergone periapical insult. The growth of microorganisms in normal culture media and their subsequent healthy growth in selective media exhibited convincing evidence of localization of the labeled organism to these areas.

The control teeth exhibited minor growth in normal media which were subcultured and identified as contaminant due to technical error or faulty seal of the access cavity. The selective media growth, however, was negative for all control teeth. The lack of growth gave strong indication that the prepared canals were free of contamination by the labeled organism.

RADIOGRAPHS

Radiographic interpretation of these specimens was limited to use
while instrumenting the canal system. Since the time evolved from start to finish would not allow for clinically evident radiographic changes, the use of post-operative radiographs was not incorporated. It must be noted, however, that the radiographic image of instruments is the factor most heavily relied upon to determine its relation to the anatomical configuration of the root canal system. On this premise, the importance of radiographic interpretation becomes paramount.

**TECHNICAL LOSS OF SPECIMENS**

Two specimens from the experimental group were lost due to separated instruments within the root canal system. One control specimen was lost due to improper trimming by the technician.

**PERIODONTAL EXAMINATION OF THE EXPERIMENTAL AND CONTROL SPECIMENS**

All animals appeared to be free of periodontal disease and caries at the time the experimental procedure was initiated. At the time of sacrifice, those specimens which were subjected to gross overinstrumentation of the periapex exhibited a disruption of the apical portion of the periodontal membrane with a consequent apical periodontitis. The control specimens which had been instrumented considerably short of the radiographic apex showed no abnormal periodontology.

**HISTOLOGY AND BACTERIOLOGY**

The specimens were sectioned in such a manner as to incorporate the apical one third of the prepared canal as well as the periapical region. The use of three stains was incorporated to give a full exposition of both histologic and bacteriologic components in the field of observation.
Those sections stained with hematoxylin and eosin exhibited classic indications of early inflammatory response. An evident increase in polymorphonuclear leukocytic infiltration and numerous dilated capillaries. Since the periapical regions of the prepared specimens had been vigorously overinstrumented, some destruction of periapical components was evident. Evidence of hemorrhage and lacerated vessels gave microscopic evidence of direct trauma to the periapical structures. In many cases, the preparation due to instrumentation beyond the apex was evident well throughout the periapical boney structures. Even though this stain was not specific for bacterial components, their presence was obvious within macrophage or phagocytic-like cellular components. Though these were very early stages of inflammatory response, the presence of macrophages is very probable. It is thought that macrophages are present even in the early stages of inflammation but are often times overshadowed by the predominance of polymorphonuclear leukocytes. Along with the destruction of many vessels, a dilation and marginal cellular activity within several vascular components was evident.

Those sections stained with Brown and Brenn as well as gram staining techniques exhibited chromatically the presence of gram positive cocci within the phagocytic components of the inflamed area.

In all sections, there appeared a highly pigmented foreign particle. It remained undefined throughout extensive examination of numerous sections. It was decided that it was some systemic foreign particulate matter possibly related to the dirt of the experimental animals. Even this foreign particle, however, remained within the scope of valid results because it also was evident in phagocytic components of the inflamed area.
CHAPTER V

DISCUSSION

The results of this experimental exercise lead us to a pointed retrospective view of the canal preparation phase of endodontic therapy. An important factor that so often determines the end result of treatment and the post-operative success or failure of treatment is interpretation. We are dependent upon interpretation in all phases of treatment from pre-operative radiographic diagnosis to post-operative biopsy result. In keeping within a purely scientific framework, we wish to keep interpretation to a minimal level. This, however, is a difficult task since our methods of treatment are often based upon sound, scientific, knowledgeable...interpretation.

The root canal system is truly a fascinating one; unlike other systems of the human organism, its anatomical configuration can vary dramatically even though its physiologic commitment from subject to subject remains consistent. For example, in treating a respiratory problem, the clinician has the advantage of a consistent and repetitive anatomy with minor variations from subject to subject. In dealing with problems of the root canal system, this consistency cannot always be depended upon; in fact, it is the failure to treat each case with a uniqueness that is soundly scientific that often causes regrettable results.

The anatomy of the root canal system, more precisely its termination at the radicular foramen, has been given close investigation by many researchers.
Studies utilizing extracted teeth have revealed the position of this foramen in relation to the anatomical apex of the tooth to be dramatically unpredictable. It should also be noted at this point that our methods for determining this anatomical structure, namely radiographic interpretation, are at best a method lacking the exactness and accuracy which is needed. It has been said and is worth repeating that a two-dimensional diagnostic aid cannot give the fullest scope of interpretation. It cannot be denied, therefore, that sole dependence upon the most obvious diagnostic visible aid to endodontic therapy could most certainly facilitate an unintentional insult of the periapical tissues.

A misinterpretation of the foramen is not always the sole cause of periapical insult. There exists a natural tapering or constriction of the root canal space in normally developed tooth structure that facilitates proper endodontic technique. The mechanical preparation of the root canal system is executed in such a manner as to facilitate this constriction and allow a more readily accessible pathway to it without disrupting it. There are techniques that utilize the cutting surfaces of endodontic instruments in such a manner as to accomplish this task in a consistent, biomechanically sound manner. There are, however, other techniques which advocate and stress the use of these instruments in such a fashion that contradicts the natural taper of the root canal system. The end result is often a reverse of what is not only desired but imperative for the successful apical obliteration desired.

The materials presently available for obliteration of the root canal system are sometimes difficult to control. If we incorporate this hazard with the previous facts pertaining to diagnosis and preparation,
it is not difficult to understand that the insult of the periradicular space is more than a mere possibility. The consequence of this probable result of root canal therapy must, therefore, be considered not only from the perspective of successful endodontic results but that of sound and acceptable biologic principal.

Optimistic students of histopathology state that inflammation is the first stage of repair. This, of course, is a fact that will not be disrupted; however, along with its reparative initiation, the inflammatory response can involve systems that are detrimental to a balanced physiologic state. The phenomenon of anachoresis as seen in this experimental exercise is a specific case in point. Here we see the inflammatory response aiding in the persistence of periapical bacteria and other foreign particulate matter. Since a significant percentage of periapical specimens of endodontically involved teeth contain bacteria and since usually no attempt is made to sterilize or directly sample the periapical lesion during conservative endodontic therapy, the existing bacterial sampling technique and sterilization of periapical tissues should be studied more closely to decide if persistent periapical bacteria could be a significant factor in conservative endodontic failures.

The tissue bacterial sections of the experimental specimens and the culture results demonstrated quite conclusively that bacteria were present in the early stages of inflammation. The origin of the bacteria in this experiment were classified; however, there are numerous other possible pathways related to endodontic therapy. Exposure to infected pulp, alveolodental periosteum lymphatics, contiguous areas of periapical pathosis, periodontal pocket extension and general blood circu-
The histologic response to bacteria in primary inflammation is reported to actively involve the polymorphonuclear leukocytes, which are supposed to be the prime phagocytes of tissue bacteria. The tissue histiocytes are reported to be the most active phagocytes in the removal of necrotic tissue, dead and degenerated cells and particulate foreign matter. They are not as actively phagocytic to bacteria as are the polymorphonuclear leukocytes. Several of the macrophages in this study which stained for bacteria appeared to be histiocytic in nature.

It should be mentioned that even though the tissue response of the dog and man to identical irritation has been proven to be identical or very similar, certain anatomic, chemical and genetic factors could possibly alter this similarity and reflect doubt upon the transference of conclusions to humans from data obtained from dogs.

The diameter of the root canal, size of the apical orifice and delta-like arrangement of the radicular foramina in dogs are anatomic factors which differ vastly from humans. The host factors related to phagocytosis, bacteriostatic and bacteriocidal mechanisms of plasma fluid exudate, and the biological environment of inflammatory site could differ in man and dog. The fact that animals may possess genetic tendencies and susceptibilities which are in no way comparable to those of humans must not be disregarded.

The importance of obtaining a negative culture before filling a root canal is still a subject of controversy. The bacterial status of periapical lesions is seldom mentioned as a significant factor in conservative endodontic therapy. If all factors indicate a well-filled root
canal and yet periapical pathosis persists, the initiation responsible is generally felt to be infection remaining in the apical dentinal tubules or in the minute radial foramina, in many instances, a periapical cyst. However, several investigators concluded that after effective sterilization of the root canal, the periapical tissues still contain bacteria, and an attempt should be made to sterilize the apical tissues before obturating the canal or periapical healing may be delayed or non-existent. It has been shown that teeth with associated periapical areas of rarefaction give significantly less successful results than teeth with no such periapical radiographic pathosis after conservative endodontic therapy. The rationale of the results of incompletely filled root canals or periapical cysts is frequently relied upon for the cause of these failures.

A periapical bacterial population combined with pulp debris in an unfilled accessory canal or an unfilled portion of the main canal could lead to persistent radiographic periapical pathology after conservative endodontic root canal obliteration and account for some of our unexplained endodontic failures.
This was an experimental exercise in which an attempt was made to detect the presence of bacteria in the periapical tissues and correlate that finding with the phenomenon of anachoresis and the histology of the periapical area.

The lower posterior teeth of healthy mongrel dogs were used. The technique employed was that of intentional insult of the periapical area by use of an endodontic instrument placed at least 2 mm past the radiographic apex.

The corresponding teeth in the opposing arch, which were instrumented 2 mm short of the radiographic apex, acted as controls. In all procedures, an attempt was made to adhere as closely as possible to clinical endodontic technique.

After a sufficient period of time which allowed for the initial inflammatory response to occur, the dogs were injected with a labeled organism (Streptococcus fecalis). This organism had been previously prepared and was a mutant strain capable of growth in streptomycin. Blood clearing time was determined by a pilot study.

At the time that blood cultures gave no indication of bacteremia, the periapex of the overinstrumented teeth were cultured by conventional endodontic means. The use of sterile paper points were utilized for this procedure.

The dogs were then sacrificed and histologic sections of the
periapex prepared. The stains utilized were Hematoxylin and Eosin, John's Hopkins Modified Gram Stain, and Brown and Brenn stain.

Histologically, the periapical response was consistent. In those teeth that had suffered overinstrumentation varying degrees of inflammatory response were evident. In some cases, there was evidence of hemorrhage and tissue laceration. The activity and components of an early inflammatory state were obviously present in all specimens.

The use of Brown and Brenn stain accentuated the presence of the microorganism. The darkly stained gram positive cocci were evident in macrophage-like structures giving indication of their fixation in the area of inflammation.

This study indicates that the phenomenon of anachoresis can be successfully demonstrated in the periapex as it has been in other systems. It emphasizes the histological consequences of periapical insult and leads the way to a possible correlation between inflammation, the presence of bacteria and the effect of these states upon successful root canal therapy.
CHAPTER VII

REFERENCES


CHAPTER VIII

APPENDIX

FIGURES
Figure 1: Photomicrograph showing isolated phagocyte containing bacterial contents, H & E stain.

Figure 2: Photomicrograph showing scattered bacterial components both extracellular and intracellular. Brown and Brenn stain.
APPROVAL SHEET

This thesis, submitted by Peter J. Lio, has been read and approved by three members of the faculty of the Graduate School.

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

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

5/14/74
Date

Franklin S. Weine, D.D.S., M.S.D.

Signature of Advisor