A Quantitative Analysis of Microleakage in Endodontic Reverse Fills

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Loyola University Chicago

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A QUANTITATIVE ANALYSIS OF MICROLEAKAGE
IN ENDODONTIC REVERSE FILLS

by

Jerome V. Pisano, B.S., D.D.S.

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

April
1976
DEDICATION

To my parents, Salvatore and Alice Pisano, whose many sacrifices made possible my many dreams, and to my wife, Noreen, who always understood and helped me in so many ways.
ACKNOWLEDGMENTS

Special thanks to the people at Argonne National Laboratory, especially Dr. Walter Kisieleski, for their interest, guidance and physical help; without their assistance this project would not have been possible.

To Dr. Marshall Smulson, who gave me the opportunity to train under a most understanding and helpful faculty, and who guided me throughout the program.

To Dr. Joseph Gowgiel for constant encouragement and ideas.

To Dr. Norman Wood who stimulated ideas that I never knew existed, and whose friendship and assistance made this study a reality.

To Dr. James Sandrik whose knowledge of dental materials and scientific method as well as his unselfish cooperation added greatly to this paper.

To Dr. Franklin Weine who has the ability to combine warm personal friendship and individual interest with teaching ability unsurpassed by anyone.
VITA

Jerome Victor Pisano was born in Chicago, Illinois, on October 14, 1946, to Salvatore Victor and Alice Rose Pisano.

He received his elementary education at St. Priscilla's school on Chicago's northwest side and at St. John Brebeuf school in suburban Niles, Illinois. He is a graduate of Niles West High School in Skokie, Illinois. In 1964, he entered Loyola University and received a Bachelor of Science degree in June, 1968. His achievements there included induction into the Blue Key National Honor Society.

In September of 1968 he entered the Loyola University School of Dentistry (Chicago College of Dental Surgery). His actions as a student leader included his serving as class president for three years and finally student body president as a senior. Graduation in 1972 included such honors as induction into Omicron Kappa Upsilon, Alpha Sigma Nu Jesuit honor fraternity and the Presidents Medallion of Loyola University.

After receiving his D.D.S., he served as a Captain in the United States Air Force at Mc Guire Air Force Base, New Jersey.

In 1974, he again returned to the Loyola University School of Dentistry to pursue a Masters Degree in Oral Biology and a specialty certificate in the Department of Endodontics.
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CHAPTER I

INTRODUCTION

Endodontic failures are kept at a minimum by exacting technique, microbiological control, a thorough understanding of pulpal and periapical disease processes, and the potential for repair in these two environments. Yet, a certain number of cases still fail, and a common recourse employed by the practitioner is surgical curettage of the periapical area, apicoectomy and reverse filling the involved roots. The sealing potential of the materials and methods used in this alternative treatment is of paramount importance to its success. If the original root canal filling is suspected of microleakage, the aim of treatment must be to improve on the seal that should exist between the tooth root canal space and the periapical area.

Clinically, the materials used as reverse filling agents are materials familiar to operative dentistry. Even gutta percha, a most popular root canal filling material has its roots in operative dentistry as temporary stopping. Cavit* is recognized as a popular temporary filling used between endodontic visits.

*Premier Dental Products Company, Philadelphia
Amalgam is probably the most widely used restorative dental material. But these materials have been adopted by those doing surgical endodontic therapy for use in an environment and in a situation for which these materials were not designed originally. It is for this reason that the sealing potential of these materials has been studied for the most part as they function in the oral environment, not the periapical region. The works to date have studied the subject of microleakage mostly on a qualitative basis leaving much to be desired in clear cut differences between materials and their sealing abilities. If the sealing properties of these materials and methods are of prime importance, they should be critically, scientifically and quantitatively determined as reverse filling agents. The purpose of this study is to quantitatively compare and contrast three different reverse filling materials and methods on extracted human tooth roots through the use of a radioisotopic technique new to the study of microleakage.
REVIEW OF THE LITERATURE

Endodontic Case Failure

As far back as 1931, Rickert and Dixon were stressing the importance of root canal obturation and tissue tolerance of the canal sealants. They, even at this early date, realized that there are many factors that may be responsible for root canal treatment failures. Specifically, bacteria could not be the only contributing factor to case failures, and that even though their importance must not be minimized, culture techniques were fraught with error. But it was in this early paper, concerned with the gross response of living tissue to implanted materials that the hollow tube theory began. It was implied that a simple hollow tube of platinum or steel implanted in living animal tissue could result in severe inflammation. Hence, the root canal, a hollow tube after instrumentation should be filled completely with a material well tolerated by the periapical tissue.

More recently, Dow and Ingle (1955) contended too that the greatest cause of failure was poorly filled root canals. To prove their point, a lab study was designed in which extracted teeth were instrumented; one-half of the sample was filled "well" and the other half was poorly filled. Autoradiography showed considerable leakage of $^{131}\text{I}$ into the canals of the poorly filled canals. Their contention was that Rickert was correct—that in
fact, in vivo, serum leaks into poorly filled canals, undergoes degradation and flows back out into the periapical tissue as an inflammatory agent. This process was purported to eventually result in granuloma formation.

Ingle's paper stressing complete canal filling, in 1956, pointed out that if, in fact, the serum leakage theory is true, bacteria do not even have to play any role at all in case failure. The periapical lesion may be sterile, yet be a mass of toxic tissue destruction products.

Rickert's hollow tube theory has since been the subject of considerable criticism and doubt. Goldman (1965) showed that tissue fluids do definitely circulate in and out of open tubes implanted in rats and guinea pigs, but no unusual inflammation persisted at the ends of the open tubes. Torneck's first polyethylene tube study (1966) seemed to show that tissue response to a hollow tube implant was not severe. His emphasis was on correlating his study with the premise that thorough debridement of the root canal, as opposed to complete obturation of the space, was more important. To dramatize this theory, he implanted tubes filled with muscle tissue debris or microorganisms in rats, in 1967. The tissue response to these contaminated channels was considerably more severe. Therefore, his key to endodontic failure reduction was thorough canal debridement.

Seltzer et al. (1967) approached endodontic failures differently by evaluating over one-hundred clinical cases.
Certain trends seemed to emerge: cases that had periapical areas prior to therapy failed more frequently than those without areas; posterior teeth with some abnormal periodontal condition failed more frequently than those without; culture results had no bearing on success or failure; and most cases that would eventually fail, failed within the first twenty-four months.

Seltzer summarized by saying that there is no general consensus on what constitutes success or failure. Therefore, making study comparisons on this subject is extremely difficult. As an example of further diversity, Mattila (1968) states that there is a propensity for other oral lesions in the same individual who has a failing endodontically treated tooth.

The controversy concerning whether endodontic clinical emphasis should be placed on debridement, disinfection or obliteration of the canal still exists. Even though, in addition to the already mentioned studies, Browne (1955), Seltzer (1965), and Kennedy (1969), all contributed considerable information refuting Rickert's hypothesis, studies from Grossman, in 1939, to more recent investigations by Strindberg (1956), Allen (1964) and Grieve, in 1972, show that considerable attention must still be given the attempt at a hermetic seal in root canal therapy even though that end may very well be impossible.
Surgical Endodontics

Luks (1956) listed several reasons for employing a root end filling technique in endodontic therapy. Included in this list were calcified canals, cemented gold posts and broken instruments. What is common to these situations is the fact that either these cases are failing or have a potential for failure. Matsura (1962) added perforations to the list of indications, and altered the type of apical preparation suggested for a reverse filling technique. Yet, he, too, implies that surgical endodontics is indicated in failing cases. Thorough and complete endodontic therapy is advocated by Sommer (1946); doing reverse fill endodontic therapy on teeth with canals that have never been attempted conventionally is contraindicated. Herbert, in 1941, advocated the removal of the "porous" apical third of the root during surgical endodontic therapy to enhance sealing this vital area of the root. He strongly believed the poorly filled canal to be the cause of treatment failure. On the contrary, Rud (1972) favors leaving as much as possible of the apical root intact before reverse filling. Periapical surgery was described as not a cure for apical inflammation, but rather an attempt to remove all necrotic debris and more completely seal the root canal.

Although the approach to endodontic surgery has changed considerably since the views of Farrar (1884) and Rhein (1890), the trend is still to create a better environment than previously
existed by thorough curettage and improving on the existing seal in the apical one-third of the root canal. Kopp (1973) has gone so far as to advocate the use of gold foil as a reverse filling material with this goal in sight. Evaluation of the betterment of the case has taken several forms. Most clinical, and probably least significant, have been radiographic follow-up studies assessing success or failure of endodontic surgeries. One thousand cases were evaluated by Rud (1972) over a four year post-operative period. It was found that most cases classified as having complete healing at one year remained in that category as did those cases showing poor healing at one year. Cases classified as incomplete healing or uncertain healing vacillated from group to group throughout the four year period. In another of Rud's 1972 papers, criteria were established for radiographic interpretation of healing. At least a one year post-operative evaluation is necessary. If healing is unsatisfactory at four years, it must be considered a failure.

Nord (1970) completed a radiographic study concerning 346 patients whose teeth were reverse filled with Cavit. A sixty-one per cent success rate was reported using this material. In general, Persson (1974) reported a range of success by several investigators studying reverse fills from eighteen to eighty-nine per cent. Persson's comparison of silver amalgam to Cavit on a one year term showed amalgam to be superior, although Cavit had the edge at the shorter six month radiographic check.
Andreason (1972) made an attempt to compare histologic findings with roentgenographic interpretation of healing after endodontic surgery. He found that the size of the lesion detected radiographically was indicative of at least the quantity of granulation or scar tissue or "inflammation" present at that time in the lesion.

**Tissue Response to Endodontic Surgery and Its Materials**

The modes of healing and the classic tissue response after endodontic surgery as described by Andreason (1972) are only of some value in this review as a base line for comparison of other tissue response studies. It is obvious that the reverse filling materials used in endodontic surgery produce some type of tissue response of their own. These materials are chosen not only for their ability to improve an apical seal in a questionable case, but certainly for their tissue tolerances.

One of the very first and classic articles concerning tissue tolerances to foreign materials was that of Dixon in 1933. This study, similar in technique to one performed three years earlier, concerned itself particularly with root canal filling materials. Materials implanted in the dorsal tissue and muscle tissue of rabbits produced various gross inflammatory responses. Since the overextension of filling material into the periapical tissue was desirable to some extent at that time, the search for a material with low inflammatory potential was a high priority.
consideration. More recently, and more pertinent to the present investigation, was the report of Feldman and Nyborg (1962). Implants of disinfected samples of silver amalgam and gutta percha were implanted into the mandibles of rabbits. On the basis of a greater capsule thickness, absence of fibrin fibers, a greater number of inflammatory cells, absence of bone apposition and abundance of macrophages around the gutta percha implants, it was concluded that there was generally a more favorable histologic response to amalgam in osseous sites. Flanders (1975) studied the tissue response of rats to zinc-free amalgam and Cavit. His criteria were similar to Feldman's, and amalgam was again found to be considerably more biologically acceptable than Cavit. Marcotte's (1975) in vivo study of twelve monkey incisor teeth reverse filled with amalgam versus gutta percha showed little difference in response in samples collected at times from three to fifteen weeks.

Microleakage and Thermal Cycling

Microleakage has been defined by Azim (1972) as "the phenomenon denoting the presence of microscopic spaces in between the tooth structure—restorative material interface." He attributes the formation of this space in general to several factors including the linear coefficient of thermal expansion, modulus of elasticity, material solubility, volumetric changes of the
restorative material, permeability of the involved tooth structures, influence of oral or body fluids and finally manipulative variables. These spaces are readily demonstrable through the use of the scanning electron microscope. Most recently, Moodnik (1975) measured defects between tooth structure and silver amalgam that varied from six to one-hundred and fifty microns. Even a case that was filled in vitro showed significant defects at the margins. Obviously, defects such as these are not recognizable through the use of clinical radiography, yet are present in most clinical cases.

The work of Nelson, Wolcott and Paffenbarger (1952) can shed considerable light on the possible significance of these spaces that exist between tooth structure and restorative material. The limit of visual acuity is roughly fifty microns. Since the scanning electron microscope can easily point out six micron defects, and the average defect between a resin filling and dentin immediately after placement is ten microns, these defects are not discernable clinically. White blood cells have an average diameter of twenty-five microns, red blood cells average a diameter of seven and one-half microns and lactobacilli are only two microns in size. Passage of these sample substances as well as thousands of smaller than fifty micron particles or molecules can be of great significance in the clinical success or failure of an occlusal restoration, or for that matter, an endodontic apical filling. Forces involved in the passage of substances
through these recognized defects between tooth and material must include capillarity, dialysis, diffusion, changes in hydraulic or gas pressures and simple thermal variation so common in the oral cavity.

Thermal cycling and dimensional changes of restorative materials was specifically studied by Jacobson (1975). The interface between glass tubes, simulating cavity preparations, and the materials tested was made part of an electrochemical cell which measured the passage of current indicative of a dimensional change in this space. Only anterior restorative materials were perused. Gilles (1975) held many of the same views on dimensional change and temperature saying that many materials perform admirably at a constant 37°C but fail to seal at fluctuating temperatures. A strip chart recorder measured the dimensional changes of materials commonly used in root end filling techniques. These materials inserted in teflon molds were thermally cycled in a dry environment. Surprisingly, Cavit showed considerably greater stability than unmodified zinc oxide and eugenol and not so surprisingly, greater sealing potential than gutta percha.

Microleakage Dye Studies

Historically, several techniques have been used to study microleakage and to try to determine which materials had the best sealing qualities. One of the oldest and most popular
techniques involved the use of a dye. Substances used have included eosin, methylene blue, methyl violet, gentian violet, hematoxylin, mercuric chloride, Prontosil, soluble red, aniline red, basic fuchsin, chromotrope 2-R, crystal violet and fluorescein (Going, 1972). Massler (1954) inserted filling materials into glass tubes, submerged the filled ends of the tubes in methylene blue and gentian violet and recorded any uptake of the dye by cotton wicks above the filling material barriers. Zinc oxide and eugenol and amalgam showed no leakage at all, even though the amalgam was purposely mishandled. Gutta percha, silicates and acrylic leaked within hours. A similar study by Parris, in 1960, using extracted teeth and aniline blue dye, showed again that zinc oxide and eugenol as well as amalgam sealed very well. Gutta percha, and this time zinc phosphate cement, leaked easily. Thermal cycling was believed to be the determining factor in his study. A portion of Going's paper (1960) dealt with the leakage of crystal violet dye around class V cavity preparations in extracted teeth. Ground sections showed the most leakage around acrylics and zinc phosphate cements, while copper amalgam showed the least marginal percolation. Tani (1969) studied anterior restorative materials using basic fuchsin dye as the tracing agent, and added the extra precaution of coating the extracted teeth (except near the test preparation site) with wax to eliminate leakage from anywhere but around the restoration in question. Barry published three methylene blue
leakage studies in 1975; one specifically dealt with the sealing capacity of amalgam, heat sealed gutta percha and a carboxylate cement when used as reverse root filling agents. He too coated the roots of the teeth, this time with a clear enamel to eliminate leakage from lateral canals or cracks. There seemed to be only small differences between the materials tested, but the carboxylate cement appeared to allow the most penetration of blue dye. One glaring fact common to all dye studies is that the results are at best qualitative between groups of samples, never quantitative. Often the results are so similar that investigator interpretation may have been a factor.

**Bacteria in Microleakage Studies**

Bacteria have been and are of considerable concern to endodontists. Their penetration between filling material and tooth structure could be of great significance. As early as 1929, Fraser had developed a technique using bacterial cultures. Glass tubes were sealed at one end which were immersed in cultures of *Bacillus*, *Streptococcus* and *Escherichia coli*. A volume of broth above the filling material was sampled and cultured for growth. Any positive growth indicated some movement of bacteria past the restorative materials. Temporary stopping seemed most permeable, while copper cements and silver amalgam formed the best barriers against the bacteria in question. Grossman (1939)
performed a similar study replacing the volume of broth with a sterile cotton wick. The materials tested included gutta percha, zinc oxyphosphate cement, zinc oxide and eugenol cement and combinations of these materials. The only material affording any seal at all was zinc oxide and eugenol. Seltzer (1955) utilized a different bacteriologic technique to study microleakage. Certain microorganisms, namely *Serratia marcescens* and *Bacillus globigii* produce an easily identifiable color when cultured at low temperature, red and orange respectively. Extracted teeth were prepared for filling under sterile conditions. Cavity preparations were filled with materials ranging from the acrylics to amalgam. Teeth treated alike were submerged in viable cultures and some samples were thermally cycled. After varying time periods, dentin shavings from under each filling were cultured and observed for the appearance of the appropriate color indicative of contamination. Samples not thermally cycled showed no leakage. Of those materials subjected to temperature changes, acrylics allowed the highest degree of contamination while only one of twenty amalgam samples allowed bacterial invasion of the underlying dentin. *Serratia* were again used by Mortenson (1963); after thermal cycling, five of twelve amalgam samples showed some leakage. In one of Parris' early studies (1964), motile bacteria which were two-hundred and fifty times larger than most dye particles were used to penetrate suspect margins. These bacteria which were approximately five-hundred millimicrons in size could
easily penetrate a marginal crevice as wide as ten thousand millimicrons as described by Nelson (1952). Even under extreme thermal cycling conditions, Cavit and amalgam both prevented bacterial contamination of sterile cotton sealed in the pulp chambers of these extracted teeth.

Again, although all of these investigations yielded variable information, the results have been criticized for the lack of quantitation. Leakage was determined on an all or none principle—a positive or negative culture.

**Microleakage Studied by Air Pressure Techniques**

One of the only quantitative methods used for the comparison of microleakage involves the measurement of air under pressure. Pickard (1965) describes a technique by which air under pressure is forced through root canals from the apical end, and the critical pressure recorded at which air bubbles under water are detected emerging from around coronal amalgam restorations. Diffusion, percolation, or microleakage per se were not studied. Rather, actual defects between the restoration and cavity walls were located through the use of this technique. Granath, in 1970, described a complicated and ingenious apparatus to measure virtually this same critical pressure. Admittedly, the test samples were subjected to nonbiologic and nonphysiologic stresses and conditions for which these materials were not designed to withstand.
Radioisotopic Research

Radioactive isotopes have established themselves as indispensable tools in biologic and physical research. They can be used as tracers in vivo as part of tagged molecules allowing the investigation of development and molecular transfer (Bartelstone, 1950). McCauley (1942) outlined one specific use in dentistry—to study the development and remodeling of tooth structure. The use of isotopes to study microleakage is removed from these objectives, but no less significant to dental research. Wainwright (1953) described the new avenues open to microleakage research through the use of isotopes. But O'Brien, in 1968, was more skeptical when he stated that unless quantitative techniques were developed and the problem of absence of controls in most studies solved, the present avenues of study were of little value.

An element's atomic number identifies its chemical and physical properties. Different atomic weights, that is, a different number of neutrons in the nucleus coupled with the same atomic number, designate the isotopes of an element. These isotope forms have a tremendous scientific advantage over their more stable elements in that they react the same chemically, but disintegrate due to their unstable nuclear structure and are detectable quantitatively. Three types of atomic particles are emitted: alpha, beta, and gamma rays. A table adapted from
Jeffay (1961) summarizes and contrasts the weights, charges and relative radioactivity of these particles.

It will be helpful in future discussions to describe at this time some of the terminology and characteristics unique to isotopes and their use. Radioactivity is classically measured in units called curies. One curie is equal to $3.7 \times 10^{10}$ atomic disintegrations per second, or $2.22 \times 10^{12}$ disintegrations per minute. The rate of decay of radioactive isotopes is measured by their half life which is the time required for the number of unstable nuclei to be reduced by decay to more stable structures to one-half the original number. This value can be helpful in selection of isotopes for long or short term studies and the

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determination of the term of their potentially hazardous effects. The specific activity of a source of disintegration is measured by comparing its activity to the unit mass, that is, in curies per gram or millicuries per gram, etc. These values must be established as a base line for comparison in quantitative analysis (Kisieleski, 1972). In general, the use of isotopes rather than chemicals has the great advantage of their being measurable in considerably smaller quantities allowing sensitive comparisons and relative quantitation if certain techniques are used (Jeffay, 1961).

Materials exposed to ionizing radiation have various interactions with the particles or rays emitted by the isotope. Alpha or beta particles have the potential to remove electrons from other atoms by electrical forcefield interaction. Once an electron is removed from an atom, that atom is left positively charged and is termed an ion. The negatively charged electron released by the ionization process can be free for some time or can be attached to another atom giving it a negative charge. Thus, an ion pair is formed, one positive and one negative. It is by detection of this ionization process that radioactivity is detected and measured (Kisieleski, 1972). It is the basis for radio-isotope leakage studies using autoradiography and scintillation counting.

Autoradiography by far has been the most popular technique in microleakage investigation. This type of research is based on
the use of low energy beta and sometimes alpha particle emitters and photographic emulsions. A photographic plate coated with emulsion can register the presence of an ionizing particle. When the emulsion, usually silver bromide embedded in a vehicle like gelatin, is struck by beta particles, the silver crystals are ionized and form a latent image which can be developed and fixed to produce black dots on the picture plate. It is important that the radiation be of short range because a source emitting long range particles can produce images far from the source atoms, therefore falsely identifying the physical location of the source (Kisieleski, 1972). Nixon (1964) in dental research, and authors of more general biologic research (Roth, 1969), have espoused the tool of autoradiography in tissue development studies. Its use in material leakage studies is also well documented.

Kapsimalis, Evans and Tuckerman (1965) described a most accurate variance of the autoradiographic principle. Their research indicated that low energy level, soft radiation emitting beta sources produced the best images on dental film or glass plates with small grain size emulsion particles giving better resolution. The sources used in the extracted teeth included $^{35}$S, tritiated glucose and tritiated proline. The earliest autoradiographic studies were performed to determine the marginal penetration around coronal filling materials. Armstrong, in 1951, used Ca$^{45}$ to compare the penetration around class V fillings, and found amalgam to be most resistant to percolation.
Crawford and Larson (1956) used the same radiation source and found marginal percolation around all materials, and that weathering of the test fillings in the oral cavity did not lessen the leakage potential. Phillips, in 1961, and Swartz, in 1961, disagreed with Crawford's contention when they showed amalgam margins leaked less after forty-eight hours of oral exposure and even less after long term salivary exposure. Going, Massler and Dute, in 1960, conducted a study incorporating over one-hundred and forty-five extracted teeth to demonstrate the marginal penetration around such materials as amalgam, gold inlays, gold foil, acrylic, silicates, zinc oxide and eugenol and gutta percha. Several beta emitters were used as energy sources including $^{35}S$, $^{32}P$, $^{22}Na$, $^{86}Rb$ and $^{45}Ca$ to determine the effects of different ion charges and possibly different chemical affinities. $^{35}S$ and $^{45}Ca$ produced the best autoradiographs while $^{22}Na$ showed the poorest definition.

Marshall and Massler, in 1961, made one of the first applications of autoradiography to the field of endodontics. Over two-hundred and sixty root canals of extracted teeth were filled with several materials using various popular methods to determine if canals can be completely obliterated, if there is a superior filling technique and how good sealers are in preventing leakage of small molecules and ions. Their contention was that canals can be made completely impervious to small ions and molecules through the use of gutta percha and the appropriate
sealer (the sealer being an important filling adjunct). They also found that $^3\text{S}^5$ gave the sharpest, most detailed autoradiographs while also demonstrating the most vigorous penetration. Holland, in 1974, used $^{131}\text{I}$ to study several sealers with gutta percha, and showed considerable differences in the type and consistency of mix of the sealers tested as it related to their sealing ability. His findings did indicate, however, that lateral condensation was superior to single cone fills in every case. Kapsimalis and Evans (1966), continuing on the premise that one of the major causes of endodontic failures may be poorly filled canals, used autoradiography to compare different filling methods, cores and sealers. Most significant were the choices of radioisotopes. $^3\text{S}^5$ was selected because it is an inorganic polar ion. Tritiated glucose was chosen because of its molecular size and non-polarity. Proline was chosen for its non-polarity and molecular size and for its unique ability to react as an acid or base when the pH is altered. Sealers were shown to have better or worse sealing potentials, but the different isotopes and different particle sizes had no effect on leakage patterns or potentials. It was shown in the case of proline that different functional groups accompanied by changes in pH caused a change in polarity which did affect the results significantly. Three studies by Avny (1970), Heiman (1971) and Taylor (1973) analyzed the diffusion and penetration of aqueous or camphorated para-chlorophenol through the use of autoradiography. The only
available reference where autoradiographic techniques specifically studied the sealing potential of endodontic reverse fillings was that of Goldberg and Frajlich (1973). $^{131}$I was shown to perfuse around apical amalgam seals into unfilled root canals and to penetrate the dentine of the canal.

A quantitative, more sophisticated use of radioisotopes in leakage studies centers about the principle of liquid scintillation counting. This principle is used to measure the soft beta radiation of usually tritium ($^3H$), carbon-14 or sulfur-35. A solvent and liquid scintillator compound are necessary to dissolve the sample. Electrons given off by beta interaction pass through matter and lose energy in the process. Some substances called phosphors can absorb the energy and emit a portion of it in the form of violet or ultraviolet light. The number of photons of visible light produced is proportional to the energy absorbed and therefore, also to the ionizing radiation present. A photomultiplier tube can transform the photons produced into a measurable pulse which can be a quantitative indication of the amount of radioactivity present (Kisieleski, 1972). Söremark, in 1961, used scintillation counting to determine the passage of Na$^{22}$ around and through acrylic facings. In 1965, Arwill used Na$^{22}$, part of a NaCl solution, to compare the permeability of fresh versus coagulated dental tissues. Quantitatively, the coagulated tissue samples were decidedly less permeable to the tagged sodium chloride. Going (1968) modified the simple use of
scintillation counting to develop yet another quantitative method of studying microleakage *in vivo* or *in vitro* of coronal fillings. A crown was fitted with the restoration of choice and then bathed in a solution of Mn(NO$_3$)$_2$. The Mn$^{55}$ was "neutron activated" to Mn$^{56}$ and an accurate determination of the volume of Mn$^{56}$ retained by the sample was measured. Zurbrigger (1975) irrigated the canals of extracted teeth with C$_{14}$-labelled chelating agent. The samples were oxidized, and the liberated carbon collected, diluted with scintillation cocktail and counted. He discovered only a 3.8% retention of labelled material by the roots. Yee's study in 1975 included only a few extracted teeth and materials not normally used to seal root canals such as silicone master cones, cyanoacrylate and polycarboxylate cements. These materials when compared to fills of gutta percha and zinc oxide and eugenol, showed no permeation in either group of Ca$^{45}$ between the canal walls and fillings.

It is well established that no matter where the emphasis lies in endodontic therapy, an attempt to seal the canal as well as possible to avoid failure at a later date is desirable. Certainly the sealing ability of a material used to better a filling already suspected of leakage is important. Techniques to study the leakage potential of restorative materials, many of which are used as reverse filling materials, have been developed, but the quantitation of data allowing accurate comparisons and
statistical significance has been lacking. It is the design of this paper to demonstrate a radioisotopic technique that can be used to specifically quantitate microleakage around or through three endodontic reverse filling materials.
CHAPTER II

MATERIALS AND METHODS

Over one-hundred and fifty human maxillary central incisors were collected for this study. All samples were supplied by oral surgeons without identification as to the age or history associated with each tooth. The teeth were immediately wiped with sterile gauze moistened with saline and then submerged and stored in baths of physiologic saline to which was added a small amount (less than one percent of the total volume) of colorless merthiolate* to control bacterial growth. The samples were collected weekly and transferred to larger storage baths of refrigerated solution. Any adherent tissue fragments were removed prior to final storage by sterile dry gauze wipings. The solutions in the larger storage baths were changed every second day to further control bacterial growth. All teeth were used within thirty days of their collection.

The anatomic crowns of the teeth were removed through the use of a 700 high speed carbide bur to facilitate engine reaming as the method of canal preparation. All canals were reamed

*Lilly--Indianapolis, Indiana
through the apex in step wise fashion with the preparation ending with a number five engine reamer. A number 10 endodontic plugger* was fitted into each canal simulating a canal filling against which a reverse filling material may be placed. Using a millimeter periodontal probe, the apex of each root was trimmed back until one and one-half millimeters of unfilled canal extended from the tip of the plugger inside the canal to the external surface of the apicoectomied root (see Appendix figure A). The cut root tip surface was not beveled and was perpendicular to the long axis of the root. A #35 inverted cone carbide bur in a high speed handpiece using water spray was inserted once into each apex to the depth of the head of the bur, approximately one millimeter, and withdrawn, creating uniform preparations to accept the reverse filling materials. The samples were allowed to adapt to room temperature while being kept moist and were again fitted with the plugger and the filling materials were placed apically. Cavit was dispensed directly from its tube and placed in the apical preparations with a #3 Woodson plastic instrument under normal digital pressure packing the material against the plugger which substituted as an existing root canal filling.

According to Wilderman (1971), the make up of Cavit is as follows: zinc oxide, calcium sulfate, glycol acetate, polyvinyl acetate, polyvinyl-chloride acetate, triethanolamine and red pigment.

Water or saliva reacting with the calcium sulfate and zinc oxide

*Star Dental Manufacturing Co., Conshohocken, Pennsylvania
induces hardening and a high coefficient of linear expansion accounting for its purported excellent marginal sealing potential. Similar fillings were made with heated gutta percha obtained from large size master cones*. The gutta percha was placed by a Woodson #3 instrument, this time warmed to manipulate the gutta percha. A doughy rather than molten consistency of gutta percha was used to minimize post placement shrinkage on cooling. Zinc free spherical silver amalgam** was the last material examined. This material supplied in compartmentalized single spill capsules, was triturated uniformly for each fill for eighteen seconds in a WIG L BUG single speed amalgamator*** and each reverse fill was placed in three small increments measured by a K-G retrofill amalgam carrier**** with intermittent condensation with a flat faced amalgam plugger. The excess material in all cases was carved flush with the flat root tip surface. The intra canal plugger was removed immediately after filling and the teeth were stored in saline baths for forty-eight hours before being utilized as test samples.

Scintillation vials with screw top caps (see Appendix picture C) were used and 1.5 ml of sterile physiologic saline was injected into the bottom of the bottle via a tuberculin syringe. This saline volume was the wash or collection bath designed to absorb any leakage from inside the root canal. The void left in the canal after removal of the plugger served as a space to

*Premier Dental Products Co., Philadelphia, Pennsylvania
**Kerr Dental Manufacturing, Detroit, Michigan
***Crescent Dental Manufacturing Co., Chicago, Illinois
****Union Broach Co., Long Island City, New York
deposit the radioactive solution. Disposable dental needles of 25 gauge and short length* had their short ends removed and were sealed over the root stump with melted Sticky Wax** to serve as a handle for each root and as a pressure vent so that active solutions were not forced through the apex as the canal orifice was sealed. This system allowed the screw tops of the vials to be sealed, closing the experimental model and isolating it from the room environment.

PILOT STUDIES

Several pilot studies ranging from six to twelve teeth each were conducted. These original studies were done to test the overall feasibility of the new technique. The first study involved six roots prepared in the previously described manner and sealed with amalgam. Ten lambda (one lambda is a microliter or one one-thousandth of a milliliter) of tritiated water was injected into each canal. This solution produced $4.78 \times 10^7$ disintegrations per minute per milliliter (DPM). One hundred percent recovery would yield $4.78 \times 10^5$ DPM for a ten lambda sample. Immediately after injection of the solution, the root stumps were sealed with the disposable needles and Sticky Wax. The apex of each root was placed in only one-half milliliter of

*Sherwood Industries, Deland, Florida
**Kerr, Detroit, Michigan
saline contained in a small glass cup placed in the bottom of each scintillation vial and the vials were sealed. One-half hour and one hour after initiation of the experiment, the teeth were removed from the wash baths. Fifteen milliliters of scintillation liquid were added to each vial and thoroughly shaken. The vials were placed in a Packard scintillation counter Model 3375 and ten minute counts were recorded. Significant amounts of radiation were detected in the wash baths encouraging other pilot studies.

In an attempt to account for one-hundred per cent isotope recovery, roots removed from the baths at the conclusion of the specified time periods were oxidized in a Packard Tri Carb oxidizer Model 306. The water was collected and diluted with scintillation cocktail and analyzed. The residue of the tooth after combustion was placed in another vial containing fifteen milliliters of counting fluid. All vials pertaining to an individual sample were counted and their values were summed. In no case was one-hundred percent recovery of the test solution realized. In many cases less than sixty per cent of the original activity was recovered, although in one case, a 90.7% value was recorded.

Lack of control was solved through the use of glass tubes sealed at one end approximating the average length and outside diameter of a human central incisor root. This model tested with tritiated water showed that this particular isotope has the
unique ability to penetrate glass. For this reason as well as other biological considerations (molecular size and physiologic value) the isotope used was changed to $^{14}C$-labelled glucose* with an activity of 5 microcuries per milliliter and a standard DPM of $1.2 \times 10^4$ per one lambda sample. Glass was impenetrable by this isotope, but oxidation recoveries were still not nearly one-hundred per cent.

Other pilot studies eliminated previously unforeseen errors such as the possibility of some material either volatilizing, or in some other way, escaping the system via the vent needle and reaching the interior of the vial in this other way contributing additional radioactivity to the wash bath that did not represent microleakage. Venting the needle through a rubber diaphragm that replaced the screw cap showed no differences between these samples and those counted in the closed system. Another significant development was the use of the same sample tooth transferred from bath to bath at distinct time intervals. The summation of detectable radioactivity from all the baths per sample gave a total leakage figure as well as an idea as to when most of the leakage occurred in each sample. Elimination of the wash bath cups and simply tripling the volume of the wash bath placed directly into the scintillation vials was the result of another pilot study, as was the reduction from ten lambda to one lambda of radioactive solution injected into each canal.

*Schwarz Inc., Orangeburg, New York
Finally, autoclaved teeth, kept moist after sterilization, were used as samples. It was suspected that bacteria could be breaking down the radioactive glucose and affecting the total recovery data. Total recovery on these teeth was the same as in non-autoclaved samples.

Based on the pilot studies, trial and error and laboratory practice of the involved techniques, a final protocol was drawn up and the experiment was conducted.

THE EXPERIMENT

Seventy-two maxillary central incisor teeth were selected at random from storage stock in physiologic saline solution. The crowns of all teeth were removed at roughly the cemento-enamel junction on the mesial and distal surfaces. All canals were engine reamed as previously described. A plugger was fitted in each canal, an apicoectomy performed and an apical preparation to receive the reverse filling agent was made as in the pilot studies. The canals were irrigated with saline to insure patency from the coronal orifice through the apical preparation.

The teeth were randomly divided into three groups of twenty-four teeth each. Each group was filled with one of the three test materials, amalgam, Cavit and heat sealed gutta percha as described earlier. One-half of the teeth in each group were coated with clear finger nail polish* as described by Crawford (1956) to minimize the possibility of leakage through indiscernable

*Chesebrough Ponds Inc., Greenwich, Connecticut
cracks and lateral canals, although a goodly portion of the more porous apex of each root had already been removed. Great care was taken not to paint the flat apicoectomied and filled surface; only lateral root surfaces were coated, but were coated twice. All samples were again allowed to stand for forty-eight hours at room temperature.

On the first day of the experiment, all canals were injected at timed intervals with one lambda of \(^{14}\)C-labelled D-glucose solution with a determined standard DPM of \(1.2 \times 10^4\). The orifice of each canal was capped with the disposable needle and wax, being careful not to occlude the canal orifice or needle opening. One and one-half milliliters of sterile physiologic saline was injected in each scintillation vial. Each sample was lowered into its wash bath, apex down, and the time recorded. All samples were transferred one hour after injection to another vial with a fresh wash bath. This was repeated at twenty-four and forty-eight hours yielding a total of three wash values per sample tooth. To each vial was added fifteen milliliters of Monophase 40 scintillation fluid*. Six glass tubes were used as controls and manipulated exactly the same way as the test samples. During the experiment, seven sample teeth were lost, mostly due to an accidental break in the seal between the root surface and needle hub. Consequently, two-hundred and ten scintillation vials as well as several standards and blanks were placed in a Packard scintillation

*Packard Instruments, Downers Grove, Illinois
counter, and ten minute counts were recorded for each vial. Blanks consisted of 1.5 ml of saline solution and 15 ml of scintillation fluid. Standards were the same make up as blanks plus one lambda of the radioactive glucose solution. Counting efficiency was determined by using $^{14}\text{C}$ toluene as a calibration standard. Efficiency was determined to be approximately 75% with a background counting rate of 50 CPM.

Results were placed in table form and converted into total percentage leakage. An analysis of variance was performed using the between within method considering the following comparisons: Was there a significant difference between any of the six groups tested? If so, which groups were significantly different from each other, and was there specifically a difference between roots coated with varnish and those left uncoated for each material?

Sample teeth randomly selected, were oxidized in a Packard Tri Carb Oxidizer to again attempt to recover one-hundred percent of the injected solution's radioactive content. The samples were individually placed in a platinum wire oxidation basket (see Appendix figure F) with the addition of equal amounts of cellulose powder and filter paper to support better combustion. The system was closed and the test samples were electronically ignited and burned for one to two minutes. The gaseous products of combustion were collected, namely $\text{CO}_2$ and water, liquified and deposited within the closed system into two vials, one designed to collect carbon labelled products. Fifteen milliliters
of scintillation fluor were automatically added and the vials were sealed and placed in the counter as were the wash samples.
CHAPTER III

RESULTS

SCINTILLATION COUNTING AND PERCENTAGES

The results of the scintillation measurements from the wash baths at one, twenty-four and forty-eight hours for all samples are listed in Table 2. Table 3 lists this data in the form of percentages of the total standard DPM count of $1.2 \times 10^4$ per lambda of injected solution.

The material groups were broken down into two groups each, those whose roots were coated with nail varnish and those left uncoated. It can be readily seen from the tables that the most significant leakage occurred between one hour and twenty-four hours. In almost all cases, the measurable radioactivity measured had begun to taper off at the forty-eight hour sample time. Glass controls showed insignificant wash bath counts indicating no broken seals and no penetration through the glass of carbon-labelled molecules.

There appeared to be significant differences between the materials tested but not between those samples with coated and unaltered root surfaces. Between within statistics affirmed these observations. An example of the calculator form of
### Table 2

#### DPM / Wash Samples

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#### Cavit Glass

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statistics used is shown in the Appendix figure G. The mean for the entire study was 5% excluding the controls. A comparison of all six groups gave an F value of 11.7, well above the value of 3.36 at the 0.01 level of significance. When each material was studied individually to determine if a significant difference, even at the 0.05 level existed between coated and uncoated roots, none could be demonstrated. Therefore, the remainder of the statistical analysis combined the sample results for coated and uncoated samples creating a larger sample size for each material tested. From this analysis it was shown that there was no significant difference between the leakage values of Cavit and amalgam, but amalgam showed significantly better sealing capabilities than gutta percha at the 0.01 level of significance. Cavit showed better sealing capacity not at the 0.01 level, but at the 0.05 level of significance. A summary of the F values and significant differences between test groups can be seen in Table 4.

SAMPLE COMBUSTION

Oxidation values for the randomly burned samples are shown in Table 5 along with the percentage wash values from Table 3. The total recovery approaches over sixty percent in less than one-half the samples, and over eighty percent only four times. A total of twenty-eight roots were oxidized.
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TABLE 4

Summary of F Values and Significant Differences Between-Within

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<th>F value 0.05</th>
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<td>mg. wt.</td>
<td>DPM/Sample</td>
<td>% RECOVERY</td>
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<td>------------</td>
<td>------------</td>
<td></td>
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<td>Total</td>
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CHAPTER IV

DISCUSSION

It is obvious that even Coolidge, in 1930, recognized the potential of surgical procedures in the correction of endodontic failures. No matter what the status of the hollow tube theory, almost all investigators agree that sealing of the root canal has great value when attempting the highest rate of endodontic treatment success.

The literature is replete with studies showing that all filling materials exhibit marginal percolation, microleakage, micromarginal leakage, fluid exchange, liquid diffusion, or capillary penetration (Going, 1972). The major problems with the studies to date is that all materials that have demonstrated marginal leakage have not been ideally compared. Quantitative autoradiography is difficult to attempt, and difficult to analyze in order to draw valid comparisons from (Baserga, 1969). Autoradiography is a qualitative tool that has been forced into action as a method of answering what appears to be a quantitative question. Scintillation counting provides a much more reliable method of leakage detection with highly reproducible data.
collection as shown in this study. The selection of the isotope appears to be critical. Tritiated water proposed problems here and has a history of difficulties associated with its use. Burke (1975) successfully used tritium to study the diffusion fluxes across human enamel. A diaphragm of enamel was placed between two diffusion cells, and different rates of diffusion were measured for canine versus incisor teeth. This penetrability and diffusion potential of tritium across enamel membranes could well be established some day across cementum or dentin in like manner. Leakage data using tritiated water therefore could be masked by diffusion through the tooth structure as well as around and through the restorative material. The penetration of glass by tritiated water in this study should be considered a clear contraindication for its use in leakage potential studies. As early as 1937, Bodecker demonstrated this porosity of tooth structure using dyes. Wainwright (1953) believes cementum and enamel are permeable to some substances selectively, but not all substances. Calcium chloride, C\textsuperscript{14}-labelled urea and radioiodine were all shown to penetrate intact tooth structure and reach the pulp. He also stated that the dentinal tubules and cellular cementum near the root apex are probably more permeable than the acellular cementum more occlusally on the root surface.

The results of this study using C\textsuperscript{14}-labelled glucose showed no through penetration of the root itself as indicated by the coated and plain samples. At least on the forty-eight hour
short term basis, all the radioactivity detected in the wash baths came from around or through the restoration placed at the apex of the root. This fact can be attributed to the molecular size and other physical and chemical properties of the glucose molecule. Also, that most porous apical portion of the roots had already been removed in the apicoectomy procedure prior to the preparation and filling.

Results showing that amalgam and Cavit sealed considerably better than gutta percha alone seem to agree with most previous qualitative studies. It should be noted that amalgam sealed better than gutta percha at a level of significance higher than that of Cavit. Parris (1964) stated that Cavit and amalgam were equally superior to gutta percha in sealing ability. This was found not to be true. Comparison with Yee's study (1975) is difficult in that his model differed considerably; the tooth's crown acted as the reservoir of radioactive material and the canals were filled their entire length.

Finally, of concern were the attempts at quantitative recovery of the isotope used. One-hundred percent recovery was never realized, and rarely were recovery values consistent. The possibility of bacteria present in the canals breaking down the glucose to some other form and ingesting or processing any of the test solution was ruled out by the pilot study on autoclaved teeth handled aseptically. Recovery for this group was the same--irregular and never near one-hundred per cent.
Two explanations seem plausible but have not been proven.

The oxidation temperature of the samples in the Tri Carb Oxidizer is said to be between 600-1000 °C. Is this temperature sufficient to break down the carbon in the compact, highly intricate tubular and calcified structure of the dental root for quantitative conversion to CO₂? If it is, is a temperature of 1000 °C actually realized in the combustion chamber, and are the combustion times long enough? Nixon (1964) said,

"... radioisotopes have been used to show that transport of ions takes place through dental tissues. This transport is two way and occurs from the pulp through dentin and enamel and also from saliva through enamel and dentin. Substances like glucose can be labelled and made radioactive and its passage through the enamel studied."

Going (1960) stated that isotopes, because of their different ion charges, have distinctive chemical affinities and therefore particular adsorption potentials and diffusion patterns through and around tooth substances and restorative materials. Wainwright (1953) showed the passage of C¹⁴-labelled urea through tooth roots in twenty-four hours. With these documented conclusions, is it not practical to assume that C¹⁴-labelled glucose could be held in a bound or converted state somewhere in the root structure that does not allow it to be volatilized and measured? Could diffusion through and adsorption to the walls of the dentinal tubules account for such results? It was shown in pilot studies prior to this final experiment that C¹⁴-labelled
glucose injected into teeth and oxidized immediately showed the highest recovery values of all. Possibly tubular penetration, binding or adsorption was not allowed to occur in such a short time. At any rate, the work of Zurbriggen (1975) would be highly suspect to error in light of the results of this experiment. He instrumented canals with a $^{14}$C-labelled chelating agent and measured, by combustion analysis and subsequent liquid scintillation counting, the amount of labelled material retained in the canals after irrigation with sodium hypochlorite. Only 3.8% of the initial activity was recovered as retained in the roots. It can now be shown that a considerably higher percentage may have been retained but never counted.

All studies to date could be termed only exercises unless some significance is attached to microleakage potential. Molecules of various substances surely diffuse around or through restorative materials. Whether this phenomenon can potentiate endodontic failures or failures of endodontic reverse fills is still only speculation. An in vivo study demonstrating this potential might add more credence to this argument, but so many factors would be involved that attributing a certain percentage of failure to one shortcoming would be impossible. It can be said that if all considerations are weighed, the best apical seal possible with a material well tolerated by living tissue should be the filling material of choice in endodontic reverse filling
techniques. Silver amalgam seems to meet these requirements better than any other material tested including Cavit.
SUMMARY AND CONCLUSIONS

Seventy-two extracted human central incisors were reverse filled with silver amalgam, Cavit and warm gutta percha in an attempt to quantitatively investigate the differences between the marginal sealing capabilities of these materials and the possible relationship between this factor and the treatment of endodontic failures. A $^{14}C$-labelled solution of glucose was measured and injected into each sample and scintillation counting and sample oxidation were performed to measure the amount of radioactive material lost around or through the test materials. The experiment clearly showed the following:

a) That microleakage of molecular size particles does occur around what appears to be clinically adequate fills.

b) That a quantitative measurement of this microleakage is possible and can be used to compare materials used as endodontic reverse filling agents.

c) That amalgam appears to be superior to Cavit and gutta percha as an endodontic reverse filling material.

d) Tritiated water is not suitable for the measurement of microleakage in this technique.
Further experimentation is necessary to consider the problem of quantitative recovery of test solutions as it pertains to this study and as it pertains to the structure and function of the tooth.
REFERENCES


**FIGURE A**

Plugger fitted into prepared canal

**FIGURE B**

Apical filling placed and plugger removed
FIGURE C

The Closed System
FIGURE D
One Lambda Syringe

FIGURE E
A One Lambda Sample
FIGURE F

Platinum Wire Combustion Basket

$F$ value is 3.32 at the 0.01 level; therefore, there are significant differences because $F_{0.01}$ is greater than 3.32.

FIGURE G: Example Between Within Statistical Design Study
Total sums of squares = \( x^2 - \frac{(\Sigma x)^2}{N} \)

\[ = 2048.6 - \frac{(319.6)^2}{64} \]

\[ = 2048.6 - 1596 \]

\[ = 452.6 \]

Between sums of squares

\[ = \frac{(\Sigma x_1)^2}{N_1} + \frac{(\Sigma x_2)^2}{N_2} + \text{etc.} - \frac{(\Sigma x)^2}{N} \]

\[ = 228 \]

Within sums of squares = Total - Between

\[ = 224.6 \]

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<th>Sums of Squares</th>
<th>Mean Square</th>
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</tbody>
</table>

F value is 3.36 at the 0.01 level; therefore, there are significant differences because 11.7 is greater than 3.36.

FIGURE G. Example Between Within Statistics Total Study
The thesis submitted by Jerome V. Pisano, D.D.S., has been read and approved by the following committee:

Dr. Joseph M. Gowgiel  
Associate Professor and Chairman  
Department of Anatomy, Loyola

Dr. Walter E. Kisieleski  
Clinical Associate Professor,  
Oral Pathology, Loyola

Dr. Marshall H. Smulson  
Professor and Chairman  
Department of Endodontics, Loyola

Dr. Norman K. Wood  
Associate Professor and Chairman  
Oral Diagnosis, Loyola

Dr. James L. Sandrik  
Assistant Professor,  
Dental Materials, Loyola

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 by the Committee with reference to content and form.

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

Date: Aug 6 1976  
Signature: Dr. Joseph M. Gowgiel