A Microbiological Investigation of Root Dentin in Pulpless Teeth

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A MICROBIOLOGICAL INVESTIGATION OF
ROOT DENTIN IN PULPESS TEETH

by

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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

JUNE
1972
CURRICULUM VITAE

Richard Bence was born in Milwaukee, Wisconsin on July 29, 1943. There he obtained his grade school and high school education.

In 1961 he entered Marquette University where he was accepted to the School of Dentistry in 1964. He graduated with the degree of Doctor of Dental Surgery in June, 1968.

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DEDICATION

I dedicate this thesis to my devoted wife, Connie, whose love, patience, encouragement, and many sacrifices have enabled me to realize my goals.
ACKNOWLEDGEMENTS

It is with sincere appreciation that I thank my research advisor, John V. Madonia, D.D.S., Ph.D., Chairman, Department of Microbiology, for his helpful suggestions and guidance in preparation of this thesis.

My gratitude is given also to Franklin S. Weine, B.S., D.D.S., M.S.D., Director of Graduate Endodontics and Marshall H. Smulson, D.D.S., Chairman, Department of Endodontics, and other faculty members for their encouragement and immeasurable contribution to my education.

I am especially grateful to Michael A. Heuer, D.D.S., M.S., who has the uncommon ability of incorporating vast knowledge with logical thought. He will continue to be an inspiration to me.
# 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>2</td>
</tr>
<tr>
<td>III. Methods and Materials</td>
<td>48</td>
</tr>
<tr>
<td>IV. Results</td>
<td>57</td>
</tr>
<tr>
<td>V. Discussion</td>
<td>68</td>
</tr>
<tr>
<td>Conclusions</td>
<td>75</td>
</tr>
<tr>
<td>VI. Summary</td>
<td>76</td>
</tr>
<tr>
<td>Bibliography</td>
<td>77</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table | Page
-----|-----
I. Teeth Treated | 49
II. Efficiency of Instrumentation in 28 Teeth | 58
III. Viable Bacteria within Dentin Sample | 61
IV. Effect of Sodium Hypochlorite Irrigation | 63
V. Second Appointment Cultures of 15 Teeth with Two Negative Post-irrigation Cultures at the First Appointment | 64
VI. 218 Comparisons of Sampling Methods | 65
VII. Comparison of Incubation Periods | 67
CHAPTER I
INTRODUCTION

The utilization of an aseptic technique, sterilization of the root canal and thorough debridement, sterilization and obliteration of the root canal are the essential prerequisites for successful endodontic treatment. Many investigators have reported that clinical success in endodontic therapy is related to the microbiological status of the root canal at the time of canal obliteration.

Nineteenth century reports reveal that dental practitioners relied on the clinical condition and the absence of pus, odor or patient discomfort as indicators of insufficient root canal preparation. Later microbiological culturing techniques were employed to establish an objective impression of root canal sterility.

Bacteriologists formulated several culture media capable of isolating the organisms present in infected root canals. As part of this application of microbiological principles, various irrigants have been used to irrigate and cleanse the root canal of necrotic debris and intracanal medicaments have been placed to suppress bacterial growth. In addition, several methods for sampling root canal contents
have been advocated. However, the reliability of bacteriologic-ical controls in root canal therapy has resulted in over sixty years of controversy.

Studies concerned with the effect of thorough chemomechanical instrumentation have shown that emphasis on adequate debridement will result in fewer infected canals. The use of nonspecific medicaments and antibiotics within the root canal have also been proposed as an effective method of root canal sterilization. However, several investigators have shown that intracanal medicaments are most effective after thorough debridement of the root canal.

Histologic examinations of extracted teeth have revealed that bacteria are present in the predentin and to a lesser extent in the dentinal tubules. The purpose of this study is to acquire further insight into the optimal technique of root canal debridement. Information regarding the presence of viable bacteria within the dentinal tubules will be acquired indirectly through examination of dentin shavings removed from the walls of the root canal. This investigation will also compare two sampling techniques for endodontic bacteriological cultures.
CHAPTER II
REVIEW OF THE LITERATURE

In 1893, Schreier advocated the introduction of kalium and natrium into the root canals to obtain disinfection. It was considered easier to convert the septic contents into an aseptic condition than to mechanically cleanse the root canal.

As early as 1901, Onderdonk recommended the use of a bacteriological test to determine the presence of infection in a root canal. He insisted that a scientific test, as the bacteriologic culture, was necessary, with the absence of periapical pain as a prerequisite for filling the root canal. Onderdonk recommended culturing the root canal after thorough disinfecting procedures to insure that an aseptic root had been secured.

Coolidge, in 1919, reinforced Onderdonk's theory that root canal problems are both mechanical and bacteriological. He conceived the idea of verifying the sterility of root canal treatment to the scientific level of treatment employed in other medical specialties. Coolidge also urged removal of pulps under aseptic conditions rather than waiting until the pulp dies and becomes infected. It was recommended
that the intracanal antiseptic and filling material should remain within the canal to avoid irritation of the periapical tissues.

Blayney, in 1928, stated that thorough instrumentation and debridement of the root canal are more important than the placement of medications. He did not list a negative bacteriologic culture as necessary for filling the root canal. However, he did point out that the filling material must hermetically seal the pulpal end of the dentinal tubules and obliterate any spaces where fluids may collect and serve as culture media.

Appleton, in 1932, discussed an earlier study by Rhein and his co-workers. He concluded that of the cases which were deemed successful by clinical and radiographic exam, those whose last culture prior to filling was negative outnumbered those whose last culture before filling was positive, at a ratio of more than two to one. He concluded that it is desirable to apply this bacteriological control whenever an effort is made to retain the tooth.

That most dentists probably do not use bacteriologic cultures to determine root canal sterility was voiced by Grossman in 1936. He enlisted the cooperation of several dentists to give their clinical impression of when a root canal was ready to fill. At that time, a culture was taken
and incubated in a hormone broth. A correct guess of the sterility of the canal occurred 58 percent of the time. Grossman asserted that the great need for bacteriological control was apparent.

Fish and Maclean, in 1936, have demonstrated that even though the bacteria can be found in the pulps of teeth, the neighboring alveolar bone is often sterile.22 Tunnicliff and Hammond in 1937, showed that the presence of bacteria in pulps is not, per se, evidence of infection.91

That endodontics must be performed only under conditions of strict asepsis was affirmed by Yates and Morse.95 The old method of determining the condition of the root canal by the absence of odor or moisture was proclaimed inadequate, in view of the existing knowledge. They pointed out that the use of a bacteriologic control is neither expensive nor complicated. Two consecutive root canal cultures were considered necessary for canal obliteration. Failure to obtain negative cultures contraindicated further root canal treatment.

Buchbinder studied 151 cultured and 94 non-cultured cases with necrotic pulps.11 A statistical analysis of the groups revealed that significantly better results were obtained when the bacteriologic culture technique was utilized. Eight percent of the cultured cases and 18 percent
of the non-cultured cases failed to show radiographic improvement. He concluded that the advantages of culturing are of a "common sense" nature.

Strindberg considered procurement of a bacteria-free condition of the root canal as the primary objective of conservative root treatment.\textsuperscript{89} Bacteriological samples should be taken from both the root canal walls and from the periapical tissue. He recommended using a coarse file to remove dentinal chips from the canal wall. These are then washed in saline which is automatically pumped out through the apex, where it is mixed with tissue fluids. The contents of the canal are then sucked up with sterile absorbent points which are immediately transferred to the cultivation medium.

After a 1940 study, Sommer and Crowley concluded that: (1) many types of bone characteristics which appear to be abnormal may be normal for certain individuals and do not necessarily indicate the presence of infection; (2) too much emphasis should not be placed on the radiograph as a diagnostic agent in condemning questionable teeth; (3) it is not possible to correlate the periapical disturbance and the specific organism possibly responsible for it; (4) in view of the rarity of proven cases of systemic involvement associated with teeth that have radiographic involvement and bacteria present, there is considerable question as to the importance
of the pulp-involved tooth as a focus for infection; (5) vitality responses do not coincide with either radiographic or bacteriologic findings.85

The effects of nine solvents on the pulps of extracted teeth were tested by Grossman in 1941.29 Chlorinated soda solution was found to be by far the most effective in vitro.

Morse and Yates, in 1941, believed that root canal therapy was not justifiable unless careful, consistent checks were made at proper intervals following the completion of treatment.61 Such checks offered an excellent opportunity for study, in light of the original pathologic condition and the types of bacteria present.

A complete physical history and thorough oral exam was considered necessary for careful case selection and every treatment was carried out under strict asepsis in order to validate the results of bacteriologic tests. Types of organisms present in each culture are recorded and two consecutive negative bacteriologic reactions are obtained before the canals are filled.

Morse and Yates re-emphasized that in light of the existing knowledge of biologic processes, the old method of determining the condition of the canal, by the absence of odor or moisture, can no longer be considered adequate.
Their report concerned 265 completed cases that had been clinically and roentgenographically followed for one to seven years from the time of placing the root canal filling. The results found at the follow-up examination were classified according to radiographic appearance and group comparisons were made. Identification of the organisms found in the positive cultures was attempted. Nearly one-third (29%) of the primary cultures were negative according to routine aerobic method. The positive cultures were about equally divided between single organisms and mixed infections. Gamma type streptococci were found most frequently, followed by *Staphylococcus albus*, *Streptococcus viridans*, and *Staphylococcus aureus*.

Morse and Yates concluded that the great majority of cases showed radiographic improvement, with complete bone replacement occurring in nearly one-third of the cases. Only 7 percent of all the cases reported failed to show improvement and in only three cases could retrogression be demonstrated. Filqueiras in 1942, stated that it is not rare to obtain cultures of streptococci from absorbent points removed from clean, dry and odorless root canals. He has also observed that, frequently, absorbent points removed from root canals saturated with pus-like exudate, when cultured for seventy-two hours showed no growth. This further verified
the belief that clinical symptoms are unreliable and unscientific as indications for root canals.

Filqueiras believed that the test for sterility of root canals by bacteriologic cultures should consist in not only demonstration of a growth or lack of growth of microorganisms in the culture medium but also a microscopic examination to determine the type of microorganisms that may be growing in the culture. It is important to know whether the microorganisms are saprophytic or pathogenic. Streptococci and staphylococci are usually considered pathogenic when found in root canals, while Micrococcus catharrlis, deptheroids and Bacillus subtilis are saprophytic microorganisms that frequently are found in the culture. The presence of these saprophytes usually indicates contamination from an improper technique either clinically or in the laboratory.

Filqueiras stated that forty-eight hours is the minimum period to leave the dressing sealed in the tooth which can be used as a test for growth and the principal bacteria in dental infections were streptococci and staphylococci. For the most accurate test for sterility of the root canals, he recommended that three negative cultures should be obtained with only a dressing of sterile saline solution employed between the culture tests.
He emphasized that sterility of the root canal does not mean cure. Cure is dependent on the patient's good health. The sterility test is used only as an indicator as to when to stop the treatment and fill the canal. It is an indicator of scientific value, but has nothing to do with therapeutics. It is proper to expect that the root canal treated under these directions and thus filled will afford a favorable prognosis. If infectious conditions persisted after the third bacteriologic test according to Filqueiras, the tooth must be extracted.

Hayes, in 1943, subjected three hundred and forty root canal cases to diagnostic procedures. Vitality tests, x-rays and bacteriological tests were made in all cases. Attempts were made to correlate the findings with each other and with those of other investigators.

It was discovered that vital pulps were primarily infected by non-hemalytic streptococci, *Streptococcus viridans* and *Staphylococcus albus*, while the non-vital pulps and the x-ray positive group contained a predominance of staphylococci and combinations of bacteria. Hayes concluded that too much emphasis on the importance of bacteria in the pulp canal is misleading.

In 1943, Grossman declared removal of debris from the canal as one of the most neglected phases of root therapy.
Clorinated soda, as a root canal irrigant, was recommended in preference to other solutions because it had been shown to be a most effective necrotic tissue solvent. In combination with hydrogen peroxide it effervesces and liberates nascent oxygen. Use of a medicament in the canal will result in a better disinfectant action if the canal had been properly cleansed by mechanical means and adequately irrigated.

From the results of previous investigations, Shay found evidence that a more efficient medium for culturing root canals was required; a medium suitable for culturing root canals that would promote growth for both aerobic and anaerobic types of organisms. He proved two media acceptable for clinical purposes. Of the 184 positive root canals cultured, 97.8 percent were positive in trypticase dextrose broth and brain heart infusion. The frequency of the appearance of organisms was found to be in this order: (1) streptococcus gamma type, (2) \textit{Staphylococcus albus}, (3) streptococcus beta type, (4) \textit{Bacillus subtilis}, (5) streptococcus alpha type, (6) \textit{Staphylococcus aureus}, (7) \textit{Lactobacillus acidophilus}, (8) yeast, and other miscellaneous organisms.

Shay explained that positive root canal cultures are not all pure; there are a few mixed cultures. He insisted that strict anaerobic conditions are not necessary for cultivation of bacteria from root canals.
A 1948 study by Ostrander and Crowley found the predominating infecting organism in root canals to be streptococci with the order of importance being: (1) *Streptococcus anhemolyticus*, (2) *Streptococcus viridans*, (3) *Streptococcus hemolyticus*. The latter organisms accounted for only a very small proportion of the infections.

Hedman, in 1951, studied fifty-six patients with upper anterior teeth having bacteria present in the root canal and in the periapical tissue. In every case, when two consecutive negative root canal cultures were obtained, no bacterial growth was found in the periapical cultures.

Grossman, in 1952, reported that in one hundred cases in which culture tubes were negative after two or three days, the tubes were incubated for an additional week at 37°C and reexamined for growth. In only one case was growth present after ten days which had not been present after incubation for 48 hours. He indicated that if growth is not present in two or three days, it is not likely to be present at all.

In 1953, Auerbach pointed out that successful endodontic results have been achieved for previously infected root canals, prior to the use of antibiotics. Simpler dressings can duplicate the results attributed to poly-antibiotics, he contended.
In his study, Auerbach obtained 44 sterile root canals in 56 infected cases, solely by canal debridement and cleansing, i.e., seventy-eight percent of the positive canals became negative without the benefit of any antimicrobial dressing.

Grossman, in a discussion of Auerbach's article, stated that mechanical debridement and canal cleansing is without a doubt the most important phase of root canal treatment. It is an axiomatic principle of surgery that all necrotic material and debris must be removed, before a wound is ready for chemotherapy.

Grossman stated further that Auerbach had reported a study of canal instrumentation plus a chemical solution, not instrumentation alone. The chlorinated soda used as an irrigant has an antibacterial effect. In a study of this kind there must be complete omission of any antibacterial agent, whether it be chemical solution or even plain tap water.

The value of taking a culture without first sealing a sterile absorbent point in the canal for at least 24-48 hours was questioned. Cultures taken several days after flooding the canal with an antiseptic solution frequently yielded a bacterial growth, if the sterile absorbent point had been sealed in the canal. Grossman also insisted that
complete treatment of the root canal cannot be consummated in less than three visits.

Stewart, in 1955, was able to render 47 of 50 infected canals sterile after an initial instrumentation and irrigation.87 A second culture taken at the second appointment yielded negative cultures in 38 of the 50 cases. An 18 percent error was evident between the two appointments. The first appointment success in rendering the growth free cultures was attributed to the antibacterial effect of remaining irrigants and an inadequate sample of microorganisms due to their decreased number. Stewart re-emphasized the statement of Filqueiras that a sterile canal does not insure that success will follow. The ultimate criteria for success was clinical responses.

Maisto, in 1955, acknowledged that a controversy existed regarding the value of bacteriologic cultures in endodontics.53 He stated that periapical pathology needs treatment regardless of the bacteriologic status of the root canal and the best criteria of success was the healing of a radiolucency. Maisto postulated that bacteriological control has no place as a routine procedure in everyday practice, although it may be useful in exceptional cases. He hypothesized that bacteria remaining in the root canal are prevented from reaching the periapical tissue by hermetically sealing
the apical foramen.

Four independent surveys on the bacteriology and pathology of the pulpless tooth were carried out by Cran in 1956. He stated that it is almost certain that many potentially pathogenic bacteria may live in the root canal of a pulpless tooth for years without producing infection. Changes in the resistance of the host produces changes in the bacterium-host relationship.

In the treatment of the pulpless tooth, Cran agreed that adequate mechanical preparation of the root canal must take priority regardless of what intracanal medication is used. Jolly and Sullivan, in 1956, stated that it is unwise to assume that a vital pulp with a carious exposure leaves a sterile root canal when the pulp has been removed. Observations of decalcified sections of dentin showed that infection of the dentinal tubules occurred; however, this was uncommon. If the canal was infected the bacteria were in close proximity to the root canal. Therefore, enlargement of the root canal by a thorough reaming and filing motion would appear to be the most effective method for removal of this infection.

Blechman, in 1957, stated that bacteriologic cultures are a time-saving procedure in treating of root canals. While the presence of microorganisms does not prove
that an infection exists, their ability to invade and multiply in the tissues of the host cannot be minimized. Blechman reported that 82 percent of 357 positive cultures contained streptococci. Fifty-three percent were pure streptococci cultures.

According to Blechman, a negative culture was obtained in the absence of bacterial growth. The culture medium will remain clear when: (1) the canal is sterile; (2) too few microorganisms are present to initiate growth; (3) the sampling is inadequate; (4) an inhibiting concentration of antimicrobial agent has been carried over; and (5) the medium will not support the growth of the more fastidious microorganisms.

That the application of bacteriologic methods to endodontic practice complements sound clinical techniques and judgement, is reaffirmed by Blechman. It serves as a continuous control and measure of aseptic technique, efficiency of drug treatment and surgical debridement. It restores confidence in the practitioner by providing objective evidence to substantiate his skill and competence.

A high incidence of streptococci, diptheroids, and micrococci were found in the root canals of 70 patients treated by Brown. Mixed infections were prevalent. Microbial forms were observed in 90 percent of the specimens.
when the phase-contrast and dark field microscopes were used to examine the initial suspension. Direct stained smear technique reveal organisms in only 71 percent of the specimens. This difference emphasized the importance of using the phase-contrast and dark-field microscopes in definitive studies. Microbial forms that were not cultivatable were repeatedly observed microscopically.

MacDonald, et al, in 1957, investigated the bacteriologic status of the pulp chambers of 46 intact teeth found to be non-vital following impact injury. Aerobic and anaerobic cultural methods obtained growth from 38 teeth with one to six strains in each tooth. A total of seventy-one strains were isolated, of which twenty-three were anaerobes. It is postulated that these organisms reached the pulp from the oral cavity via the lymphatics and blood vessels of the periodontium.

In 1958, Ingle and Zeldow worked with sixty-five teeth that were found to be infected at the initial appointment. Immediately after complete mechanical instrumentation, the presence of sterile distilled water irrigation, 13 of the 65 infected teeth (20 percent) yielded a 48 hour negative culture. At a subsequent appointment and without intracanal medication, only 3 (4.6 percent) of the original 65 infected teeth yielded two successive negative cultures; 95.4 percent
of the canals remained infected.

They verified that mechanical instrumentation and irrigation with sterile distilled water proved to be a very inefficient method of sterilizing the root canal and that the negative culture does not necessarily imply sterility of the root canal. The authors suggested that a critical re-evaluation of the entire problem of the so-called "negative culture" and "sterile" root canal is needed.

In 1959, Hobson described experiments which indicate that neither organisms, antiseptics nor antibiotics appear to penetrate to any extent into the dentinal tubules from the root canal. A number of freshly extracted teeth were placed in culture media infected with mixed mouth organisms, and incubated for varying times. They were fixed in 10 percent formalin, sectioned and stained by Gram's method, to observe the depth of penetration of bacteria into the dentinal tubules from the pulpal end.

There was a surprisingly small degree of penetration into the tubules and organisms were rarely found beyond the zone of predentin, most of them remaining in the pulp chamber and canal.

Leavitt, et al, discovered that the bacterial flora of infected root canals presented a much wider and different variety of organisms than that disclosed by one of the media
commonly used in endodontics (dextrose broth). Trypticase soy broth plus 0.1 percent agar, a relatively new aerobic-anaerobic culture medium, disclosed anaerobes in percentages comparable to those shown by strict anaerobic laboratory techniques, and at the same time was shown to be particularly sensitive for most aerobic growth.

In 1959, Winkler and Van Amerongen discussed the bacteriologic results from more than four thousand root canal cultures. Streptococci formed 61 percent of the isolated organisms and seem to be the pathogens. All other organisms were considered to be chance contaminants.

The chance of carrying bacteria from the carious cavity into the root canal during the opening of the canal is relatively great. Consequently, they believed that many cultures from sound teeth will be positive due to this type of contamination.

The permeability of root dentin was studied by Marshall, et al, in 1960. Freshly extracted teeth immersed in 0.025 percent gentian violet dye showed with striking clarity a penetration of the dye into all areas of the dentin in the crown and in the root but no penetration at all in the apical area of the dentin.

Shovelton and Sidaway obtained cultures from previously unexposed root canals of 147 teeth upon opening.
Growth was obtained from 110 of the canals and a high frequency of isolation of obligate anaerobes was noted. There was no apparent correlation between the radiographic appearance of the periapical tissues and the bacteriological status of the root canal before treatment. It was suggested that rarefaction of bone in the apical region in the absence of demonstrated infection in the root canal was due to irritation from productions of protein decomposition following death of the pulp.

In 1961, Engstrom and Frostell performed a bacteriological investigation on 36 teeth with non-vital pulps, the pulp cavities of which were intact. Teeth with superficial carious lesions, restorations or enamel fractures were accepted providing the pulp cavity was surrounded by hard dentin. The samples were cultured aerobically and anaerobically on solid and in liquid media. Smears were taken from the pulp debris for microscopic examination. All the samples were taken from the root canals when the pulp chambers were opened for the first time. Microorganisms were found in 26 teeth; cultivated from 21 teeth and in the other 5 found in smears. Eleven out of the 26 infected teeth had intact crowns, i.e., no fractures or caries were present.

In the discussion, Engstrom claimed that the literature on the bacteriology of the non-vital pulp is partly
Stewart, et al, noticed from the results presented in previous papers that the repeated flushing of the canal with hydrogen peroxide and sodium hypochlorite seemed to yield a higher percentage of growth-free cultures than when the hypochlorite solution was used alone and the canal was then flushed with sterile distilled water.

Crawford and Shankle examined root canal specimens from 57 teeth by aerobic and anaerobic culture on agar media. They realized that root canals may contain microorganisms not detected by culturing and recommended using phase-contact and dark-field microscopy for detection. Crawford stated that although there is no available information from controlled studies to indicate which type of microorganisms commonly cause post-operative difficulties and which cause failures after canal obliteration, culturing is accepted as an advisable procedure.

In 1961, Seltzer, et al, referred to medications as possible tissue irritants. They questioned whether medications reduce inflammation, stimulate repair or prevent or lessen pain. Thorough debridement of the root canals appeared more important as it removed large numbers of microorganisms as well as necrotic tissue and inflammatory products, allowing body defenses to become adequate for healing in spite of incomplete sterilization.
Sciaky and Sultizeneau stated that if the selection of teeth for endodontic treatment is not limited to the first stages of infection, but also includes gangrenous teeth, 9 out of 10 involved teeth will be infected. Out of all infected teeth, more than ten percent are caused by purely anaerobic or a combination of anaerobic and aerobic microorganisms. Thus, purely aerobic media do not fulfill the needs for the determination of sterility of root canal environment if the need for sterility is taken so as a prerequisite. Furthermore, close to 60 percent of the infected canals contain only one type of microorganism, 30 percent contain two types. Of all microorganisms, both in single and combined cultures, streptococci were the most frequently found.

Sommer, et al, in 1962, declared that the main purpose of root canal therapy was to determine whether infection was present and, if present, to eliminate it so that the tooth may be restored to a condition impervious to future infection. Infection was defined as tissue damage caused by microorganisms.

A positive culture may indicate either infection or contamination. A negative culture indicates that microorganisms are absent or present in such small numbers that they cannot initiate growth in culture media. One of the most common reasons that false negative cultures are obtained
is that the dentist does not insert the inoculum point the entire length of the canal. Failure to sample material from the apical third of the canal may result in negative cultures even though bacteria are present, as this area is more apt to contain organisms than is the remainder of the canal.

Oliet, in 1962, conducted a preliminary clinical study of 98 endodontic cases which were completed in an effort to evaluate the use of a culturing technique in root canal therapy. The assumption that a better prognosis can be expected when a root canal is filled following negative cultures seems to be consistent with previous thoughts on this subject. Cases with a negative culture did better regardless of location, patient age or original diagnosis. Oliet recommended routine use of a proper culturing technique in order to obtain the most favorable prognosis possible for the patient.

Fox and Friedman stated that taking a bacteriologic culture requires less than two minutes, and its proper execution expediates completion of almost all root canal cases for the general practitioner. A survey was conducted to demonstrate that the average practitioner usually requires four visits to complete endodontic treatment on a supposedly infected pulpless tooth. It appeared that the institution of bacteriologic culturing techniques would permit completion
of at least 85 percent of all endodontic cases in two visits with a minimal expenditure of efforts and expense. They emphasized that asepsis, chemomechanical preparation of the root canal and its proper filling are prime requisites for successful endodontics.

Sicher, in 1962, stated:

The course of the dentinal tubules is somewhat curved, resembling an "S" in shape. Starting at right angles from the pulpal surface, the first convexity of this doubly curved course is directly toward the apex of the tooth. In the root and in the area of incisal edges and cusps, the tubules are almost straight. Over their entire lengths, the tubules exhibit minute, relatively regular secondary curvatures that are sinusoid in shape.

The ratio between surface areas at the outside and inside of the dentin is about 5 to 1. Accordingly, the tubules are farther apart in the peripheral layers and are more closely packed near the pulp. In addition, they are wider near the pulpal cavity (2 to 3 microns) and become narrower at their outer ends (1 micron). The ratio between the numbers of tubules per unit area on the pulpal and outer surfaces of dentin is about 4 to 1. Near the pulpal surface of the dentin, the number per square millimeter is said to vary between 30,000 and 75,000. There are more tubules per unit area in the crown than in the root. 84

Nichols found 49 percent of 134 contaminated root canals yielded negative bacteriological specimens after careful reaming and filing, followed by irrigation with antiseptic fluids. 64 He attributed the higher percentages quoted by others as probably due to their more frequent irrigation of the canal.
The findings of Nichol's study support the conclusion of Ingle, (1958) that the reduction in bacterial population resulting from cleansing of a contaminated root canal is to some extent associated with the antiseptic effect of the fluid used in irrigation. Neither the periapical status nor the type of irrigant used revealed any apparent difference in the incidence of negative cultures.

According to Burnett and Scherp, if pathogenic streptococci are to be demonstrated by direct plating of a throat swab, at least 1000 viable streptococci must be obtained on it before they can be demonstrated. For isolation and identification, the streptococci must initially be obtained on a selective medium, then transferred to an appropriate broth and plated on blood agar, and finally identified by immunological analysis.

Garber, in 1963, stated that wiping out the root canal with paper points prior to taking the cultures reduces the inoculum, thereby reducing the accuracy of the culture results. His experiment proved that the first and second paper points placed into the canal gave the most reliable indication of bacterial growth. The third and fourth points were not as reliable as the first two.

Zeldow and Ingle reported on 89 single canal teeth treated by faculty members at the University of Washington.
The canals were enlarged until clean white dentinal shavings were obtained. All cases were filled at the second appointment regardless of bacteriological status. The success or failure of the cases was based on clinical and radiographic observations two years after treatment.

Eighty-three percent of those cases filled in the presence of cultivable bacteria and 93 percent of those having negative cultures were judged successful. From Zeldow and Ingle's study it is apparent that the mere presence of bacteria is not the determinant of success or failure. Virulence and numbers of microorganisms contained in the infected root canal, periapical area, or both, were apparently the primary antecedents.

Grahnen and Krasse, in 1963, used three groups of single canaled non-vital teeth to study the effects of instrumentation and flushing with various agents. The flushing agents used in the different groups were saline, a quaternary ammonium compound (Biosept), and a polyantibiotic (Nebacetin).

With the use of saline as a flushing agent, bacteria disappeared from the root canal in 48 percent of the cases at the second appointment. The corresponding figures for the quarternary ammonium compound and the antibiotic were 54 and 81 percent respectively.
Gurney proclaimed that it is a well established fact that bacteria in a tooth are seldom found solely in one locality, as the pulp chamber or a canal, but instead are found throughout the whole tooth substance, especially the dentin tubules. They can and do migrate toward the cementum, regardless of pulp vitality. It follows, then, that a pulp chamber and canal shown to be sterile at one time may not necessarily be sterile at another time.

Large numbers of bacteria are removed during withdrawal of the soft tissue, and with the first increments of dentin as the canal wall is filed and reamed. According to Gurney, as enlargement of the canal proceeds, the number of bacteria per unit area in the dentin tubules decreases, but at the end of instrumentation significant numbers still remain in the tubules.

Gurney considered sodium hypochlorite and hydrogen peroxide as helpful irrigants during canal instrumentation. The hypochlorite solution, in addition to its penetration enhancement, has a wide and effective antimicrobial spectrum, is fast acting, dissolved pus and necrotic tissue exudate, dissolves certain high molecular weight proteins, dissolves inorganic material under certain conditions and is a powerful deodorant.
Heuer, in 1963, stated that the biomechanical considerations of root canal preparation and hermetic sealing of the root apex are the prime requisites for success in endodontic therapy. The employment of bacteriological controls and other considerations are of secondary importance.

Seltzer, et al, studied 64 teeth of three dogs which were subjected to endodontic procedures with culture controls. Periapical repair around canals which yielded a prefilling positive culture were compared with the repair around canals which yielded a prefilling negative culture. The exact role of the presence of infection within the root canal has yet to be determined as there was no significant difference in repair between the two groups. The most severe inflammatory responses appeared to occur as a result of overfilling the root canals with filling material or sealer.

Repair of the periapical tissues did not occur uniformly in all teeth that were treated, regardless of the culture results; and chronic inflammation persisted in the periapical tissues of dogs for at least one year following the endodontic procedures. Seltzer reaffirmed that the role of the host is therefore of critical importance in the end result of treatment.

Engstrom, et al, in 1964, studied 899 endodontic cases, of which 14.2 percent yielded positive cultures at the
fill appointment. Further investigation showed that 24.1 percent of the cases with persisting infection were later declared unsuccessful while only ten percent of the cases with negative cultures failed. Engstrom concluded that the presence of persisting infection at the time of fill adversely affected the prognosis.

The occurrence and distribution of bacteria within infected root canals and surrounding dentin was investigated by Shovelton. Ninety-seven teeth with non-vital pulps were extracted and fixed in formalin. After decalcification, the teeth were prepared in serial transverse and longitudinal sections. From each tooth alternate sections were stained with hematoxylin and eosin and with a modification of Gram's stain.

The number of tubules containing bacteria and their depth of penetration were recorded for each section. Over 77 percent had bacteria penetrating into the tubules to varying degrees while 18.6 percent had bacteria present in the root canal but no dentin penetration was observed. It was not uncommon for bacterial invasion to be found only in the predentin. A few sections had bacteria as far as half-way through the thickness of the dentin, but no sections showed bacteria to the cementum. In general, in acutely infected
teeth where bacterial invasion of dentin was present it was much less extensive than in teeth with chronic infection.

Shovelton noticed less dentin invasion around lateral canals than around main canals. Eighteen teeth showed a tendency for buccal and lingual invasion rather than uniform distribution around the root canal. Teeth with root canal preparations showed no organisms in the canal, but did show bacteria to be present deeper into the dentin.

Shovelton expected that mechanical preparation of the root canal would remove most of the organisms from the tooth since bacteria were found most frequently in the root canal and just within the dentin surrounding the canal. Those present deep in dentin would remain even after the most vigorous cleansing of the canal.

Bender, et al, reported the results of root canal treatment in 2,335 teeth evaluated after six months and 706 teeth evaluated after two years. The results were subjected to statistical analysis in an attempt to uncover a significant relationship between success and repair in teeth with and without areas of rarefaction, positive or negative cultures, methods of filling canals and other variables.

On the basis of radiographic evidence alone, the prognosis for successful repair was less favorable in teeth with rarefaction, regardless of the bacteriological status of
the root canal (88.8 percent success in teeth without rarefaction, 77 percent success in teeth with such areas). Of the root canals which had previously yielded a negative culture, 16.6 percent yielded positive cultures immediately prior to filling the canal.

Bender, et al, found no statistically significant difference between success of repair in teeth yielding positive or negative cultures prior to filling. It was observed that overfilling of the root canal gave the lower percentage of success (69 percent) and canals filled flush with apex showed the better results (87.4 percent).

Masterson, in 1965, outlined the steps to be taken to insure the adequate mechanical preparation of the root canal. However, because of the irregular morphology of the root canals, he declared that chemical debridement is also advisable. The technique to be employed in undertaking debridement of the canal and periapical tissues was presented. Masterson suggested that thorough mechanical preparation and chemical debridement may be sufficient therapy for the vast majority of pulpless teeth and that it is as successful as apicoectomy.

Seltzer and Bender stated that debridement of the root canal appeared to be essential part of endodontic therapy, because without debridement there can be no cure.
agreed that eliminating infection from human tissue appears to be a reasonably sound goal and should be a part of endodontic therapy. However, many cases are rated successes without attainment of a negative culture. Seltzer and Bender questioned whether microorganisms can actually be eliminated from an infected root canal, i.e., attainment of sterility.

In 1965, Hobson concluded that our bacteriological knowledge of the infected canal and periapical tissue is very unsatisfactory, since existing methods of investigation are primitive. The main tool is the culture which has been shown to be inadequate at best, and thoroughly misleading at worst. Bacteriological sampling of root canals is frequently inadequate leading to false negative culture results. All organisms will not grow in the same medium or a culture tube may be teeming with bacteria and yet not appear turbid to the eye.

In reviewing the literature, Hobson noticed that there is a lack of knowledge on the connections between the pathological condition, the clinical signs, and symptoms and the infecting organisms. She explained further that the bacteriological condition of the canal and periapical tissues alone does not give a full picture of the case and other factors must be involved such as: (1) the number of organisms
present, (2) the virulence of the organisms and (3) the resistance of the host.

Kakehashi, et al, observed the changes resulting from untreated experimental pulp exposures in germ-free rats as compared with conventional rats with a normally complex microflora. While devitalized pulps, apical granulomas and abscesses were found in conventional animals, pulpal healing resulted in the gnotobiotic animals. These results indicate that the presence or absence of microbial flora was the major determinant in the healing of exposed rodent pulps.

Ingle reported in his text that although mechanical cleansing alone will only sterilize 4.6 percent of infected root canals, it is still the primary method used to remove the majority of debris and bacteria from the canal. Instrumentation coupled with irrigation is particularly effective; however, complete sterilization is dependent upon intracanal medication.

Ingle suggested that the practical method for determining sufficient canal preparation is to enlarge until clean, white dentin chips are removed, which clinging to the apical 4 to 5 mm. of the instrument. The tip of the instrument should be inspected for the color and consistency of the cuttings each time it is removed from the tooth, and should
be cleaned with a sterile cotton roll each time before returning to the tooth.

Engstrom and Lundberg, in 1966, examined the frequency with which one or two negative bacteriologic tests in connection with root canal therapy were followed by a positive one.²⁰ The study consisted of 236 teeth on which root canal treatment was to be performed. From each tooth a bacteriological specimen was taken at three consecutive sessions; the first of these three was always negative, while the others were negative or positive.

In 66.1 percent of the teeth none of the three tests was positive. In 21.2 percent (50 out of 236) the second was positive, and in 16.2 percent (30 out of 186) the third test was positive after two negatives. The difference between the last two percentages was not significant. Therefore, the probability of a positive culture was the same whether it had been preceded by one or two negative tests.

Melville and Birch investigated the microbial floras of the root canal and the periapical area of a series of teeth.⁵⁸ These floras were compared with each other and with the flora of the labial bone plate overlying the apical area exposed by flap reflection.

They discovered that anaerobic as well as aerobic culture medium was essential in a study of the microbiology
of the periapical area. The periapical area may be sterile, even when the bacteria are present in the pulp canal. When periapical or labial plate flora did exist it was usually identical with or formed part of the root canal flora.

Marshall and Savaie studied the root canals of four groups of 25 extracted teeth each which were inoculated with known microorganisms: *Serratia marcescens*, *Bacillus cereus*, *Staphylococcus albus*, or a mixture of all three. Twenty-five teeth were left untreated as controls.

After the teeth had been incubated for 72 hours, samples were taken by means of a sterile paper point. The canals were premoistened with saline solution or peptone or left dry. Positive cultures occurred in 93 percent of the canals moistened with saline, 65 percent were positive when moistened with peptone and 36 percent of the dry canals were positive, while the 25 untreated canals remained negative.

Luebke, in 1967, stated that true sterility in the pulp cavity is a rarity. Nevertheless, he considered the reduction of microbial contamination of the root canals to a clinically acceptable level to be a realistic prerequisite to successful endodontic therapy.

Luebke suggested the use of 5 percent sodium hypochlorite as an effective intracanal irrigant. He visualized irrigation as a lavage of the pulp chamber and root
canals, even to the point of debriding lateral canals and furrows in the dentinal walls. Effective irrigation was assured if only clean dentin cuttings are found on files used for canal enlargement.

Matusow, in 1967, stated that microorganisms have been isolated from diseased pulp canals and periapical areas with varying frequency, depending on case selection and identification procedures. Most studies indicated that streptococci were the predominant microbial forms isolated.

It was postulated by Matusow that there are three general routes by which microbes are able to gain access to diseased pulpal and periapical tissues: (1) direct access via the dentinal tubules proximal to dental decay, fractures, erosion, etc., (2) periodontal access via apical and lateral foramina of the root, (3) vascular access in which blood-borne bacteria can localize at the site of injured and diseased pulpal and periapical tissues (anachoresis).

Infection was defined as the invasion of the tissue by microorganisms in such a manner as to produce a pathogenic reaction. The mere presence of microbes does not constitute infection per se, as the body lives in coexistence with a wide spectrum of microbes. However, when this balance is upset with injury to the tissues, infection can result.
Matusow stated that the numerical reduction of infectious microorganisms during endodontic debridement may allow host defense mechanisms to eliminate residual microbes, as the factor of host resistance often plays an important role in pulpal infection and its pathologic periapical sequelae. Matusow felt that assuming the tooth is free from clinical symptoms, one negative bacteriologic culture, after debridement, should provide a sufficient criterion for final obturation and recommended a minimum incubation of 48 hours at 37° C.

Grossman and Oliet, in 1968, reported on bacteriologic samples from the coronal and radicular portions of the same pulps of 771 teeth. In each case, the cultures were correlated with the clinical diagnosis. In addition, the cultures of the coronal portion of the pulp were correlated with those of the radicular portion. The cultures were in agreement 77 percent of the time, tempting one to make the deduction that the bacteriologic status is the same throughout the pulp in most cases.

Bartels, et al, reported the results of a study to determine the causative factors of 196 endodontic cases having pain and swelling during treatment. Trypticase soy broth with 0.1 percent agar was used as a medium for bacteriologic cultures and smears were stained by Gram's method and examined
for microorganisms and the presence of leukocytes. The cultures were incubated at least 10 days if no growth appeared. They concluded that cultures should be incubated more than the customary 48 hours, preferably one week, before they are discarded as showing no growth of microorganisms.

In 75.4 percent of the cultures examined mixed microbial species were found; Streptococcus mitis and Streptococcus salivarius occurring most frequently. Non-isolation of microorganisms was attributed to: (1) a root canal inoculum which contained too few microorganisms to initiate growth, (2) death of the microorganisms as a result of metabolic products of the predominant streptococci present in some of the specimens, or (3) the antibacterial activity of the inflammatory reaction. They were unable to establish any relationship between clinical symptoms and any specific microorganisms. This conclusion agrees with that of Crawford and Shankle in 1961. 17

According to Blechman, one of the more important objectives in endodontics is the elimination of microorganisms. 9 At present, the only available means of determining whether this objective has been attained is bacteriologic examination of the content of the root canal.

Meat or vegetable infusion broths with appropriate added enrichments and 0.1 percent to 0.2 percent agar are
regarded as suitable culture media. Among the more widely used media are brain heart infusion broth or trypticase soy broth containing 0.1 percent agar, thioglycollate broth, cooked meat medium and glucose ascites medium. Blechman stated that root canal cultures should be incubated at 37° C for 48 hours before they are examined for growth and kept for a minimum of one week if no growth has occurred.

Blechman insisted that although significant numbers of microorganisms and large masses of infected soft and hard tissue are removed during the processes of debridement, canal enlargement, and irrigation with antimicrobial agents, it remains for antimicrobial adjuncts in the form of germicidal irrigants and between-visit medicaments to complete the destruction of the microorganisms. Bacteriologic studies of the effect of mechanical preparation and irrigation of infected root canals suggested that a significant number of these canals can be rendered sterile by careful and effective procedures.

Myers, et al, in 1969, studied the incidence and identity of the microorganisms present in the root canals of treated teeth with culture reversals, i.e., those that previously had produced negative cultures. Two hundred fourteen previously treated pulpless teeth were cultured at the time of root filling and the organisms present were
identified. The observations reported were: (1) Canals scheduled to be filled following one negative culture yielded 25.9 percent positive cultures; (2) those filled following two successive negative cultures produced a reversal rate of 13.2 percent; (3) streptococci were present in the thirty-six out of forty-eight positive cultures; (4) extraoral contaminants comprised 18.6 percent of the total positive cultures; (5) there was about a 20 percent chance of a culture's being positive at the time of filling, regardless of the bacteriologic status of the culture taken at the beginning of treatment.

Torneck considered the relationship of microorganisms to endodontic disease to be divided into two phases: (1) that of microorganisms, actually establishing disease within the pulp and periapical tissue, often considered a primary effect, and (2) that of microorganisms, preventing or impeding post-treatment repair, considered a secondary effect. He stated that not all organisms or their by-products will have this influence and that a time factor is required for this effect to be manifested.

Torneck concluded that the presence of microorganisms is one of the etiological factors which can delay or prevent healing in the periapical tissues following treatment of the root canal. However, the effect of these microorganisms seems
to secondary and cumulative, i.e., they must co-exist with some other factor that would prevent repair. And not all microorganisms or groups of microorganisms or their by-products will have this influence. Even among those that do have this influence, a critical number must be present.

Serens and MacDonald reported, in 1969, that wiping out the root canal with paper points before taking a culture apparently reduces the inoculi; thereby significantly reducing the accuracy of the culture results. The first paper point used to sample a canal gives the most reliable indication of bacterial growth. This study indicates that a combination of three points sampling the same canal is the most desirable combination; the fourth point did not appear to be significantly important. The results reported agree with previous findings by Garber.

From his study of the microorganisms within infected roots, Homma, et al, reconfirmed the predominance of streptococci, which were isolated in 100 percent of the infected root canals of 18 extracted teeth. They also compared two bacteriological sampling techniques in 18 clinical cases. One hundred percent positive cultures were obtained in the thioglycollate media from cotton pellets sealed into the root canals between appointments, while 51.9 percent positive
cultures were obtained from paper points used to wipe the canal walls.

The dentin of the canal walls was also analyzed for bacterial presence. Dentin substance was collected on hand reamers and transferred to 1.0 ml. of sterile saline solution. 0.1 ml. of this suspension (which was regarded as the contents of the infected root canal) was used to inoculate thioglycollate medium and Sabourand's broth. Streptococci were isolated in 16.7 percent of the canals aerobically, and 33.3 percent anaerobically from this dentinal substance.

The purpose of Olgart's study, in 1969, was to investigate whether bacteriologic examination of root canals, made immediately after conservative treatment at the first appointment, was reliable for detection of infection. If this would be the case, such a sample could replace the one taken at the next appointment, thus reducing the overall number of visits by one.

After initial samples had been taken from the apical part of the root canals of 207 teeth, the canals were prepared mechanically with files and rinsed with sodium hypochlorite solution. When the canal preparation was finished, the canals were dried and sodium thiosulfate solution was introduced for one minute as a specific inactivating agent against the antibacterial solution used. Bacteriological samples were then
taken after which sterile, unprepared paper points were sealed into the teeth under temporary restorations.

At the second appointment, the enclosed paper points were removed for bacteriological examination, and samples of the root canals were taken conventionally using a bacteriological sampling fluid. In 84.5 percent of all cases, the first appointment samples showed agreement with samples taken at the second visit. The commonest causes for disagreement were in cases with positive first samples and negative second samples (11.6 percent of all cases). In 3.8 percent of all cases, negative first samples were followed by positive second samples. Thus, if one relies upon one sample, the risk of a persisting infection at a root filling procedure at the second appointment is 3.8 percent.

Shih, et al, in 1970, completed a laboratory study to appraise the bactericidal efficiency of sodium hypochlorite used as a root canal irrigant. A laboratory tube dilution study showed 5.25 percent sodium hypochlorite (Clorox) to be a powerful germicide. *Streptococcus faecalis* and *Staphylococcus aureus* suspended in 0.1 ml. of Bacto-ascitic fluid were killed in 30 seconds when added to 5 ml. of sodium hypochlorite diluted 1:1,000 (0.00525 percent sodium hypochlorite).

Experimental studies with a simulated clinical irrigating procedure showed that only full-strength Clorox
had a 100 percent sterilizing effect in root canals inoculated with *Streptococcus faecalis* and *Staphylococcus aureus*. Two-day and seven-day post-irrigation culture results revealed high reversal rates in the group irrigated with full-strength Clorox or in the group irrigated with Clorox diluted 1:10. The 7-day reversal rates were greater than the 2-day rates.

Shih, et al, concluded that a negative culture report after debridement indicates that the bacterial population in the root canal may be highly reduced, not that the canal is sterile. For a final assessment of the bactericidal efficiency of sodium hypochlorite, he recommended that a clinical study be carried out.

Morse, in 1970, stated that within the last few years, certain studies have produced results that tend to disagree with the concept of microbiologic cultures being an important component of endodontic treatment. It is essential to reduce the microbial population to as low a level as possible to ensure that the host-parasite relationship will be a favorable one for the host. Microbiologic evaluation is indicated only in those cases of acute apical abscess and cellulitis in which a sample from the exudate is placed into a transport media and sent to a microbiologic laboratory to determine the organisms present and their sensitivity to antibiotics. Yet, he believed that the culture as presently
constituted is irrelevant in routine endodontic therapy.

According to Grossman, the object of biomechanical preparation is to cleanse the pulp chambers and root canals of pulp remnants, foreign debris, infected or softened dentin; to remove obstructions; to enlarge the canal so as to receive the maximum amount of medicament or antibiotic; to smooth the canal wall in order to improve contact of the medicament with the infected canal surface; and also to prepare the canal walls so as to facilitate eventual obturation of the root canal. He stated that the importance of biomechanical preparation of the canal cannot be stressed too much. Many dentists have relied principally on drug therapy rather than on thorough cleansing and irrigation of the root canal.

Regarding bacteriologic culturing, Grossman lists several satisfactory media, including brain heart infusion broth, trypticase soy broth, and glucose ascites broth. He felt that culture is more sensitive than a smear, provided sufficient material is obtained for culturing. The presence of turbidity in a tube of culture medium indicates growth of microorganisms.

Morse considered the completeness of canal fillings to be the most important consideration for successful endodontic therapy. In 1971, Morse continued his attack on microbiological cultures in root canal therapy. He
recommended the utilization of host-parasite interaction as part of the determination of "when to fill" a root canal.

Senia, et al, in 1971, studied the solvent action of sodium hypochlorite on pulp tissue of extracted teeth. The mesial root canals of extracted human mandibular molars were prepared in a standard manner. One of the root canals was treated with full strength Clorox, while the other was irrigated with normal saline solution as a control. Both 15 and 30 minute irrigation times were used.

Cross sections of the roots were made at 1 mm., 3 mm., and 5 mm. levels from the apex and, after staining, were examined microscopically. Since the canals were not adequately debrided and cleaned in the apical 5 mm. by standard endodontic techniques, Senia, et al, concluded that the value of sodium hypochlorite as an irrigating agent for dissolving pulp tissue in the apical 3 mm. of narrow root canal is questionable.
CHAPTER III
MATERIALS AND METHODS

Thirty-three patients, ranging in age from 10-67 years, were selected from those registered at Loyola University School of Dentistry. A medical history was obtained from each patient and an oral examination performed.

The criteria for case selection for this study included:

1. Any individual with systemic disease or who had received any antibiotics within two weeks was eliminated.

2. The presence of a tooth that required endodontic treatment;
   a. that provided a field of operation that could be isolated with a rubber dam and disinfected,
   b. that allowed direct access to the root canal and could be enlarged to the apical constriction,
   c. with bacteria in the root canal.

The teeth treated were classified according to their root canal's communication with the oral bacterial flora and the presence of vital pulp tissue. (Table I)
### TABLE I

**TEETH TREATED**

<table>
<thead>
<tr>
<th>Tooth Location</th>
<th>Communication with the Oral Cavity</th>
<th>Pulp Vitality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open Canal</td>
<td>Caries</td>
</tr>
<tr>
<td>Maxillary Anteriors</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Maxillary Bicuspids</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mandibular Anteriors</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Mandibular Bicuspids</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>
Standardized instrument tray set-ups, including all intracanal instruments and absorbent paper points, were sterilized. Endodontic reamers* and carbide burs were dry heat sterilized at $320^\circ F$ for 2.5 hours. The remaining instruments were autoclaved at $270^\circ F$ and 30 pounds of pressure for ten minutes.

BBL dehydrated fluid thioglycollate medium was used for test tube cultures and BBL dehydrated brain heart infusion agar** was used for plating in petri dishes.

The patients were divided into two groups and the following procedures were carried out for each patient.

**GROUP A**

The tooth and area were anesthetized with 1.8 cc of 2 percent xylocaine with 1:100,000 epinephrine if any patient discomfort was experienced. In thirty cases, the tooth was isolated from the oral cavity by the placement of a rubber dam and secured with a suitable rubber dam clamp. The application of prophylaxis paste with a rubber cup removed gross debris and dental plaque from the tooth surface. Both the tooth and surrounding rubber dam were disinfected by one minute applications of 3 percent hydrogen peroxide,

* Kerr Manufacturing Company, Detroit, Michigan
**Division of Bioquist, Cockeysville, Maryland
70 percent isopropyl alcohol and Bactine.* Carious debris, if present, was removed with a spoon excavator.

Entry into the pulp chamber was accomplished with sterile carbide burs and an excavator was used to debride the pulp chamber. A sterile absorbent paper point was removed from a covered petri dish and inserted into the root canal for 15 seconds; then immediately transferred to a tube of thioglycollate culture medium. In each case, a barbed broach was inserted into the pulp in an effort to remove pulp tissue for culturing.

A reamer was placed into the root canal to an accepted average tooth length (or until the apical constriction was encountered) and a radiograph was taken to establish a "working length" approximately one millimeter from the radiographic apex of the tooth. Movement of silicone discs on the instrument shaft allowed a set of consecutively larger diameter reamers to be adjusted to the same "working length". Reamers were selected rather than files because more dentin substance appeared to remain on the reamers.

Beginning with the smallest size reamer that retained some observable dentin sample on the instrument, the instruments were rotated clockwise several turns and

*Miles Laboratory, Inc., Elkhart, Indiana
examined for canal contents on their surface. The sample was transferred to the culture tube by either loosening the sample from the reamer with a sterile instrument, allowing the material to drop into the tube (10 cases) or by fracturing the instrument to allow the portion with the sample to drop into the tube (20 cases). A sterile absorbent point was then placed into the canal for 15 seconds and examined for the presence of root canal debris or dampness prior to its placement in the thioglycollate medium. If debris or dampness were not apparent, the tip of another point was moistened in sterile distilled water and reinserted into the canal for 15 seconds prior to placing it in the culture tube.

The next larger size reamer was then used in the root canal in the same manner as the previous instrument and the dentin cultured. A paper point culture followed. This sequence of dentin and paper point culture continued until the canal was enlarged to three instrument sizes larger than that size which first exhibited some resistance to rotation.

An irrigant was not used during instrumentation. Each instrument size and culture tube number were carefully recorded on a data sheet.

The root canal was then irrigated with 2 cc. of sodium hypochlorite (5.25 percent)* and allowed to remain

*The Clorox Company, Oakland, California
flooded with the irrigant for 5 minutes. A reamer was moved in a gentle pumping action within the canal to insure that the irrigating solution had contacted the entire prepared surface.

After five minutes, the canal was dried with sterile absorbent points, irrigated with 2 cc. of sterile distilled water and redried with absorbent points. Two more cultures were obtained. An absorbent point (the tip moistened in sterile water) was used to obtain the first and rotation of the largest size instrument previously used secured the dentin sample. Both the absorbent point and the portion of the instrument with the dentin sample were placed in culture tubes. Each tooth was sealed with a dry cotton pellet and Cavit.* The patient was scheduled to return in one week.

At the second appointment, the tooth under treatment was isolated with a rubber dam and the area disinfected as at the first appointment. Access to the root canal chamber was accomplished with a sterile bur and the cotton pellet was removed with a barbed broach. A sterile absorbent point was inserted into the canal for 15 seconds and transferred to the thioglycollate medium. A sterile reamer of the largest size used at the initial appointment was rotated in the canal until a dentin sample was observable on the instrument. The

*Premier Dental Products Company, Philadelphia, Pennsylvania
instrument portion with the sample was then placed in a culture tube.

After the root canal was irrigated with sodium hypochlorite and dried, an intracanal medicament was placed and the canal was sealed in the usual manner. Endodontic treatment was completed at a subsequent visit.

GROUP B

Six patients with maxillary anterior teeth requiring root canal treatment were selected. After the teeth were isolated, disinfected and an access preparation completed, as in Group A, an initial absorbent point sample from the canal was obtained. Two dentin samples were obtained in the following manner. Consequently, larger reamers were employed until dentin shavings were observed on the instrument. These shavings were transferred to a sterile container which was also used to collect dentin shavings dropping from the canal during the following instrumentation and also from larger instruments. When the sample appeared to exceed one milligram (considered the minimal measurable quantity) a second vial was used to collect all subsequent dentin shavings either falling from the canal or on the larger reamers. The two samples were appropriately labeled for further laboratory study.
As described for Group A the canals were irrigated with sodium hypochlorite, dried, rinsed and re-dried. Absorbent point and dentin samples were obtained to inoculate thioglycollate media prior to sealing the canal with a sterile cotton pellet and Cavit. The second appointments were identical to those for Group A at which absorbent point and dentin samples were cultured. Endodontic treatment was completed at a later visit.

Laboratory Procedures

The thioglycollate cultures (Groups A and B) were incubated at 37° C and examined at 48 hour and five day intervals for the presence of bacterial growth. If any cloudiness or turbidity of the medium was observed, the culture was recorded as positive.

The dentin samples collected in the sterile containers were weighed on a Torbal Model EA Electric Balance by obtaining the difference between the weights before and after placing the contents in three milliliters of sterile distilled water. The water and dentin sample suspension was then placed in an ultrasonic vibrator for two minutes in an attempt to reduce the particle size.

A dilution series was obtained by pipetting one millimeter of solution from each 3 ml. sample into 9 ml. of sterile water. One millimeter of the latter solution was
then transferred into another 9 ml. of water. One millimeter of each of the three dilutions was transferred to a sterile petri dish and brain heart infusion agar was added, swirled and allowed to solidify and placed in an incubator at 37° C under aerobic conditions. The plates were examined for growth after 72 hours and the number of bacterial colonies present was recorded.
CHAPTER IV

RESULTS

The bacteriological analysis of the root canals of the teeth selected gave the following results:

Every tooth revealed the initial presence of bacteria in the root canal using an absorbent point and thioglycollate culture media. The number of negative dentin sample and paper point cultures after enlargement of the contaminated canals was observed. On the data sheet the final reamer used in each canal was designated No. 1 with the next smaller instruments being No. 2 and No. 3, respectively. In every case the No. 3 reamer was the first instrument to exhibit some resistance within the canal.

Twenty-eight of the 30 teeth treated produced some positive cultures from the canal contents attached to the reamers used prior to reamer No. 3. However, the dentin sample collected on reamer No. 3 produced no bacterial growth after a 48 hour incubation period in 9 of 28 teeth (32.1 percent). After five days 5 of 28 cultures (17.9 percent) were negative. Corresponding absorbent point samples yielded 14.3 and 10.7 percent negative cultures, respectively, after 48 hour and 5 day periods. (Table II)
### TABLE II

**EFFICIENCY OF INSTRUMENTATION IN 28 TEETH**

<table>
<thead>
<tr>
<th>Incubation Period</th>
<th>Number and Percent of Teeth with Negative Cultures</th>
<th>Final Instrument 1</th>
<th>Next smaller size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>Pt</td>
</tr>
<tr>
<td>48 hour</td>
<td>No.</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Percent</td>
<td>32.1</td>
<td>21.4</td>
</tr>
<tr>
<td>5 day</td>
<td>No.</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Percent</td>
<td>14.3</td>
<td>14.3</td>
</tr>
</tbody>
</table>
Graph I

EFFICIENCY OF INSTRUMENTATION IN 28 TEETH

Percent of teeth with negative dentin sample cultures

Percent of teeth with negative absorbent point cultures

FINAL REAMER

NEXT SMALLER SIZES

ABS. POINT CORRESPONDING WITH FINAL REAMER

NEXT SMALLER SIZES

48hr. 5 day
The dentin sample from reamer No. 2 provided cultures with no bacterial growth in 9 of 28 teeth (32.1 percent) after 48 hours and in 4 of 28 teeth (14.3 percent) after 5 days. The corresponding absorbent points resulted in 10.7 and 7.1 percent bacteria-free samples.

The final reamer produced the identical number of cases with negative cultures as reamer No. 2: 32.1 and 14.3 percent. The absorbent point cultures were free of culturable microorganisms in 6 of 28 teeth (21.4 percent) at 48 hours of incubation and in 4 of 28 teeth (14.3 percent) at 5 days.

Dentin and absorbent point samples collected prior to reamer No. 3, i.e., Nos. 4 and 5, provided fewer growth-free cultures than with any of the larger instruments.

(Graph I)

The six teeth in Group B gave the following data:

(Table III)

A. The first samples taken from the earlier instrumentation, averaged 3.2 mg. of dentin substance while the second taken from the final instrumentation averaged 4.0 mg. The range was 1.3 mg. to 7.1 mg.

B. Four of the 12 samples produced an insufficient number of bacterial colonies to be considered representative and are, therefore, reported as
TABLE III

VIABLE BACTERIA WITHIN DENTIN SAMPLE

Group B

Number of Bacteria per Milligram of Dentin Sample

<table>
<thead>
<tr>
<th>Case No.</th>
<th>First Sample (from early instrumentation)</th>
<th>Second Sample (from final instrumentation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(&lt; 2.7)</td>
<td>(&lt; 3.3)</td>
</tr>
<tr>
<td>2</td>
<td>697.4</td>
<td>26.2</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>(&lt; 1.0)</td>
</tr>
<tr>
<td>4</td>
<td>1928.5</td>
<td>807.5</td>
</tr>
<tr>
<td>5</td>
<td>(&lt; 4.3)</td>
<td>3.4</td>
</tr>
<tr>
<td>6</td>
<td>79.5</td>
<td>47.5</td>
</tr>
</tbody>
</table>

Average Sample

First Sample: 3.2 mg.
Second Sample: 4.0 mg.
less than a countable number. As a result, in two of the six teeth it was not possible to determine which sample contained more bacteria per milligram of dentin sample.

C. Four of the six teeth displayed more culturable bacteria in sample one than in sample two.

After irrigation with sodium hypochlorite, 22 of 24 teeth produced cultures of dentin samples that were free of bacterial growth after five days incubation. Twenty-eight of 31 absorbent point cultures were negative after 48 hours while only 23 remained negative three days later. (Table IV)

Eight of 15 teeth which produced negative dentin sample cultures after sodium hypochlorite irrigation at the first appointment, yielded positive dentin sample cultures at the second appointment. Twelve of 15 cases producing negative post-irrigation absorbent point cultures yielded positive cultures one week later. (Table V) (Clinical judgement required that several teeth not be sealed with Cavit at the first appointment. No further bacteriological samples were sought in these cases.)

In order to compare sampling methods, the cultures of dentin samples were compared to those of corresponding absorbent point samples. In 33 teeth, 218 comparisons were made. (Table VI) After an incubation period of 48 hours,
TABLE IV

EFFECT OF SODIUM HYPOCHLORITE IRRIGATION

Number of Teeth
Post-irrigation Culture

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Negative</th>
<th>Positive</th>
<th>Total No. of Teeth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48 hour</td>
<td>5 day</td>
<td>48 hour</td>
</tr>
<tr>
<td>Dentin</td>
<td>23</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>Percent</td>
<td>95.8</td>
<td>91.7</td>
<td>4.2</td>
</tr>
<tr>
<td>Absorbent Point</td>
<td>28</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>Percent</td>
<td>90.3</td>
<td>74.2</td>
<td>9.7</td>
</tr>
</tbody>
</table>
TABLE V
SECOND APPOINTMENT CULTURES OF 15 TEETH WITH TWO NEGATIVE POST-IRRIGATION CULTURES AT THE FIRST APPOINTMENT*

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>No. of Teeth with Negative</th>
<th>No. of Teeth with Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentin</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Percent</td>
<td>46.3</td>
<td>53.3</td>
</tr>
<tr>
<td>Absorbent Point</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Percent</td>
<td>20.0</td>
<td>80.0</td>
</tr>
</tbody>
</table>

*Both absorbent point and dentin sample cultures were negative.
**TABLE VI**

218 COMPARISONS OF SAMPLING METHODS*

<table>
<thead>
<tr>
<th>Absorbent Point Cultures</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident Period</td>
<td>Incidence Period</td>
<td></td>
</tr>
<tr>
<td>48 hour</td>
<td>5 day</td>
<td>48 hour</td>
</tr>
<tr>
<td>136</td>
<td>159</td>
<td>48</td>
</tr>
<tr>
<td>Percent</td>
<td>62.4</td>
<td>72.9</td>
</tr>
</tbody>
</table>

| Number of Dentin Sample Cultures | 31 | 21 | 3 | 3 |
| Comparisons in Disagreement    | Percent | 14.2 | 9.6 | 1.4 | 1.4 |

*218 Dentin Sample Cultures were compared with corresponding absorbent point cultures in 33 teeth.*
136 (62.4 percent) of the dentin sample cultures were in agreement with positive absorbent point cultures, while 31 (14.2 percent) dentin sample cultures disagreed. The five day comparisons were 72.9 percent in agreement and 9.6 percent in disagreement.

The 48 hour comparisons also revealed 48 (22.0 percent) dentin sample cultures conforming with negative absorbent point cultures, while after five days 35 (16.1 percent) of the cultures concurred. Disagreements were observed in three (1.4 percent) comparisons at both observation periods.

The agreement of all the dentin and absorbent point comparisons at the 48 hour and 5 day periods were 84.4 percent and 89.0 percent, respectively. The disagreements were, therefore, 15.6 percent at 48 hours and 11.0 percent after 5 days.

Of the 504 dentin and absorbent point samples obtained 346 (68.7 percent) demonstrated visible signs of bacterial growth after 48 hours. (Table VII) Forty (7.9 percent) did not display bacterial growth until the five day observation. The other 118 cultures (23.4 percent) remained negative at both observations. Of the 386 positive cultures, 346 (89.6 percent) showed visual signs of bacterial growth within 48 hours.
TABLE VII
COMPARISON OF INCUBATION PERIODS

<table>
<thead>
<tr>
<th>Incubation Period Necessary for Positive Culture</th>
<th>Negative Cultures after 5 days Incubation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 hour</td>
<td>5 day</td>
<td></td>
</tr>
<tr>
<td>No. of Cultures</td>
<td>346</td>
<td>40</td>
</tr>
<tr>
<td>Percent</td>
<td>68.7</td>
<td>7.9</td>
</tr>
<tr>
<td>No. of Teeth</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>Percent</td>
<td>97.0</td>
<td>48.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>118</td>
</tr>
<tr>
<td></td>
<td></td>
<td>504</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>97.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>
CHAPTER V
DISCUSSION

That a definite relationship exists between pulpal and periapical disease and certain bacteria has been adequately demonstrated by Kakehashi, et al, and reported by others. Several clinical studies have approached a similar conclusion by correlating the presence of culturable bacteria within the canal prior to root canal filling with treatment successes determined at recall appointments. In each study the results indicated that negative cultures at the fill appointment showed a greater percent of successes than positive cultures.

As a result of these studies the attainment of negative cultures became increasingly important. Strindberg and Sommer, et al, considered procurement of a negative culture the primary objective of root canal therapy and Filqueiras insisted that tooth extraction was the only alternative if obtaining a negative culture failed.

For the above reasons, several investigations were performed to determine the type of microorganisms in the root canal, their location and elimination. In every study reviewed, streptococcus were found to predominate with other organisms playing a secondary role.
Jolly and Sullivan were the first to report infection of the tubules in decalcified sections of root dentin. They reported that bacteria were found in close proximity to the root canal. Later, Chirnside studied the ability of microorganisms to invade the odontoblastic processes of crowns. His findings confirmed earlier reports that bacteria are able to transverse the entire length of the dentinal tubules.

The presence of bacteria within the dentinal tubules apparently became a concern of Shovelton who examined their relationship to the root canal in histologic sections. He found that bacterial invasion of dentin generally occurred at all levels of the root or in none of the sections. Jolly and Sullivan and Shovelton agreed that bacterial invasion of the predentin was common while there was considerably less invasion of calcified dentin. In some teeth bacteria were observed in some of the tubules as far as halfway through the thickness of the dentin. However, in no section did bacteria reach the cementum.

This investigation confirms Shovelton's histological findings. After minimal root canal enlargement and no irrigation nearly one-third of the teeth studied were rendered free of culturable bacteria in the dentin samples. A quantitative study of the bacteria in the dentin samples...
showed that more bacteria were present in the predentin and more superficial dentin than in the deeper sample.

Finding viable bacteria in the dentin adds significance to the results of others whose histological observations showed bacterial invasion of dentinal tubules. However, there is no reason to believe that infected tubules play a significant role in endodontic therapy. It would appear that the cementum prevents bacterial invasion of the tubules from one end and after root canal treatment is completed the filling material and sealer secures the canal end. Entombed bacteria are likely to exhaust their nutritional supply within the tubule and cease to multiply.

The utilization of a germicidal irrigant to cleanse the root canal has been advocated for years. This study confirms the previous reports that sodium hypochlorite is a very effective adjunct to mechanical enlargement and debridement. Nearly seventy-five percent of the infected canals treated had microorganisms eliminated or reduced to an uncultivable number by irrigation with full-strength Clorox. Utilization of the irrigant during canal enlargement should increase its debriding and germicidal effectiveness.

That over ninety percent of the dentin samples and nearly 75 percent of the absorbent point samples were recorded
as negative after irrigation further verifies that few, if any, living bacteria were in the calcified dentin. Since the dentin and absorbent point samples were taken at the same time and the dentin sample was larger, it is even more meaningful that the dentin sample produced over 17 percent fewer positive cultures. It is likely that the positive dentin sample cultures were due to contamination from the canal debris which consistently showed more positive cultures, as it was impossible to sample the dentin without including canal contents.

Throughout the years, clinicians and investigators have professed that root canal debridement is the most important phase of endodontics. Blayney explained that canal debridement is more important than intracanal medications. Later, Grossman stated that removal of debris is not only the most neglected phase of treatment but a necessity for effective medication. Filqueiras recommended tooth removal if bacteriological evidence of debridement could not be demonstrated.

There have been several criteria for evaluating whether a root canal has been sufficiently debrided. The attainment of a negative culture has been considered an indicator by some, while others rely on the presence of clean dentin shavings on the enlarging
no culturable bacteria, or more correctly, too few to produce turbidity in a culture tube.

It has been stated that cultures taken at one appointment may not agree with those of subsequent appointments. Reports have indicated that negative cultures after debridement of infected canals are frequently followed by positive cultures at the next appointment, providing no intracanal medications are employed. Ingle reports that only 3 of 13 teeth produced two consecutive negative cultures. Other studies by Engstrom and Lundberg, Myers, et al, and Stewart showed that 66.0, 74.1 and 76 percent, respectively, remained "sterile". This study produced two consecutive negative cultures in only 3 of 15 teeth (20 percent) when absorbent point cultures were examined. However, when only the dentin was sampled, 46.3 percent remained negative. Canal enlargement and sodium hypochlorite irrigation were able to reduce the number of bacteria so that most cultures were negative; however, bacterial multiplication reoccurred.

A comparison of 218 corresponding absorbent point and dentin samples revealed 24 disagreements after five days incubation. It is interesting to note that in 21 disagreements (87.5 percent) the culture of dentin was negative and the absorbent point culture was positive. These results
reaffirm the histologic findings of Shovelton\textsuperscript{82} and Jolly and Sullivan\textsuperscript{47} regarding bacterial penetration into the dentinal tubules. In view of these findings, examination of the canal contents rather than dentin has proven to be more reliable for detection of endodontic infections.

It is generally accepted that 48 hours is the minimum incubation period for endodontic cultures.\textsuperscript{31,57} Blechman recommends one week as a minimum.\textsuperscript{9} In this study forty-eight hour and five day readings were compared. Of 386 positive cultures, forty (10.4 percent) required 5 days of incubation for bacterial growth to become visible. Of the 158 negative cultures at the 48 hour reading, forty (25.3 percent) contained culturable bacteria as viewed three days later. Accordingly, one of four negative cultures at 48 hours could be regarded as a "false negative".
CONCLUSIONS

1. Canal enlargement without irrigation can render root canals free of culturable bacteria.

2. More viable bacteria are present in the pulp canal, predentin and adjacent calcified dentin than in the deeper dentin.

3. Sodium hypochlorite (5.25 percent) is an effective germicidal irrigant in root canal disinfection.

4. Enlargement and irrigation of infected root canals usually results in "sterile" canals. However second appointment cultures are most often positive.

5. Absorbent point cultures of the canal detect bacteria more frequently than do cultures of the dentin.

6. Over one-fourth of the endodontic cultures which appear negative after 48 hours of incubation are likely to become positive upon further incubation.
CHAPTER VI
SUMMARY

Thirty-three patients with infected root canals were selected for endodontic therapy and the canals were enlarged under aseptic conditions without the use of an irrigant. Cultures of the dentin substance removed showed that 21.4 percent of the canals were rendered bacteria-free and that fewer viable bacteria were detectable during the later stages of canal enlargement.

Sodium hypochlorite was found to be effective as a germicidal irrigant after canal enlargement; producing "sterile" canals in nearly 75 percent of the teeth. However, 53.3 percent of the supposedly "sterile" canals produced positive cultures at the next appointment.

A comparison of cultures of the dentin with those of the canal contents showed that agreement occurred 89 percent of the time. Disagreements indicated that more culturable bacteria are present on absorbent point cultures than within the dentin removed on reamers.

A comparison of incubation periods of 504 cultures indicated that over one-fourth of the endodontic cultures which appeared negative after 48 hours became positive after 5 days.
BIBLIOGRAPHY


The thesis submitted by Dr. Richard Bence has been read and approved by members of the Department of Oral Biology.

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.

May 10, 1972
Date

Signature of Advisor