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The Effect of Eugenol and Eugenol-Containing Root Canal Sealers on the Microhardness of Human Dentin

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THE EFFECT OF EUGENOL AND EUGENOL-CONTAINING ROOT
CANAL SEALERS ON THE MICROHARDNESS OF HUMAN DENTIN

by

Glenn M. Biven, B.S., D.D.S.

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

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CURRICULUM VITAE

Glenn Mamo Biven was born in Honolulu, Hawaii on the 4th day of July 1935. He attended Iolani School in Honolulu, and graduated in June, 1954.

After four years at Creighton University College of Arts and Sciences, Omaha, Nebraska, he received his Bachelor of Science degree in June, 1958, and entered Creighton University School of Dentistry that fall. He graduated from the dental school in June, 1962, with the degree of Doctor of Dental Surgery.

Following graduation from dental school, he entered the United States Army Dental Corps and has served at Fort Lewis, Washington; Seoul, Korea; and Fort Sam Houston, Texas. In 1969, he was accepted to the Graduate School, Loyola University matriculating in the Department of Oral Biology, and to the Department of Endodontics as a resident.

He is a career officer in the United States Army with the rank of Major.

DEDICATION

To my wife, Lucille, and my children, Petrina and Gentry, whose loyal support and sacrifices have contributed to my goals.

To my mother, Carnation K. Biven, for her love, encouragement, and inspiration in making this all possible.

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CHAPTER 1

INTRODUCTION

Through the years, men especially versed in the field of endodontics have been concerned with the perplexity of exactly obliterating a root canal. It is a most important phase in endodontic therapy that the root canal space be entirely filled with some inert and hermetically sealing agent to attain success.

Ingle¹ simply states that: "The primary objective of operative endodontics must be the development of a fluid-tight seal at the apical foramen, and total obliteration of the root canal space." This idea of a totally obliterating and hermetically sealing the root canal has been expressed by many authors^{2,3,4,5,6} in a number of ways.

Grossman⁶ has characterized the various acceptable filling materials as cements, pastes, plastics, and solids.

Currently, the methods most frequently employed to fill root canals involves the combination of a bulk filling material consisting of a rigid or plastic point used in conjunction with a cement or sealer. Whether or not the cement or sealer itself would bring about the hermetic seal is not a matter of

conjecture, for it is the combination that properly fills the root canal.

The requirements and characteristics of a good root canal cement has been enumerated by Grossman⁶ and Sommer et al.⁹ as follows:

- 1) It should be tacky when mixed to provide good adhesion.
- 2) It should provide a hermetic seal.
- 3) It should be radiopaque.
- 4) It should be tissue tolerant.
- 5) It should be bacteriostatic.
- 6) It should not shrink.
- 7) It should set slowly.
- 8) It should be insoluble in tissue fluids.
- 9) The powder particles should be very fine for easy mixing.
- 10) It should be soluble in a common solvent for easy removal if necessary.

The ideal root canal cement or sealer that will satisfy all these requirements has yet to be formulated. For the various commercially available root canal sealers, there are no set standards of quality or performance with which they must comply. Root canal sealers are currently outside the

scope of the Food and Drug Administration of the Federal Government and do not come under the auspices of either the Council on Dental Therapeutics or the Bureau of Materials and Devices of the American Dental Association, although the latter body may at some future date concern itself with the physical properties of root canal sealer, if not their histopathological potentials. In general, very little basic research precedes the introduction of a root canal sealer into the dental market place, and it is up to the consumer to discover the discrepancies between promotional patter and clinical performance, and then attempt to compensate for shortcomings in the different products that they desire to utilize.

The majority of the studies of root canal sealers deal with connective tissue responses to them, and a limited number of the investigations have been concerned with the physical properties of root canal sealers. This study will undertake the investigation of an unknown property of root canal sealers. It is the purpose of this study to determine the effect(s) that eugenol and eugenol-containing root canal sealers have on sound dentinal surfaces. By means of comparative microhardnesses of pretreated and posttreated dentinal surfaces of the root, the effects of various sealers will be measured by determinating the alteration these materials produce in the

hardness of dentin.

CHAPTER 2

LITERATURE REVIEW

During the past two decades, tremendous strides have been made in the scientific approach to endodontics. At the Second International Conference on Endodontics held in Philadelphia in 1958, Ingle and Levine¹⁰ demonstrated the fact that there was a total absence of true formulation of endodontic instruments and filling materials alike. The analysis of their reported data led the way to the resolution of some of the major problems relating to the uniformity of sizing in endodontic instruments and solid core filling materials. We are currently approaching a point of ultramodern refinement of the design and construction of the instruments required in the practice of endodontics, but we have yet to begin resolving the problems relating to endodontic filling materials.

The clinical methods of preparing the pulpal space for receiving a root canal filling material, the method of bacterial control, and the method of obliterating the root canal are many and varied, and are justifiably so. As Grossman¹¹ reminds us, the fact that there are several methods of filling

canals is in itself an indication that no one method will suffice in all cases. The canals may be wide or narrow, long or short, round or ovoid, straight or curved, and all combinations thereof. The methods used in arriving at a good root fill may be considered immaterial provided the pulpal space is totally sealed and obliterated.

There have been many materials, literally hundreds, used in the attempt to find the root canal filling material ideal for all circumstances. Grossman⁶ gives a partial list of these that runs the gamut from amalgam to wood. Coolidge and Kesel¹³ groups the root canal filling materials into:

- 1) Antiseptics combined with a vehicle.
- 2) Cements and plastic materials.
- 3) Gutta-percha used separately or in combination with other substances.
- 4) Metals.

Grossman has stated in 1963¹⁴ and in 1966⁶ that the next great advance in endodontics will be in the field of root canal filling: a more simple and accurate filling material. Since that time, there have been no major advances in this area.

The use of a solid or plastic point in combination with a cementing material is by far the most popular means of obliterating a root canal at present (1971). Whether the more uni-

versally used gutta-percha cone or the less acceptable silver cone is used, it is felt by most practitioners that these points alone do not adequately seal a root canal and they must be used in conjunction with a sealer or cement.

The need for a root canal sealer or cement can be clearly shown when one looks at cross-sections of the roots of teeth prepared for filling. The normal irregularities of the pulp canal are almost never completely eliminated during the canal preparation. Haga¹⁶ in 1967 pointed out that at 2mm. and 6mm. levels from the apex, the root canal files cut only on three walls of many of the root canals he studied. Gutierrez and Garcia¹⁷ demonstrated through microscopic and macroscopic investigations that a high incidence of prolongations of the root canal, closely resembling the fins of a fish, were never touched by the root canal instrumentation. The occurrence of transverse or horizontal intra-radicular canals may persist and may interconnect the normally occurring central root canals at varying levels of the tooth. Accessory canals also occur at different levels of the root, and they communicate with the periodontal ligament. Employing sealers or cements facilitates the complete obliteration of these internal irregularities in pulp canal anatomy found in many teeth.

The history of root canal cements goes back to 1856, when

Sorel^{11,13} introduced a cement consisting of a saturated solution of magnesium chloride and magnesium oxide. In 1883, Sir John Tomes¹¹ recommended using paraffin as a root canal filler. Paraffin was easy to use, but it tended to shrink. In 1920, Brady¹⁸ used a neo-balsam sealing agent which he claimed neither shrunk nor absorbed moisture. In 1929, Grove¹⁹ employed the use of "neutrolite", a carbolated resin which he described as being an impervious liquid sealer which hardens at body temperature. Also in 1929, Buckley²⁰ utilized a material called "dentinoid", a physical mixture of calcium phosphate, barium sulfate, with resins and antiseptics added to provide more plasticity when used in combination with lead points. In 1931, Rickert²¹ formulated a zinc oxide-eugenol cement which is still available commercially, and widely used in clinical practice. In 1936, Grossman²² developed a cement consisting of zinc oxide, Staybelite resin, and silver. Both the Grossman and Rickert sealers met most of the postulated requirements of the ideal root canal sealer. Schmitt²³ introduced a polyvinyl resin, Diaket, in Europe in 1951. Waechter²⁴ reported Diaket was a keto-complex in which the neutral organic agents reacted with basic salts or basic metal oxides. These polyketones united with the metallic substances in the sealer to develop cyclic complexes which are insoluble in water, but

soluble in special organic solvents and in chloroform.

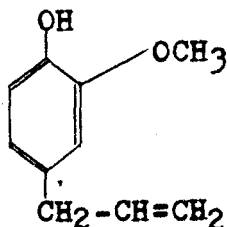
The formula for Wach's compound, a root canal sealer, was published in 1955²⁵ and again in 1958²⁶, although it had been in use for over 30 years. This cement is essentially a zinc oxide and eugenol mixture with most of the eugenol replaced by the Canada balsam. In 1957, Schroeder²⁷ demonstrated that AH-26, an epoxy resin, is well tolerated by the periapical tissues, and has good adhesive properties, contracting only slightly while hardening. Then in 1958, Grossman²⁸ developed a nonstaining root canal cement. This cement is also a zinc oxide-eugenol mixture that has been modified to slightly retard the setting time. Although reasons were published for the various additives, these reasons were not substantiated by experimental evidence.

The present day root canal cements are composed of various substances which when first combined are soft and adhesive, but later become hard as the result of chemical reactions between certain specific substances and in addition to the loss of excess liquid portions by evaporation and side reactions with other substances extraneous to the cement itself. Nielson²⁹ stated that in order for a root canal cement be effective, it must have a reasonable coefficient of expansion. It should also be impenetrable by bacteria and be resistant to both

physical and chemical influences, while in situ.

A. Zinc Oxide and Eugenol Cements

Eugenol is an aromatic oil which has a methoxy group ortho to a phenolic hydroxy group. It is the essential constituent of oil of cloves, cinnamon, and other spices. It is also called eugenic acid or caryophilic acid.

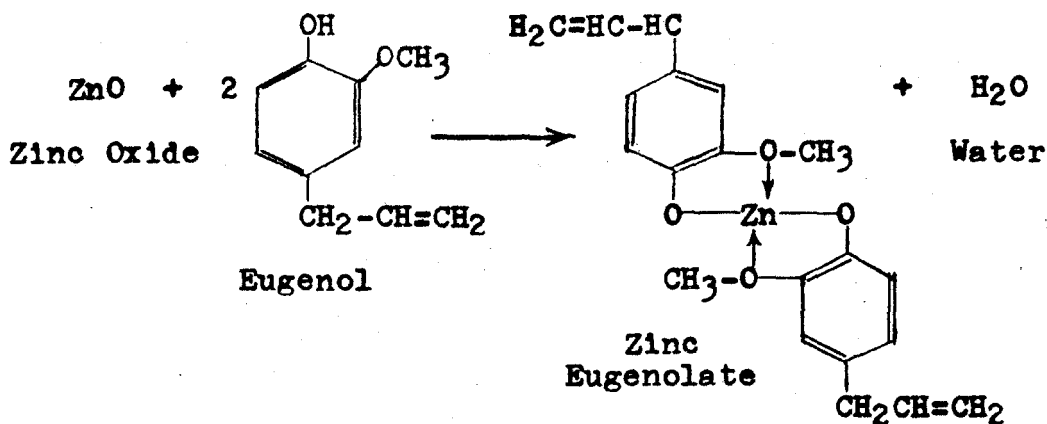


Eugenol

Molnar³⁰ reports that oil of cloves had been used for the treatment of dental caries as early as the 16th century, but it was not until 1873, that Chrisholm³¹ described the preparation of zinc oxide-oil of cloves. Since eugenol makes up 85% of oil of cloves, and it alone is the agent responsible for the therapeutic effects, it has been substituted in dental preparations for oil of cloves.

The nature of the setting reaction of zinc oxide and eugenol has been studied by many investigators^{32,33,34,35,36}. The setting reaction of zinc oxide-eugenol involves both a physical and chemical processes. Copeland et al.³⁴ points out that the set cement resulting from mixes of zinc oxide and

eugenol consists of zinc oxide embedded in a matrix of long, sheath-like crystals of zinc eugenolate. Since zinc is a bivalent element with a coordination number of four, it is likely to form a chelate. With eugenol having a replaceable hydrogen and a nearby donor in the oxygen of the ortho-methoxy group, the chelate formed here would show two molecules of eugenol and one molecule of zinc.



Upon seeing the setting reaction of zinc oxide and eugenol, one may wonder how a set mass of the cement could contain an equimolar mixture. Most mixtures do not contain an equimolar mixture of zinc oxide and eugenol, and even if the mixture did contain such equal proportions, Copeland et al.³⁴ have shown through extraction procedures that there is unreacted zinc oxide as well as free eugenol. Their explanation from their findings is that the sheath-like zinc eugenolate crystal act as a matrix for the zinc oxide, and the excess eugenol is

sorbed by the zinc eugenolate and possibly by the unreacted zinc oxide. In 1958, Smith³⁵ demonstrated that during the setting reaction of the zinc oxide and eugenol cements, a concurrent sorption of eugenol took place. Hardening of the mixture is due to the zinc eugenolate formation while the unreacted eugenol that remained trapped tends to weaken the mass. And in 1967, Molnar³⁷ reported the progressive decrease in free eugenol in mixtures of 2 hours to 10 years. He also showed that the greater the amount of agitation, the less free eugenol was present.

Besides the studies performed on the nature of the setting reaction of zinc oxide and eugenol, many investigators have attempted to perfect the formula of the cement, while a considerable number of researchers studied the effects of additives to the basic mixture. Molnar and Skinner³² showed that zinc acetate and resin reduced the setting time. Weiss³⁸ and Messing³⁹ indicated that the addition of polystyrene and zinc acetate would produce a cement which had a slow setting time and a high crushing strength. The strength of the cement can be highly increased by various additives such as polystyrene, hydrogenated rosin, and ortho-ethoxybenzoic acid (EBA)^{32,38,39,41,43,44,45,46,47}.

There are many other factors which affect the setting time

of zinc oxide and eugenol cement. Smith⁴⁰ found that the method of how the zinc oxide was prepared was closely related to its setting time. Wallace and Hansen³⁶ and others^{35,41,42} demonstrated that an increase in temperature and humidity during mixing resulted in a decrease in setting time. Smith⁴⁰ also made comment on the effect of the manner of spatulation as regards the setting time. He stated that the longer and more vigorously the mixture was spatulated, the greater the decrease in setting time. Norman et al.⁴³ demonstrated that the setting time of the cement was increased by decreasing the particle size of the zinc oxide.

B. Root Canal Cements or Sealers

Today, the most commonly used root canal cements or sealers are the modified zinc oxide and eugenol mixtures. An ideal root canal cement or sealer should fulfill the necessary requirements enumerated in the introduction of this paper, but as yet, none satisfies them all.

The mode of reaction in the setting of zinc oxide and eugenol is probably the mode of reaction in the setting of root canal sealers since zinc oxide and eugenol are the basic constituents. Through the use of various additives to the basic zinc oxide and eugenol, different authors have been able to vary the setting time, the solubility, the sealing property,

the strength, and the dimensional stability of their preparations of a root canal sealer.

In the past two decades, the drive for a more scientific approach to endodontics have lead men to investigate the physical properties of root canal sealers. In 1954, Seidler¹⁵ reported a great amount of shrinkage on setting of all the sealers, although he did not use any evidence to justify his observations. The following year McElroy²⁵ did a volume and a porosity study of nine sealers and their modifications. He concluded that the chloropercha combined with gutta-percha and Callahan's chloroform-rosin combined with gutta-percha had the greatest volume change; while Wach's filling material, Neo-balsam, and Rickert's sealer demonstrated the least change in volume of those examined. In the porosity study, he found Wach's filling material to be least porous, and chloropercha to be the most porous.

The staining effect of the precipitated silver on tooth substance in both Rickert's²¹ and Grossman's²² root canal sealers prompted Grossman²⁸ to develop a nonstaining cement which contained zinc oxide, staybelite resin, bismuth subcarbonate, barium sulphate, eugenol, and oil of sweet almond. The staybelite resin attributes adhesiveness. The bismuth subcarbonate gives it better radiopacity. The oil of sweet

almond retards the setting time. There was no scientific data presented on the adhesiveness or the retardation of the setting time.

In 1958, Stewart⁴⁸ did a study on the permeability and tensile strength (or "adhesive property" as Higginbotham⁵⁰ describes it) of Diaket, the new Grossman sealer and the Kerr antiseptic pulp canal sealer. He concluded that there was no penetration by a test dye when Diaket was used, but that the new Grossman and Kerr sealers showed sealer-tooth interface penetration by the test dye to a depth of 0.5mm. While in another part of the study he showed Diaket to have the greatest tensile strength. In the tensile strength studies, he used orthodontic wires sealed in tubes. After the sealer was permitted to set for one week, a Scott tester was used to record the necessary pounds of pressure required to withdraw the wire from the tube.

Higginbotham⁵⁰ studied some of the physical properties of five popular commercial root canal sealers: setting time, film thickness, solubility, radiopacity, and sealing ability. Diaket, Tubli-Seal, and Kerr sealer had about 21 to 23.5 minutes setting time, while no results were obtained for Proco-Sol and Kloroperka N-Ø. Film thickness varied from an average of 0.83 mm. for Tubli-Seal to 0.433mm. for Diaket. The solubility of

the materials in water for one week ranged from 0.11% (Proco-Sol) to 0.72% (Kloroperka N-Ø). The sealing ability was determined by the use of radioactive isotopes, Ca^{45} . The antiseptic pulp canal sealer and Proco-Sol showed better sealing properties at 1 month than at 1 day. It was believed to be related to the slow setting time of the materials.

In 1965 and 1968, Curson and Kirk^{51,52} tested different sealers for sealing ability, retentive strength, setting time, and working properties. The sealing ability was tested utilizing the dye penetration method. It was found that the fortified zinc oxide and eugenol cement, Tubli-Seal, Kerr Sealer, and AH-26 produced an excellent seal for the 30 day period. The strength test was studied using standard steel posts cemented into a standard size canal preparations. These were separated on the tensometer after varying intervals. Fortified zinc oxide and eugenol cement and Diaket strongly retained the posts. The setting time was determined according to the method of the American Dental Association specifications for dental cements. The materials were tested dry and with moisture to simulate a periapical situation. They showed that the more commonly used root canal sealers set in about two hours or less, and the addition of moisture decreased the setting time in most cases by one-half. They concluded that most sealers produced

less satisfactory results after 30 days, although they felt that all the zinc oxide and eugenol cements and AH-26 were suitable root canal sealers.

Further studies on the comparative physical properties of Rickert's sealer and the new Grossman sealer were conducted by Isasmendi⁵³. He judged the cement set when it resisted penetration of the explorer point. At room temperature and humidity, Grossman's sealer set in three hours, and Rickert's sealer in twenty minutes. At 37°C in water, Grossman's sealer set in four hours, while Rickert's sealer set in 30 minutes. In testing the water absorption of the cements, he prepared mixes which set at normal room conditions. The samples were immersed in distilled water for 48 hours. He found Grossman's loss 2.78% of its weight, and Rickert's sealer loss 0.99% of its weight. He concluded that Rickert's sealer was superior to Grossman's in all respects.

In some of the most recent studies concerning the physical properties of root canal sealers, Weiner⁵⁴ investigated the setting time at various temperatures and relative humidities, and the dimensional changes after setting. In evaluating the setting time, the different mixes were subjected to nine combinations of environmental conditions. All of the specimens showed decreased setting time with increased temperature and

relative humidity, with the exception of Proco-Sol Radiopaque Silver cement in a few of the test conditions where after three months the sealer was still unset. In the evaluation of the dimensional changes, the different sealer specimens were placed in glass micropipettes and visual and photographic observation were made at varying intervals. After 90 days, linear dimensional changes were seen only in Kerr sealer, Tubli-Seal, and Roth 601. Volumetric loss was made by comparing the average volume change with the original volume calculated. The loss of volume increased with the increase of time. Kerr sealer was the only cement that did not show volume loss after 30 days whereas most sealers demonstrated a greater loss of volume after 30 days. Values for Proco-Sol nonstaining sealers were not obtained because the cement did not set completely in the pipette.

The use of radioactive isotopes have been utilized by many investigators^{55,56,57,58} to study the sealing ability of the various root canal cements. Dow and Ingle⁵⁵ had concluded that poorly filled root canals did not reveal leakage, and this could lead to an endodontic failure. It may be concluded from the different studies using radioactive isotopes that the best seal is obtained when a well-fitted solid core was cemented with a zinc oxide and eugenol sealer.

In 1970, Messing⁵⁹ used a high molecular weight fluorescent dye to study the sealing properties of chloropercha, Kerr sealer, AH-26, and silver amalgam in conjunction with a solid core filling material. A low molecular weight fluorescent dye was not used because it passed through the walls of the teeth and gave false positive reports. In all of the specimens, no dye penetrated into the dentin because of the high molecular weight. It was decided that the different methods and materials used produced a hermetic seal without any penetration of the dye provided that a careful filling technic was followed.

Dentinal Response

One can conclude from a review of the literature, that there have been several investigations on the physical properties of root canal sealers, and numerous studies on connective tissue responses^{7,8,12,52,53,56,57,65,66} to these sealers. However, there have been few, if any, investigations relative to the effects of root canal sealers on dentin.

Dentin is a calcified connective tissue through which run dentinal fibers which usually are noncalcified. The dentinal fibers are protoplasmic processes of the cells that remain in the pulpal cavity. The mature dentin is composed of about 21% organic material and 67% inorganic material largely in the form

of tricalcium phosphate. The root canal sealer used in a careful total obliteration of the pulpal space is in contact with sound dentin. Mjör⁶² in 1962 studied the effects of zinc oxide and eugenol on dentin in situ. First bicuspid teeth which were to be extracted for orthodontic purposes were used in this study. He made preparations well into the dentin of several teeth, and covered the pulpal floor with a thick mixture of zinc oxide and eugenol, then filled the rest of the preparation with amalgam. Some of the teeth were left unoperated as controls. Using the Kentron microhardness tester on the unpolished teeth section after varying intervals, he found a statistically significant increase (2.9 KHN) in the microhardness of the zinc oxide-eugenol covered dentin. It was felt that the increase in hardness was due to an increase in the mineralization of the dentinal tissue. Fusayama and Maeda⁶³ investigated the effect of pulpectomy on dentin of adult dogs. One of their findings was that no significant difference could be observed in the hardness of the dentin between the devitalized teeth with the pulp chambers left open and those obliterated with a zinc oxide and eugenol cement and a gutta-percha core. However, no details on the method of hardness testing was given, nor were the areas of dentin examined identified.

To evaluate the effect of eugenol and root canal sealers

containing eugenol on the microhardness of dentin, the methods of testing must be well described as well as the areas of dentin to be examined must be well delineated.

There have been a number of methods used to determine the hardness of tooth structure. Craig and Peyton⁶⁵ found that the microscratch or microindentation was the preferred method since the hardness of enamel and dentin has been shown to have large local variations. The following year Craig, Gehring, and Peyton⁶⁶ undertook the study to correlate the microhardness of dentin with differences in structure. In this investigation, they stated that:

Measurements taken with the 10-gram load were found to show less experimental variation than those obtained with smaller loads and were considered to be more representative of microhardness values than those obtained with lesser loads because of the heterogeneous nature of dentin.

They used a 15-second time load cycle. The results of the study showed dentin adjacent to the root canals to be softer than the rest of the dentin, and the majority of the root dentin had the same microhardness as the dentin in the center of the crown.

In studies by Craig et al.⁶⁶ and Peyton et al.⁶⁷, it was emphasized that the Knoop hardness measurements were taken from the same teeth since the hardness of dentin is likely to vary considerably in similar areas in different teeth. And more

precise data can be obtained from the same tooth in the adjacent areas of comparable indentation. Because of this highly heterogeneous nature of dentin, the comparative values reported by Mjör⁶² of an increase of 2.9 KHN can hardly be statistically significant.

Rotberg and deShazer⁶⁴ in 1966 reported on the complexing action of eugenol on sound dentin. Rectangular samples of dentin were immersed in eugenol and in water. At periodic intervals, the samples were removed and the liquid phase was examined for calcium content. The comparative values were 21.5mg./100ml. of calcium in the eugenol, and 2.8-4.1mg./100ml. of calcium in the distilled water. In another investigation of this study utilizing a von Kossa method of staining treated dentin to measure its calcium content, less calcium was found in the eugenol-treated samples than in the distilled water-treated samples. Samples treated with ethylenediaminetetraacetic acid were devoid of any stain, indicating that the calcium had been completely chelated. In the third portion of their experiment, their zinc oxide and eugenol mixtures were applied to dentin sections. Later, these mixtures were removed in toto from the dentin and the samples stained by the Glycoxal bis(2-hydroxynil) method of Kashiwa and Atkinson. The surface next to the dentin stained red, indicating calcium

present, while the surface not exposed to the dentin did not stain at all. Rotberg and deShazer concluded that the "softening" of sound dentin beneath a zinc oxide and eugenol mixture might be explained by the complexing of the mixture with calcium. Since the more commonly used root canal cements or sealers are modified zinc oxide and eugenol mixtures, it is suggested that some similar interaction may take place between the sound dentinal walls of the root canal and the sealer. Upon retreatment of an endodontic case, or in the case of a restorative procedure that is intimately connected with the core root canal filler, one not infrequently finds silver cones that are very loose. Does this mean that the complexing action of eugenol on sound dentin causes it to physically soften?

With Rotberg and deShazer⁶⁴ pointing out the fact that eugenol has the ability to complex with calcium, and the fact that Molnar³⁷, Copeland et al.³⁴, and Smith³⁵ have shown that there is much free eugenol in zinc oxide and eugenol mixtures, it would seem appropriate to investigate the effect that eugenol and eugenol-containing root canal sealers have on sound human dentin.

CHAPTER 3

METHODS AND MATERIALS

A. Selection and Preparation of Specimen

Freshly extracted mature permanent teeth were collected from the Oral Surgery Department, Loyola University School of Dentistry, Maywood, Illinois. The random selection of root specimens was completed on sufficiently large roots which demonstrated some hemorrhage or gelatinous pulp remnant in the canal upon sectioning. The specimens included samples of all types of teeth with the exception of mandibular centrals and laterals. These were not used because of their narrow mesio-distal width. The fact that a tooth had either caries or restorations did not affect its selection for the experiment since only the midportion of the roots were utilized in the study.

Immediately upon the removal of these teeth, they were placed in sterile normal saline solution, and shortly thereafter the roots were sectioned horizontally, midway between the cervical area and the apex of the tooth, using a high speed air rotor handpiece with water spray. The apical portions were

used for the experiment. The sectioned surface of the root was left exposed while the rest of the root was embedded in Buehler plastic for cold mounting utilizing the Buehler Standard Specimen Mount Press*. The specimens were mounted to facilitate stabilization and easy handling during polishing and microhardness testing.

The resin mounted root specimens were polished on the Buehler Fine Grinding Apparatus* at low speed using emery polishing paper starting with grit #1, then progressively to 0, 00, 000, and finally effecting the final polish with 0000. During the polishing, care was taken not to overheat the specimens. This was accomplished by applying the blocks to the polishing wheel for only short intervals and alternately dipping the specimen into room temperature distilled water. The face of the block was then dried with paper towel, and returned to the polishing wheel. At the conclusion of the polishing on the Buehler Fine Grinding Apparatus, the block was then refined on the Buehler Standard Metallographic polisher using a microcloth and a thin slurry of levigated alumina (15 micron) and distilled water at 550 rpm. This polishing procedure resulted in an adequate surface for microhardness

*Buehler, Ltd., Evanston, Ill.

testing. In the case where the specimen was to be treated with various sealers, a carefully prepared trough was placed around and contiguous with the tooth specimen using a #4 round burr on the high speed handpiece. This procedure was initiated to prevent contact of the sealers with the resin block. A small round burr (#2) was used to countersink into the root canal to simulate biomechanical preparation of the root canal. The act of countersinking into the root canal helped remove necrotic debris and also much of the predentin that is usually removed in root canal therapy.

B. Method of Microhardness Evaluation

The method utilized to evaluate the microhardness of the dentin was similar to that utilized by Craig et al.⁶⁶ The Kentron micro hardness tester with the Knoop diamond indenter was used in this study. It was determined by Craig et al.⁶⁶ that a 10-gram load exhibited less experimental variation than measurements of smaller loads. They also employed a 15-second time load cycle in applying the Knoop diamond indenter to the specimen of dentin.

Utilizing the 10-gram load at the 15-second time load cycle, it was necessary to view the indentations at 50X magnification for proper transcription. When measuring indentations, the

makers of the Kentron micro hardness tester recommends that the objective used show a length of not less than 200 nor more than 700 filar units. The 50X magnification satisfied this requirement. The ocular piece contained a filar micrometer for determining the filar units of the indentation. These filar units can be converted to microns by multiplying them by the factor of .2068. With this measurement in microns, reference is then made to standard tables prepared for each tester by the manufacturer that will in turn convert the measurement of each indentation to Knoop hardness numbers.

Due to the heterogeneous nature of dentin, it was decided that in order to acquire valid comparative measurements, they would be made as close as possible to each other, and yet not be influenced by adjacent marks. It was empirically determined that indentations to be compared side to side be made approximately three widths apart (approximately 40 microns). If the marks were compared length-wise, end to end, they were separated by not less than one indentation width. Initial indentations were placed approximately 200 microns from each other so as to provide sufficient margin for subsequent comparative microhardness measurements.

C. Materials utilized to affect Sound Dentin

The following materials and preparations were selected for study:

A. Eugenol, U.S.P.*

B. Root canal sealers

1. Proco-Sol radiopaque silver cement**

Powder:

Precipitated silver	17%
Zinc oxide, U.S.P.	45%
Hydrogenated resin	36%
Magnesium oxide, U.S.P.	2%

Liquid:

Eugenol	90%
Canada balsam	10%

2. Kerr pulp canal sealer***

Powder:

Zinc oxide (arsenic free)	34%
Silver (molecular) C.P.	25%
Oleo-resins	30%
Dithymol diiodide	11%

Liquid:

Oil of cloves	78%
Canada balsam	22%

*The J. Bird Moyer Co., Inc., Philadelphia, Pa.

**Proco-Sol Chemical Co., Inc., Philadelphia, Pa.

***Kerr Manufacturing Co., Romulus, Mich.

3. Wach's root canal sealer*

Powder:

Zinc oxide powder A.R.	184.0 gm.
Calcium phosphate tribasic	36.81gm.
Bismuth subnitrate	64.41gm.
Bismuth subiodide	5.52gm.
Magnesium oxide, heavy	9.21gm.

Liquid:

Canada balsam	222.00cc.
Clove oil, U.S.P.	66.66cc.
Eucalyptol	5.67cc.
Beechwood creosote	5.67cc.

D. Procedure for Determination of Microhardness Values

In all of the procedures involving the various root canal sealers, the following measures were pursued:

1. The use of a sterile and clean glass slab and spatula was utilized to mix the different materials.
2. The materials were mixed according to the recommendations of the manufacturers.
3. After the initial microhardness measurements, the specimens were abundantly covered with the sealers on the sectioned dentinal surfaces only.
4. The sealer covered specimens were stored in an analytical oven (low gradient)** at 37°C at approximately 90% relative humidity.

*King's Specialty Co., Fort Wayne, Ind.

**Sargent-Welch Scientific Co., Chicago, Ill.

5. After a three week interval, the specimens were removed from the oven, and the root canal sealers or cements were flicked off the dentinal surface with a #23 dental explorer probing into the center of the root canal. In removing the bulk sealer, care was employed to avoid marring the dentinal surface. The residue sealer was removed with xylol swabs and immediately swabbed with 70% alcohol. The specimens were then dried with the air syringe.
6. Comparative microhardness readings were obtained from areas adjacent to the initial measurements.

There were two root specimens used as controls in this study. They were mounted in a sample block and polished in the manner previously described. The specimens were placed in an analytical oven at 37°C at approximately 90% relative humidity for three days. This period permitted the specimen to adjust to the environment standard for the experiment. After wiping the sectioned surfaces with 70% alcohol, thirty-six initial microhardness readings were recorded. Twenty-one indentations were made on specimen #1, and fifteen on #2. The sample block containing the specimens were returned to the oven for a three week interval. The comparative microhardness measurements made on these control specimens were made adjacent to each other.

Two resin blocks with three and four root specimens respectively were used for the eugenol treatment portion of the study. The teeth in this group were transected at mid root, and the sectioned coronal portion utilized in the study. The crowns of the teeth were embedded in the mounting resin with the specimens protruding approximately 5-7mm. This arrangement prevented contact of the resin with the eugenol. The eugenol could have caused the mounting resin to dissolve. The specimens were polished and a total of 115 initial control indentations were made on the specimens, and then recorded. Each root specimen averaged 15 to 19 initial indentations. The surfaces of the specimens were then covered with a thin layer of fresh eugenol in a clean and sterile petri dish. The petri dish was placed in the oven for a predetermined interval.

After two and four weeks of contact in the eugenol, the specimens were removed and wiped dry. The sectioned surfaces were washed with 70% alcohol and thoroughly dried with the air syringe. Microindentation measurements were made on the two week samples on the left and the four week on the right of the initial measurements.

In that part of the study concerned with the effect of Proco-Sol radiopaque silver cement, three root specimens were prepared in the usual manner and subjected to a total of 99

initial hardness measurements. Each specimen received from 28 to 42 initial measurements.

Subsequently a smooth and creamy mixture of one capsule of Proco-Sol cement powder and one drop of the Proco-Sol cement liquid was spatulated as recommended. A drop of the catalyst was added and thoroughly mixed with the cement. The mixture was applied to the dentinal surfaces of the exposed sectioned roots, care being exercised to restrict the cement to only the dentinal surfaces of the block. The sample block was then placed in the oven. After a three week interval (time period determined from the results of preliminary studies, page 34), the block was removed from the oven and the root canal cement removed from the specimens. Immediately thereafter, the specimens were swabbed with 70% alcohol, and comparative microhardness readings were recorded.

The three root specimens employed in the Kerr pulp canal sealer portion of the study received a total of 95 preliminary microhardness measurements; each of the specimens had from 22 to 45 initial readings. One capsule of Kerr pulp canal sealer powder was mixed with one drop of the required liquid to make a smooth and creamy mix. Generous amounts were placed over the sectioned surfaces of the specimens. The samples were stored in the oven at standard temperature and humidity, and at the

end of a three week interval, they were removed and the sealer was cleaned from the specimens. Comparative microhardness measurements were made for each initial reading.

In the last portion of the study, Wach's root canal sealer was studied by the manner described above. A total of 74 initial microhardness measurements were recorded on three root specimens. One drop of the liquid was combined with sufficient powder to make a smooth and creamy "mix", which after spatulation, would string one inch from the glass slab to the spatula. The sealer was placed on the sectioned dentinal surface, and the specimens stored in the oven for the three weeks. Hence the hardened sealer was removed, and comparative microhardness measurements were obtained from areas adjacent to the initial readings.

CHAPTER 4

RESULTS

In the preliminary studies with eugenol, it was found that the interaction between eugenol and human dentin as evaluated with the micro hardness tester attained a steady state of increased hardness at approximately three weeks. Values recorded after that time interval, up to six weeks, did not appear to fluctuate much despite the weekly change of fresh eugenol. Furthermore, the sealers or cements hardened quite rigidly in less than three weeks while in the oven at the experimental conditions. Consequently, the three week interval was selected as the appropriate length of time required for this study.

The results obtained from the control root specimens showed a minimum change in microhardness values (Table 1). The mean of the 21 initial microhardness recordings of specimen #1 was 53.48 KHN with a standard deviation of 8.19. The mean of the 15 initial values of root specimen #2 was 47.35 KHN with a standard deviation of 8.69. The values obtained from root specimen #1 after the three week interval demonstrated

TABLE 1
 MEAN COMPARATIVE MICROHARDNESS DETERMINATIONS (KHN)
 CONTROL TEETH

Tooth Specimen No.	Mean Microhardness Values (KHN) Time (Weeks)		Increased Microhardness ΔH_3 (KHN)
	H_0	H_3	
1	53.48 \pm 8.19 (n = 21)	52.13 \pm 7.67 (n = 21)	-1.35
2	47.35 \pm 8.69 (n = 15)	48.41 \pm 9.56 (n = 15)	+1.06

a mean of 52.13 KHN and a standard deviation of 7.67, thus showing a decrease in hardness of 1.35 KHN. Specimen #2 demonstrated a slight increase in hardness of 1.06 KHN.

In the part of the study which involved the interaction of eugenol and the root dentin, seven root specimens were utilized. Typical rows of the microhardness values are shown in Table 2. The mean comparative microhardness determinations of each tooth specimen is shown in Table 3. The mean of the initial recordings of all the specimens was 48.61 KHN with a standard deviation 9.35. The mean value of all the specimens at the two week interval showed 58.83 KHN with a standard deviation of 10.19. The mean value of all of the microhardness recordings at the four week interval was 60.37 KHN with a standard deviation of 9.65. These results are shown in Table 8.

Table 4 demonstrates typical rows of microhardness values for Proco-Sol radiopaque silver cement. The results of this portion of the study are shown in Table 5. The results here demonstrated a vast increase in hardness of dentin after being in contact with the cement. The mean of the initial readings of specimen #1 was 43.26 KHN with a standard deviation of 7.54, and this value increased to 50.40 KHN with a standard deviation of 6.12, thus yielding an increase of 7.14 KHN in the difference of the means. Root specimen #2 had a mean of 47.70 KHN

TABLE 2
TYPICAL ROWS OF MICROHARDNESS VALUES
EUGENOL

Row	Field	Readings (KHN) Time (Weeks)			Increased Microhardness	
		H ₀	H ₂	H ₄	ΔH ₂ (KHN)	ΔH ₄
1	1	40.06	55.79	57.84	15.79	17.78
	2	45.70	62.80	66.30	17.10	20.60
	3	54.07	62.80	59.75	8.73	5.68
	4	47.38	63.87	63.87	16.49	16.49
	5	44.94	55.36	62.80	10.42	17.86
	6	44.94	56.24	58.78	17.86	13.84
	7	48.44	55.79	56.81	7.35	8.37
	8	38.24	41.20	54.49	2.96	16.25
	9	41.00	52.02	53.23	11.02	12.23
	10	37.02	51.23	49.60	14.21	12.58
	11	28.78	40.40	37.02	11.62	8.24
	12	28.78	31.51	37.02	2.73	8.24
2	1	43.39	75.54	71.85	32.15	28.46
	2	46.26	49.66	51.23	2.90	4.97
	3	36.90	55.36	57.61	18.46	20.71
	4	42.44	55.79	55.79	13.35	13.35
	5	55.36	62.80	65.24	7.44	6.88
	6	32.06	37.02	43.39	4.96	11.33
3	1	67.54	71.21	74.17	3.67	6.63
	2	56.81	62.80	68.13	5.99	11.32
	3	49.16	54.07	60.24	4.91	11.08
	4	40.10	63.87	64.69	23.77	24.59
	5	54.49	60.24	68.73	5.75	14.24

TABLE 3
MEAN COMPARATIVE MICROHARDNESS DETERMINATIONS (KHN)
EUGENOL

Tooth Specimen No.	Mean Microhardness Values (KHN)			Increased Microhardness	
	H ₀	Time (Weeks) H ₂	H ₄	ΔH ₂ (KHN)	ΔH ₄
1 (n = 28)	46.49±8.52	58.30±9.34	59.20±9.23	11.80	12.70
2 (n = 33)	49.06±4.92	63.45±4.97	63.33±5.43	14.39	14.27
3 (n = 24)	59.14±8.48	75.74±9.12	74.19±6.66	16.60	15.05
4 (n = 17)	48.51±12.52	55.34±13.35	59.66±11.61	6.83	11.15
5 (n = 6)	43.43±6.07	52.04±7.70	52.43±7.29	8.61	9.00
6 (n = 8)	46.78±12.22	55.98±10.44	61.51±9.33	9.20	14.73
7 (n = 4)	46.84±12.72	50.96±16.42	52.28±18.00	4.12	5.44

TABLE 4

TYPICAL ROWS OF MICROHARDNESS VALUES
 PROCO-SOL RADIOPAQUE SILVER CEMENT

Row	Field	Readings (KHN) Time (Weeks)		Increased Microhardness ΔH_3 (KHN)
		H_0	H_3	
1	1	49.16	56.24	7.08
	2	49.16	52.42	3.26
	3	53.23	59.75	6.52
	4	51.23	60.74	9.51
	5	45.70	54.49	8.79
	6	42.44	50.85	8.41
2	1	41.30	54.07	12.77
	2	58.31	62.80	4.49
	3	60.24	65.81	5.57
	4	44.30	63.87	19.57
	5	38.77	40.40	1.63
	6	32.47	41.84	9.37
3	1	34.85	42.74	7.89
	2	50.85	57.37	6.52
	3	31.32	37.50	6.18
	4	30.20	37.02	6.82
	5	36.19	41.00	4.81
	6	54.49	62.80	8.31
	7	55.79	62.28	6.49
	8	43.06	47.38	4.32

TABLE 5
 MEAN COMPARATIVE MICROHARDNESS DETERMINATIONS (KHN)
 PROCO-SOL RADIOPAQUE SILVER CEMENT

Tooth Specimen No.	Mean Microhardness Values (KHN) Time (Weeks)		Increased Microhardness ΔH_3 (KHN)
	H ₀	H ₃	
1 (n = 27)	43.26 \pm 7.54	50.40 \pm 6.12	+ 7.14
2 (n = 41)	47.70 \pm 7.55	54.48 \pm 7.45	+ 6.78
3 (n = 29)	45.94 \pm 9.53	52.31 \pm 9.97	+ 6.37

for the initial recordings with a standard deviation of 7.55, and the mean comparative value three weeks later was 54.48 KHN with a standard deviation of 7.45, thus a resulting increase of 6.78 KHN. Meanwhile, specimen #3 had a mean value of 45.94 KHN and a standard deviation of 9.53 for its initial readings, and a mean three-week value of 52.31 KHN with a standard deviation of 9.97, thus an increase of 6.37 KHN.

Typical rows of microhardness values for the segment of the investigation that employed Kerr pulp canal sealer are shown in Table 6. This portion of the study revealed a demonstrable increase in microhardness values (Table 7). Columns 2 and 3 of Table 7 represent the mean comparative microhardness determinations in Knoop hardness numbers. The increases in hardness of each of the root specimens were found to be 8.91 KHN, 9.76 KHN, and 6.99 KHN respectively.

In the last part of the study, Wach's root canal sealer was placed on three root specimens. The results of the 75 initial recordings gave a mean of 48.77 KHN with a standard deviation of 7.46. The mean comparative three-week interval value was 51.35 KHN with a standard deviation of 6.82. The difference in hardness of the two means showed a somewhat smaller increase of 2.58 KHN.

TABLE 6
TYPICAL ROWS OF MICROHARDNESS VALUES
KERR ROOT CANAL SEALER

Row	Field	Readings (KHN)		Increased Microhardness ΔH_3 (KHN)
		Time (Weeks) H_0	H_3	
1	1	44.62	53.23	8.61
	2	49.90	57.37	7.47
	3	54.92	65.81	10.89
	4	54.92	68.13	13.21
	5	55.36	62.80	7.44
	6	41.00	47.73	6.73
2	1	49.16	66.38	17.22
	2	54.49	64.69	10.20
	3	56.24	61.25	5.01
	4	54.07	63.87	9.80
	5	55.36	63.87	8.51
	6	50.85	61.25	10.04
	7	34.42	44.62	10.20
3	1	61.86	69.96	8.10
	2	60.74	69.34	8.60
	3	58.31	66.38	8.07
	4	31.14	46.03	14.89
	5	26.10	40.70	14.60

TABLE 7
 MEAN COMPARATIVE MICROHARDNESS DETERMINATIONS (KHN)
 KERR ROOT CANAL SEALER

Tooth Specimen No.	Mean Microhardness Values (KHN) Time (Weeks)		Increased Microhardness ΔH_3 (KHN)
	H_0	H_3	
1 (n = 26)	49.29 \pm 7.09	58.20 \pm 7.52	+ 8.91
2 (n = 44)	48.03 \pm 9.12	57.79 \pm 8.59	+ 9.76
3 (n = 22)	52.26 \pm 5.17	59.25 \pm 4.78	+ 6.99

A summary of the mean comparative microhardness values, in Knoop hardness numbers, of dentin due to the interaction of eugenol and eugenol-containing root canal sealers could be found in Table 8.

The analysis of the results showed a statistically significant increase in the microhardness of human dentin when exposed to eugenol and eugenol-containing root canal sealers. The use of the t-test was employed to test the null hypothesis that the mean of the difference is equal to zero. At the $\alpha=0.01$ level, the results were statistically significant (see Table 9)

TABLE 8

SUMMARY OF MEAN COMPARATIVE MICROHARDNESS VALUES (KHN)
OF HUMAN DENTIN DUE TO EUGENOL AND EUGENOL-CONTAINING
ROOT CANAL SEALERS*

Time (Weeks)	Percent Eugenol Content				
	Eugenol 100 (n = 120)	Proco-Sol ~90 (n = 97)	Kerr ~78 (n = 92)	Wach's ~22 (n = 74)	Control 0 (n = 36)
0	48.61±9.35	45.63±8.21	49.86±7.13	48.77±7.46	50.42±8.44
2	58.83±10.19	-	-	-	-
3	-	52.40±7.85	58.41±6.96	51.35±6.82	50.27±8.62
4	60.37±9.65	-	-	-	-
ΔH_3	~+11.00**	+6.77	+8.55	+2.58	-0.15

*Mean of the mean values of each tooth specimen.

**The mean value for a three week interval with eugenol was interpolated from the two and four week mean values.

TABLE 9

STATISTICAL ANALYSIS UTILIZING THE t-TEST
EVALUATION OF MICROHARDNESS INCREASE OF AFFECTED DENTIN

Material (n)	Mean* Δh_3	Mean Δh_3 Control	t-Value	Probability
Eugenol (120)	11.53 \pm 5.94	-0.37 \pm 3.44	10.51	P<.01
Proco-Sol (97)	6.91 \pm 3.65	-0.37 \pm 3.44	12.37	P<.01
Kerr (92)	8.87 \pm 4.27	-0.37 \pm 3.44	13.53	P<.01
Wach's (74)	2.62 \pm 3.51	-0.37 \pm 3.44	6.66	P<.01

*Mean of all the recordings of each group of specimens.

CHAPTER 5

DISCUSSION

In 1966, Rotberg and deShazer⁶⁴ observed a slight to moderate softening of sound dentin beneath clinically applied zinc oxide and eugenol mixtures in the preliminary work prior to their study of the action of eugenol on sound dentin. Although no scientific data was shown how the dentin was analyzed for softening, it would seem possible that loose particles of zinc oxide and eugenol on the dentinal surface mixed with moisture could have been mistaken for softened dentin. Further in their investigation, they showed the progressive removal of calcium from dentin. They employed the von Kossa staining technic for identification of the relative amounts of calcium remaining in the various dentin sections after being treated with water, eugenol, and a 5% solution of disodium ethylenediaminetetracetic acid (EDTA). They also utilized a modified spectrophotometric method for determination of calcium. The third method that they used to analyze for calcium was the Glyoxal bis(2-hydroxynil) method of Kashiwa and Atkinson. In their investigation, they demonstrated the ability of eugenol

and zinc oxide - eugenol mixture to progressively remove calcium from dentin. They thought that the softening of sound dentin beneath a zinc oxide and eugenol mixture could be caused as a result of the ability of eugenol to complex with calcium. The results obtained in the present investigation disclosed an increase in the microhardness of human dentin, which would be contrary to the findings of Rotberg and deShazer⁶⁴ if their use of the word "softening" was to indicate the decrease in hardness of dentin. But the present results would be consistent with the results of Mjör⁶² which showed slight increase in microhardness of dentin due to the effect of zinc oxide-eugenol.

It was shown by various investigators^{34,35,37} that there is much free eugenol available in zinc oxide and eugenol mixtures. The fact that there is a large amount of free or unreacted eugenol present in zinc oxide and eugenol mixtures over the period of years, leads to the premise that modifications of these mixes do contain much free eugenol. When root canal sealers that are basically zinc oxide and eugenol mixtures are utilized in endodontics, these sealers do come in contact with dentin and probably interact with it. The results of this study positively indicate that with the increase of eugenol content in the various root canal sealers, there is a corresponding increase in microhardness of the dentin. This finding and an

examination of the composition of root canal sealers; and in association with the results of Mjör⁶² and Rotberg and deShazer⁶⁴ strongly suggest that eugenol is the material which brings about the changes in microhardness of dentin.

In his study of the effect of zinc oxide and eugenol on dentin in situ, Mjör⁶² showed an average mean increase of 2.9 KHN. However, he compared entirely different teeth with each other and areas in the same teeth but remote from each other. The findings of Mjör⁶² coincide with the assumption that dentin hardness is related to the viability of the dental pulp and its biologic response to cavity preparation and filling materials. The mineralization of the dentinal tubules in a viable tooth is a normal biologic response to various irritations. Nevertheless, Craig et al.⁶⁶ and Peyton et al.⁶⁷ emphasized that in order to obtain more precise data, comparable measurements must be taken in adjacent areas of the same field due to the highly heterogeneous nature of dentin. Hence, the comparative values reported by Mjör⁶² can be considered less than reliable. In demonstrating the variations in microhardness from areas adjacent to the pulp to areas in the vicinity of the cementum in transverse root sections, Craig et al.⁶⁶ showed values from 56 to 69 KHN with a mean of 62 KHN. In a 2mm. distance with seven readings, it was readily apparent that a comparative

reading be taken within 300 μ of the initial reading for a significant value to be obtained. The comparative recordings acquired in the course of this present study were taken approximately 200 μ from each other. The findings of this investigation are in accord with those of Craig et al.⁶⁶.

From the results of Table 8, the difference in hardness after three weeks shows Proco-Sol to be somewhat out of context in relation to the rest of the materials since its eugenol content is greater than that of Kerr, and yet its increase in hardness is slightly less. The most likely explanation for this slight deviation from a smooth curve would be a possibility of the different components of the various sealers as well as the particle size of the material. The reaction of the particular component with eugenol could be the factor which causes less eugenol to be free to interact with the dentin. The particle size of the material plays an important role. The smaller the particles in the mixture, the more surface will be available for the eugenol to react with and therefore less free eugenol will be available to interact with the dentin. Consequently, a conjecture could be made that if mixtures of liquids with increasing percentage of eugenol are mixed with equal amounts of a zinc oxide of uniform particle size, there would be a linear relationship between the gradual increase in micro-

hardness values and the increasing percentage of eugenol content of the mixes as they interact with dentin.

The results obtained in the part of the study which involved Wach's root canal sealer showed a slight increase in microhardness. The average mean difference in hardness after three weeks demonstrated a 2.58 KHN. It would seem logical that the low increase in hardness would be consistent with the low percentage of eugenol in this sealer. The different microhardness readings had a wide variation. However, approximately 80% of the recordings showed a significant increase in microhardness values.

Some explanations for this increase in the microhardness values of dentin that have been exposed to eugenol and eugenol-containing root canal sealers would now be considered.

The structural nature of dentin consists mainly of odontoblastic processes within the dentinal tubules and intercellular substances of mineralized apatite crystals, hypomineralized areas, and varying amounts of organic elements and water. Dentin consists of 70% inorganic material and 30% organic matter and water. Therefore, in order to discuss the findings of this study, there must be some speculation of the action of eugenol and the root canal sealers that contain eugenol on either the organic or inorganic substances of dentin, or the combination

of these materials.

A tentative explanation for the increased hardness of human dentin due to eugenol and eugenol-containing root canal sealers may be the effect of the eugenol on the organic portion of dentin. As shown in a previous study⁶⁴, eugenol does have a complexing action with calcium, thereby removing calcium from dentin. This may be a secondary factor in the inducement of increased hardness of dentin. The primary factor may be the "coagulating" effect of eugenol on collagen. As mentioned before, mature dentin is composed of approximately 20% organic material. It would seem feasible that the eugenol-effect initiates a high volume loss of water from the dentin, and thereby altering the ratio of water to calcium. As the effect of eugenol further causes increase in "coagulation", there is a tendency for a state of equilibrium in concentration in acquiring more water to compensate for the increased amount of calcium. The calcium now tends to bind with the protein within the dentinal tubules and its interconnecting branches. Furthermore, with the number of tubules per square millimeter on the pulpal surface of dentin varying from 30,000 to 75,000, it would seem possible for the calcium-protein salt formed to increase the remaining hydroxyapatite structure strength by forming a rigid lattice frame within the dentin.

The increased hardness of dentin through the action of eugenol and eugenol-containing root canal sealers as a result of this study could also be interpreted as an effect caused by eugenic acid. Eugenic acid is a weak acid, but stronger than carbonic acid. The action of eugenic acid on the hydroxyapatite and uncalcified substances is possibly the breakdown of the calcium salts in such a manner that a loss of carbonates take place as eugenic acid replaces the weaker carbonic acid of dentin. With the loss of carbonates, there is a rise in the pH, and thus remineralization or reprecipitation of minerals from the dissolved apatite takes place which in turn possibly creates a more rigid dentin. The reprecipitate could possibly occur peritubularly or within the dentinal tubules. The idea of this weak eugenic acid reacting with dentin is consistent with the slow changes that take place as evaluated by microhardness testing.

How the increase in hardness of the dentin could affect the physical property of brittleness of the dentin could be speculated at this time. Dentin has a modulus of elasticity that varies with the age of the tooth. With the increase of calcification of the tooth, the relative amount of the inorganic component rises and the relative amount of the organic material decreases and there is a gradual loss of elasticity. The

application of eugenol or eugenol-containing root canal sealers to dentin increases the hardness of dentin. Whether this increase is due to the effect of eugenol on the organic materials of the dentin or the remineralization of the dentin caused by eugenic acid, it could be speculated that there may well be an increase in the brittleness of dentin under these clinical circumstances.

It has been reported by different investigators^{68,69,70} that there seems to be a failure of zinc oxide and eugenol to produce a calcific bridge of secondary dentin at the site of pulp exposures. However, in dentin with near pulpal exposures, there is a mineralization in the viable tubules due to the normal biologic response of the odontoblastic processes to the irritation caused by the zinc oxide and eugenol. In the case of pulp exposures, the failure of a calcific bridge to form indicates that the uncalcified available components for hydroxyapatite in the surrounding dentin binds with eugenol or the impurities of eugenol. This lack of calcification in the form of reparative dentin at the site of exposures points to the fact that there could be insufficient calcium present, and the "remineralization" of dentin overlying unexposed pulps is related to an overabundance of calcium and is rather a reprecipitation of this mineral.

CHAPTER 6

SUMMARY

The use of root canal sealers or cements in conjunction with a rigid or plastic master point has become the most popular means of obliterating a root canal. There have been studies that have investigated the connective tissue response to these materials, and a few studies that have evaluated the physical properties of these materials. The effect of eugenol and several eugenol-containing root canal sealers on human dentin was analyzed by the microhardness testing in this study. Sound dentinal surfaces from the midsection of the roots of freshly extracted teeth were subjected to eugenol, Proco-Sol radiopaque silver cement, Kerr pulp canal sealer, and Wach's root canal sealer for varying time intervals. Teeth used as controls were subjected only to the environmental conditions, and not to the various materials. The comparative microhardness readings of the control dentin did not show a significant difference in the experiment time period, whereas the dentin that had been exposed to eugenol, Proco-Sol, Kerr, and Wach's sealers demonstrated a statistically significant increase in microhardness values.

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APPROVAL SHEET

The thesis submitted by Dr. Glenn M. Biven 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 requirement for the degree of Master of Science.

May 20, 1971
Date

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