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Histopathologic Comparison of Two Commonly Used Root Canal Sealers

Henry Bruce Feinberg
Loyola University Chicago

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HISTOPATHOLOGIC COMPARISON OF TWO
COMMONLY USED ROOT CANAL SEALERS

by

Henry Bruce Feinberg

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

Henry Bruce Feinberg was born in Springfield, Illinois on August 6, 1943. He attended Butler Grade School until moving to Gary, Indiana in 1953. He attended Glen Park Grade School and graduated from Lew Wallace High School in June, 1961. He entered Indiana University and graduated with the degree of Bachelor of Science in Dentistry in June, 1965.

In September of 1964, the author entered Indiana University School of Dentistry and was graduated with the degree of Doctor of Dental Surgery in June, 1968. For the next seven years, he practiced general dentistry, three years in Gary, Indiana, and four years in Lansing, Illinois.

He began graduate studies in the Department of Oral Biology and clinical training in Endodontics under Dr. Franklin Weine at Loyola University in August, 1975.

Dr. Feinberg is married to the former Deborah Gubitz of Munster, Indiana.
DEDICATION

To my parents, Marvin and Helen Feinberg, and to my wife, Deborah, all of whose love, help, and encouragement has brought me through the various phases of my formal education.
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To my advisor, Dr. Franklin Weine, whose friendship since dental school and guidance during graduate training have been of unmeasurable value. For this opportunity, I offer my sincere appreciation.

To Dr. Marshall Smulson and Dr. James Sandrik, whose advice has been most helpful, I offer my thanks.

I offer my sincere thanks to the Department of Endodontics for making the past two years possible.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. REVIEW OF RELATED LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>III. MATERIALS AND METHODS</td>
<td>19</td>
</tr>
<tr>
<td>A. PART ONE</td>
<td>19</td>
</tr>
<tr>
<td>B. PART TWO</td>
<td>21</td>
</tr>
<tr>
<td>IV. RESULTS</td>
<td>25</td>
</tr>
<tr>
<td>A. PART ONE</td>
<td>25</td>
</tr>
<tr>
<td>B. PART TWO</td>
<td>27</td>
</tr>
<tr>
<td>V. DISCUSSION</td>
<td>32</td>
</tr>
<tr>
<td>A. PART ONE</td>
<td>32</td>
</tr>
<tr>
<td>B. PART TWO</td>
<td>33</td>
</tr>
<tr>
<td>VI. CONCLUSIONS</td>
<td>38</td>
</tr>
<tr>
<td>VII. SUMMARY</td>
<td>39</td>
</tr>
<tr>
<td>VIII. REFERENCES</td>
<td>41</td>
</tr>
<tr>
<td>IX. APPENDIX</td>
<td>49</td>
</tr>
<tr>
<td>A. TABLES</td>
<td>50</td>
</tr>
<tr>
<td>B. FIGURES</td>
<td>56</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

The dental supporting structures must remain or return to a state of acceptable health in order for endodontic treatment to succeed (51). The periapical structures provide a vital, dynamic area capable of various physiologic functions, a most important of which is repair. Pulp degeneration may lead to periapical breakdown which cannot be expected to repair unless sound endodontic treatment is performed.

First, the thorough removal of affected pulpal and necrotic debris from the root canal system must be accomplished (13)(79). By careful preparation of the root canal space, the debris is eliminated and microbiological control is brought about in favor of the patient (13)(79). These preparation procedures also provide a receptacle so that the tooth will receive the root canal filling (79). Complete obliteration with optimum seal of the foramina is the desired final requisite for predicted periapical repair (13)(29)(79).

Although debridement and obturation are necessary for success, the materials used in endodontic treatment must be compatible with living tissue (57)(75)(79). It stands to reason that materials with the lowest inflammatory potential be used
to seal the root canal space.

Current theory calls for ending the root canal preparation and filling at the cemento-dentinal junction (40)(79). It is still possible, and in many situations inevitable, that root canal sealer will be expressed beyond this ideal termination (7)(15)(62). The sealer then becomes an implant with which the periapical tissues must contend.

The purpose of this study is to evaluate the tissue response to certain popular root canal sealers.
CHAPTER II

REVIEW OF RELATED LITERATURE

In 1929, Blayney (8) noted that if root canal obturation materials extruded through the apex, a foreign body reaction occurred. He stated that a better result will be obtained if the root canal is not filled beyond the apex.

Rickert (60) first mentioned his formulation marketed as Kerr's Root Canal Sealer in the late 1920's. He warned not to instrument beyond the dento-cementinal junction and not to force sealer beyond that point when obturating the canal.

Hall (32) in his early endodontic book, recommended Kerr's Root Canal Sealer, but only after the apex has been plugged with a combination of chlororosin and gutta-percha. Again, the idea was to prevent sealer from contacting vital tissue.

Rickert and Dixon (16) performed the first histologic testing of Kerr's Sealer. Rabbits were used and it was determined that subcutaneous implants were preferable to intramuscular. Kerr's sealer was encapsulated by connective tissue and therefore was determined to be inert. They felt the reaction was milder than that which they observed for chloropercha or gutta-percha.
They also claimed clinical success with Kerr's sealer by many dentists for a period of several years. They mentioned that it is inevitable that the sealer will contact vital tissue and therefore, root canal sealers must be tolerated by tissue.

In a subsequent report, Dixon and Rickert (61) tested several different dental materials both intramuscularly and subcutaneously in the rabbit. The surgery was described as dorsal incisions with blunt dissection of pockets into which the samples were placed, and the wounds were then sutured with black silk. The Rickert's formula root canal sealer was claimed to give a similar reaction as that to glazed porcelain, and a milder reaction than the sealer components individually. At that time, fifteen years of clinical experience for the sealer had been recorded. It was then proposed that it was better to overfill than underfill the canal.

In 1933, Boulger (9) implanted human root apices filled with gutta-percha into the thighs of rats. It was concluded that gutta-percha was well tolerated, but no mention of root canal sealer was made.

Dixon and Rickert (17) reported a histologic study of Rickert's sealer in dogs' teeth in 1938. They concluded that a small amount of inflammation promotes regeneration of cementum and periodontal membrane.
The first tissue tolerance study using monkeys was published by Bernier and Canby (4) in 1943. They observed that vitallium was well tolerated in the monkey jaw.

Ostrander (57) indicated that, in addition to sealing the root canal, a sealer must be well tolerated by the tissues and not be absorbable by body tissues, which would result in a delayed leakage. The use of Rickert's formula sealer was advocated.

In 1955, McElroy (48) reported the formulae of Rickert's and Wach's sealers. He found that both Wach's and Rickert's, when used in conjunction with a gutta-percha filling core, had much less volume change than chloropercha. McElroy listed ten desirable qualities for a root canal filling material, summarized as: non-irritating, free from volume change, radiopaque, adaptable to canal walls, sterilizable, insoluble in tissue fluids, non-staining, easily manipulated, readily removable, and non-toxic.

Hunter (36) stated that the least reactive materials should be used for endodontic fillings. He implanted silver points, gutta-percha points, and Rickert's sealer into the tibias of guinea pigs. The materials were observed at one, two, and six month intervals. He concluded that all three materials were compatible with bone, however, Rickert's sealer was considered to be non-permanent because it was seen to undergo phagocytosis.
In 1958, Stewart (74) reported on three root canal sealers, including Kerr's and Diaket. He found all three were well tolerated in rabbit soft tissue. In the antimicrobial portion, tensile strength testing and dye penetration experiments, Diaket was the most inhibitory to organisms, the strongest, and showed the least leakage. Stewart felt that the other two sealers had more desirable properties, but Kerr's has stood the test of time.

In 1958, McElroy and Wach (49) reported several clinical cases where overfills of Wach's paste occurred. In most cases, the overfill was resorbed and the cases remained successful, some having been followed for 22 years. They reported that Wach's paste had been used successfully for about thirty years and stated that there was no need to correct apical radiolucent areas with surgical intervention.

Mitchell (50) suggested that the supramuscular, subdermal rat tissue was excellent for the biological screening of dental materials. A good correlation of histopathologic response to that of humans was noted. He also recommended ether anesthesia for rats and sacrifice periods of 2-4 days, 14-16 days and four weeks.

In 1961, Marshall and Massler (45) reported on their radioisotope leakage experiments. They used autoradiography for eleven different root canal filling materials and combinations; and six different isotopes were used. Wach's was
found to be almost as good of sealing agent as Kerr's and both were superior to the other two sealers tested. The authors noted that although they used a single gutta-percha cone with sealer technique, it probably would have been better to have used a multicone technique. They concluded that sealers are essential for effective root canal obliteration.

Torneck (76) implanted root canal medications in polyethylene tubes into the backs of hamsters. Eugenol, a common component of most root canal sealers, was found to elicit a localized, but nevertheless intense, inflammatory infiltration.

In 1962, Feldman and Nyborg (21) reported on implanting silver amalgam spheres and gutta-percha rods into the mandibles of ten rabbits. When the rabbits were sacrificed after three months, they found that the amalgam was better tolerated than the gutta-percha. A thicker connective tissue capsule was seen around the gutta-percha. It was more common to find bone trabeculae in contact with the amalgam than the gutta-percha and there were more macrophages and exudate cells associated with the gutta-percha. They also found a greater occurrence of resorption lacunae in the bone associated with the gutta-percha specimens. Later, these authors (22) reported a similar experiment involving the implanting of pure silver spheres and
spheres of a root canal sealer. The results showed that a much larger capsule was associated with the sealer implant. Based on the results of the two experiments, one might hypothesize that the sealer would show less favorable biologic response than the gutta-percha, if they had been directly compared.

Guttuso (31) investigated ten endodontic materials by implanting samples in rat ventral abdominal, dorsal interscapular, and dorsal pelvic areas. Two of the four sealers tested were Diaket and Kerr's, but Wach's was not tested. Animals were sacrificed so that 2 day, 16 day, and 32 day histologic sections could be observed. Of the ten materials, Kerr's and Diaket gave the least severe reaction. He concluded that Kerr's and Diaket are good root canal sealers, however, Kerr's has the property of potentially staining the endodontically treated tooth.

Rappaport, et al (59) used four methods to assess the biologic toxicity characteristics of ten endodontic filling materials. In addition to implants in rat subcutaneous connective tissue, the materials were tested for toxicity on a tissue culture of HeLa cells, and sealer components were tested for irritation in the conjunctival sac of rabbits' eyes. The fourth part of the study was for inhibition of certain bacteria. Diaket was seen to give a severe reaction
in the rat, while Kerr's sealer was better tolerated. Similar results were noted in the tissue culture portion. As might have been expected, Diaket was superior as a bacteriologic inhibitor. The authors concluded that irritation retards healing.

Wach's and Kerr's were directly compared along with eight other root canal sealers by Maurice, et al (47), in 1965 for antibacterial effectiveness. They used five different microorganisms and found that Wach's and Kerr's were among the very best inhibitors.

Greenberg (26) introduced a new root canal sealer to dentistry in 1965. His sealer shared a few components with Wach's and Rickert's and he cited McElroy and Wach with regard to the resorbability of extruded sealer.

In 1966, Kapsimalis and Evans (39) used various polar and non-polar isotopes for autoradiography to check the sealing ability of certain filling methods and root canal sealers. Wach's appeared to seal better than Kerr's when they were used with laterally condensed gutta-percha or with silver points augmented by accessory gutta-percha.

Muruzabal, et al (53) have reported on the reaction of the rat molar periapical area to endodontic overfilling. Kerr's was studied along with several other sealers including Diaket. Muruzabal and Erausquin (54) also used Diaket in subsequent
studies. The authors (55) have also developed a standardized method of evaluation so as to compare the results of their various experiments. Erausquin and Muruzabal (18)(19)(20) have reported other experiments using their uniquely standardized technique. They have studied Kerr's, Diaket, and many other root canal sealers.

Seltzer, et al (65) studied the anatomy of the primate periapical area. Sinai, et al (70) studied the periapical reaction to pulp extirpation in the Macaca mulatta. Sacrifice was so that specimens of one week, three weeks, one month, six weeks, three months, and six months were obtained. Seltzer, et al (67) reported that better healing was obtained if instrumentation and filling were carried out short of the apex than if the teeth were overinstrumented and overfilled. Seltzer, et al (66)(69) have shown the histopathologic results of instrumentation beyond the apex in other reports. Seltzer, et al (69) have also compared overfilling and underfilling of instrumented root canals.

Higginbotham (34) studied Kerr's, Diaket, and three other root canal sealers. He examined setting time, film thickness, solubility, radiographic density, and sealing ability. Kerr's was the most radiopaque, had reasonably low solubility in both neutral and acidic media, and seemed to
have adequate working time compared to the others. It was noted that the sealing ability was better at one month than at one day. Higginbotham concluded that condensation of the root canal filling of gutta-percha and sealer combination is essential for a good seal.

In 1968, Curson and Kirk (14) reported on the setting time, sealing ability, tensile strength, and tissue reaction of eight root canal cements. Rickert's formula and Diaket were both good sealing agents, both had good strength, and both were associated with multinucleated giant cells in the tissue reaction study. They found that the unset materials were more irritating than after they had set. Since only a 6 1/2 week time period was used in the tissue reaction portion, the authors stated that long-term study was needed.

Browne and Friend (11) implanted five root canal sealers and silver amalgam in polyethylene tubes into the flanks and back of the necks of rabbits. Several observation periods of 2 days to 12 months were used. Diaket and silver amalgam initially showed severe reactions, but the amalgam samples were free of inflammation after four weeks, while a severe reaction, including multinucleated giant cells, persisted for over twelve months with the Diaket. These authors (24) found essentially the same results when they repeated the experiments in rabbit bone.
Antoniazzi, et al (1) reported on the sealing ability of three root canal sealers including Kerr's. When sealer alone was studied, Kerr's was the most resistant to dye penetration; however, when a core of gutta-percha was used with the sealers, Kerr's was second best. The authors state that an in vitro investigation such as theirs, does not give any indication of the biologic aspects. They felt that more than sealing ability must be taken into account to justify the use of a root canal sealer.

Frank and Abrams (23) reported two cases of endodontic implants from humans that they were able to examine histologically. Diaket was used to cement one of the implants and they found considerable foreign-body response after 44 months. They concluded that the inflammatory reaction was from the sealer, not from the implant, and that Diaket is very slowly eliminated from the body.

Gutierrez, et al (30) implanted sterile tubes of dentin filled with gutta-percha and sealer into rabbit subcutaneous tissue. They found a better histologic result if the sealer did not protrude from the tube and therefore it did not contact the tissue.

Langeland, et al (41) felt that a basic requirement of a root canal sealer is that it should be non-irritating to
periapical tissue. They suggested that the material be placed in polyethylene tubes and be implanted in rat subcutaneous connective tissue for preliminary screening. The periapical area of rhesus monkey teeth and ultimately the human periapex are necessary for complete evaluation of biocompatibility. In a later report, Langeland (42) concluded that all sealers are irritants in their freshly mixed state; some become relatively inert; all sealers are resorbable; sealer components may be transported to other areas of the body away from the implant site; a solid or semisolid core should fill as much of the root canal system as possible; and finally, that no or minimal sealer should touch the pulp or periapical tissue.

Spangberg (72) felt that experimental root canal sealers should be freshly prepared and placed in teflon caps. He placed the filled caps into holes in the mandibles of fifteen male guinea pigs and sacrificed them at two and twelve weeks. In a later report, Spangberg (73) found that Kerr's sealer was the least toxic of materials tested on HeLa cells.

In 1970, Weisman (80) studied the flow rate of ten root canal sealers, including Kerr's and Diaket. No clinical correlations can be drawn.

Oynick (58) has stated that the implant method of studying sealers does not reproduce the root canal filling
procedure, however, implanting of sealer samples is suitable for initial screening. He felt that the most suitable animal group for studying periapical tissue response is the primate.

Davis, et al (15), in 1971, reported a study involving instrumentation and overfilling to various lengths in dogs teeth. A better response was always seen from an underfill than an overfill, with no significant difference between the earlier sacrificed dogs and the one killed after one year. What might be the most important finding of the study was the histologic finding of sealer in the periapical area even in some cases of radiographic underfills. In this case, the biocompatibility of sealers is most important even if one is filling root canals "short".

Using filled canals with silver cones and gutta-percha in dogs. Bhaskar (5) concluded that underfills are better than overfills.

In 1971, Wiener and Schilder (81), reported on setting time of Kerr's and seven other root canal sealers. They found that heat and humidity decreased setting time. The following month, these authors (82) reported on dimensional changes of sealers. They found that all nine sealers shrank. They felt that standards for study and American Dental Association Specifications should be developed.
In 1972, after his dye penetration study, Grieve (27) found Kerr's showed significantly less leakage. He used loose fitting silver points and felt that the results might have been different had a tight fitting silver cone or condensed gutta-percha been used. Grieve (28) later reported on Diaket and several other sealers. Several compared fairly well to the Kerr's from the previous report. Grieve felt sealing was important, however, tissue toxicity, manipulative ability, and absence of dentin staining properties also must be taken into account. He points out that Kerr's sealer stains dentin.

Blair (7), in 1972, reported on sealing ability based on his dye penetration study. He concluded that a sealer is necessary in conjunction with either gutta-percha or silver cones to reduce leakage. He noted that it was inevitable for excess sealer to be expressed through the apical foramen.

Barker and Lockett (3) have reported on experimental overfills of dogs' root canals with a sealer containing zinc oxide, precipitated silver, eugenol, resin, and Canada Balsam. They generally found fibrous encapsulation, very little inflammation, and very little phagocytosis.

Safer, et al (63) have tested the pulpal response of rhesus monkeys to Kerr's sealer with and without certain additives.
Biven, et al (6) have examined the change in microhardness of human dentin to certain eugenol-containing root canal sealers. Kerr's sealer showed more microhardness increase than Wach's. This was speculated to be due to the amount of free eugenol available from each sealer.

Although Clayton (12) was studying sealing ability of apically packed dentin chips in dogs' teeth, he used laterally condensed gutta-percha with Wach's paste to obturate the root canals. In cases where sealer was accidentally expressed through the apical foramen, he found an initially severe response which later showed fibrous encapsulation. He concluded that excess sealer appeared to inhibit hard tissue formation, but it did aid in sealing against dye penetration.

Holland, et al (35) reported on their implantation of polyethylene tubes containing various root canal sealers. The rat subcutaneous tissue response was generally one of fibrous connective tissue capsule formation around the Wach's sealer. They also found a moderate infiltration of macrophages, lymphocytes, and neutrophiles associated with one of the Wach's specimens at the 30 day sacrifice.

Ingersoll (37) has compared the long term antimicrobial properties of Rickert's formula to two other root canal sealers.
Brewer (10) has used male rhesus monkeys to compare conventional endodontic treatment to paraformaldehyde paste fillings. In 1975, he reported inflammation if either were exposed to periapical tissue, but the reaction was always more undesirable to the paraformaldehyde paste, which showed bone necrosis and ankylosis.

Rising, et al (62) reported on the histologic reaction of three root canal filling materials in primate periapical areas. Laterally condensed gutta-percha with Rickert's sealer was used as the control and was interpreted as being well tolerated. Excess material was found in the periapical area no matter how carefully the teeth were prepared or filled.

Nicholson, et al (56) studied the rat subcutaneous tissue response to two formulations of gutta-percha. They used dorsal quadrants as the implant sites. Glantz, et al (25) have used bluntly dissected subcutaneous tissue pouches in rats to study dental implant materials. Wolfson and Seltzer (83) have injected samples of various gutta-percha formulations into the fascial plane of the dorsal subcutaneous tissue of rats. Wolfson and Seltzer stained their histologic sections with hematoxylin and eosin and Masson's trichrome for evaluation.

Tagger (75) reviewed current endodontic filling materials and felt that Rickert's formula and Wach's were
acceptable, but they should be used with a gutta-percha core. 
He also felt that the silver in Rickert's might be deleterious 
due to staining and possible foreign body reaction. He 
commented that highly irritating materials must not be used 
and that biological testing of sealers is more difficult than 
assessment of physical properties.

In 1976, Martin, et al (46) studied silver amalgam 
compared to stainless steel in rats. They used sacrifice 
periods of 2 days, 14 days, and 30 days.

Antrim (2) used radiolabelled tissue culture cells to 
measure toxicity of root canal sealers. He found Rickert's 
to be the least toxic of the materials tested.

Finally, Wasilkoff and Maurice (78) surveyed prac-
ticing dentists and found that Kerr's was the most popular 
root canal sealer. Wach's sealer was used by half as many 
dentists as used Rickert's formula. The likelihood exists 
that some dentists used both, and possibly other sealers as 
well.
CHAPTER III

MATERIALS AND METHODS

A. PART ONE:

The connective tissue response to three root canal sealers and a chrome-cobalt material* was tested in the following manner. Pellets of sealer approximately 4 mm. diameter by 1.5 mm. thick were prepared in a silicone rubber mold and allowed to harden. Wax patterns were made in the same mold and cast in the chrome-cobalt metal. These were then trimmed and electrolytically cleaned. The metal pellets were autoclaved; but due to the nature of the sealer pellets, they were maintained in a clean environment during and after manufacture.

Three white, male Sprague-Dawley rats** weighing approximately 500 grams each were utilized for this part of the experiment. They were treated and housed in the animal research room at Loyola University School of Dentistry.

The rats were anesthetized with ether in a large glass jar until they became inactive. They were then transferred to the operating table. Surgical anesthesia was maintained with

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**Locke Erikson Laboratories, Melrose Park, Illinois
a small glass jar containing ether saturated gauze sponges positioned near the rat's nose.

The dorsal fur was trimmed and the skin scrubbed with alcohol sponges. Four separate incisions were made through the skin of each rat, one incision in each quadrant. A pellet of Kerr's Sealer* was placed in the front left quadrant of each rat. A pellet of Wach's Sealer** was placed in each front right quadrant. Pellets of Diaket*** and chrome-cobalt metal were placed in the posterior left and right quadrants respectively in each animal. The surgical wounds were closed with 5-0 black silk suture, one suture per incision.

One rat was sacrificed at four days, the second at fourteen days and the last at thirty days. The pellets and surrounding tissue was excised and immediately placed in 10% buffered formalin for fixation. After fixation, the tissue was trimmed and the pellets removed to facilitate sectioning.

The trimmed blocks were dehydrated in ethanol and embedded in paraffin. Sections were cut at 6 microns through where the pellet had been. They were mounted on glass slides, deparaffinized, half were stained with hematoxylin and eosin and half with Masson's trichrome connective tissue stain (43).

*Rickert's Formula, Kerr Manufacturing Co., Romulus, Michigan
**King Specialty Co., Ft. Wayne, Indiana
***Premier Dental Products Co., Philadelphia
The stained histologic sections were examined with a light microscope to evaluate the amount and type of inflammation present. The results of part one are recorded in Chart II.

B. PART TWO:

To simulate the periapical reaction to sealer that may be extruded through the foramina during root canal filling procedures, part two was designed and carried out. Two rhesus monkeys (Macaca mullata) were obtained, treated, and housed at the Loyola University Medical Center Animal Research Facility. Both monkeys were male, one young adult, the other estimated to be a mature adult.

For each treatment, the animal was injected intramuscularly with phencyclidine hydrochloride*. A 1.0 cc dosage was sufficient to immobilize the animals in order to remove them from their cages and prepare them for general anesthesia.

A subcutaneous injection of atropine sulfate** was administered to decrease oral secretions. 1.0 cc was given at the time the Sernylan was administered. In some instances it was necessary to give another 1.0 cc during the endodontic procedure.

*Sernylan, Bio-Ceutic Laboratories, Inc., St. Joseph, Missouri
**Atrosed, Burns-Biotec Laboratories Division, Chromalloy Pharmaceutical, Inc., Oakland, California
The monkey was placed on the operating table and an intravenous injection of pentabarbitol sodium* was given to effect, usually about 1.5 cc. Pre-treatment radiographs were taken and developed. A spring-retained mouth prop was positioned over the cuspid teeth.

The teeth were isolated so as to approximate aseptic technique for endodontic treatment. Gauze packs, cotton rolls, the mouth prop, and atropine injections all aided in isolation. The incisal and occlusal surfaces were reduced on those teeth to be treated with a crosscut fissure bur in a high speed air handpiece. This same bur was then used to enter the pulp chamber and complete the access cavity.

Attempt was made to hand excavate the pulp chamber and #08 or #10 files** were placed in each canal. Radiographs were taken to establish the canal length measurement. The apex was penetrated by at least 2 millemeters and the canals and periapical area enlarged to the #25 file. The preparation was then enlarged to usually a #40 file, one millemeter short of the radiographic apex. The preparations were then flared, making sure that the apical foramen was kept patent by intermittently going back to the #25 file. Copious amounts of sterile physiologic saline were used for lubrication and

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*W. A. Butler Company, Columbus, Ohio
**AAE Standardized Instruments
irrigation during debridement.

The canals were dried with sterile paper absorbant points. Standardized size gutta-percha master cones* were fitted one millemeter short of the radiographic apex.

Sealer was introduced into the periapical areas with a #25 file by pumping action and counter-clockwise rotation. The gutta-percha master cones were coated with sealer and seated with a pumping motion. The canals were then obliterated with laterally condensed technique (79) using conventional sized accessory gutta-percha cones**. Immediate post-operative radiographs confirmed the presence of extruded sealer in the periapical area.

Excess gutta-percha was removed to the cervical area, retention enhanced with the crosscut fissure bur and a lingual or occlusal amalgam placed to seal the access opening.

The monkeys were operated on one quadrant each at preselected intervals so that upon sacrifice, specimens of one week, one month, three months and six months would be obtained. Sacrifice was done by intravenous injection of 12.0 cc of Beuthanasia-D Regular***. The jaws were sectioned and removed and immediately placed in 10% buffered formalin solution.

*Premier Dental Products Co., Norristown, Pennsylvania
**Mynol, Inc., Broomall, Pennsylvania
***Burns-Biotec Laboratories Division, Chromalloy Pharmaceutical, Inc., Oakland, California
Unnecessary hard tissue was removed with high speed burs to facilitate fixation. After one week, the sections were rinsed in running water for 24 hours.

A sodium citrate-formic acid solution was used to decalcify the specimens (52). During decalcification the specimens were further trimmed to facilitate block embedding. Decalcification was completed in forty-five days and the sections were then subjected to another 24 hour running water rinse. The trimmed blocks were dehydrated in ethanol, embedded in paraffin, and sectioned at 6 microns. Longitudinal serial sections through the endodontically treated teeth and apical area were mounted on glass slides. The sections were deparaffinized, hydrated, and stained alternatively with hematoxylin and eosin and Masson's trichrome connective tissue stain (43).

The stained histologic sections were examined using a light microscope. The sections were evaluated according to the amount and type of inflammation present in the periapical tissues. Also noted was cementum, dentin, and bone resorption or apposition, in addition to the organization of connective tissue.

The radiographs were evaluated for evidence of periapical rarefaction and sealer resorption. The findings are recorded in Charts III-VI.
CHAPTER IV

RESULTS

A. PART ONE

On the days of their sacrifice, the rats were examined and appeared to be in good health. Although suture removal was not included as part of the procedure, all sutures were missing from the incision sites in all of the animals.

There was external evidence of good healing having begun at four days. Microscopically, the pattern was similar for all four materials implanted. Tissue edema, capillary engorgement, necrosis of muscle, and cellular infiltrate were seen in varying degrees around all four materials. The inflammatory cell infiltrate mainly consisted of polymorphonuclear leukocytes, histiocytes, lymphocytes and plasma cells.

At the fourteen day sacrifice, healing appeared complete with a good amount of the hair regrown over the surgical sites. Microscopically, there was evidence of new collagen and granulation tissue formation surrounding the implants. There was some acute cellular inflammatory response, but lymphocytes and plasma cells were the most predominant cell types. Numerous new capillaries were observed throughout the repairing tissue. The pellet of Rickert's sealer was seen to have a small epithelium lined communication to the skin surface with
epithelium surrounding the implant for a short distance. The Wach's pellet had a small microabscess containing many polymorphonuclear leukocytes. There was some necrotic debris around the Diaket pellet. The connective tissue developing around the Durallium pellet appeared more mature than the tissue surrounding the sealer pellets.

The skin looked completely healed and the hair was virtually fully regrown at thirty days. After sacrifice, it was discovered that the pellet of Kerr's sealer was missing from the implant site; however, small fragments of sealer remained in the tissue and could be seen by the unaided eye. The microscopic picture had very few acute inflammatory cells but still had a good number of lymphocytes and plasma cells associated with the four materials. Particles of Kerr's sealer were being phagocytized and more red blood cells were associated with the Kerr's than the remaining pellets. The connective tissue capsule around the Diaket was less mature than that seen around the Kerr's or the Wach's and the capsule surrounding the Durallium was more fibrous than any of the others.

The relative response is illustrated in Table II.
Each monkey was examined at the termination of the experiment. They both appeared healthy and had been eating as they had been prior to having the dental procedures performed. The amalgam seals were intact in all of the operated teeth. The soft tissues were examined for evidence of pathology and Monkey A had a draining sinus tract and localized swelling located on the lingual periapical area of the mandibular right lateral incisor. It had been noted that this tooth had a non-vital pulp with a small apical radiolucency with considerable apical resorption seen in the pre-operative radiographs. Radiographic observations made at sacrifice were compared to pre-operative and immediate post-operative films.

1. Radiographic Changes

After six months, there generally was radiographic evidence of resorption of extruded sealer. On teeth that were overfilled with gutta-percha and sealer, the gutta-percha appeared to remain intact, while the sealer that previously surrounded it was no longer seen in the radiograph. The one possible exception was on a mandibular molar that still had some Kerr's sealer evident.
The three month observations were similar to those from the six month period in that there was radiographic evidence of sealer resorption with gutta-percha overfills remaining intact. Again, one overfill of Kerr's sealer was seen to remain, this time associated with a maxillary central incisor.

The one month observations showed a range of minimal to substantial resorption of the extruded sealer. The notable exception in this time category was the non-vital mandibular right lateral incisor in Monkey A that had been grossly overfilled. There was evidence of most sealer still remaining and a definite increase of the periapical radiolucency to several times its pre-operative size.

The sealer overfills at one week were virtually unchanged from that seen in the radiographs taken immediately after the root canal fillings were placed.

Examples of the immediate post-sacrifice radiographs are seen in Figures 5 and 6.

2. Histology

A reasonably consistent histopathologic pattern was seen for each time period observed in each monkey. Histologically, sealer was seen in almost every specimen. The results will be considered for each sacrifice time period and for each monkey individually.
a. **Six Month Specimens**

Monkey A showed minimal inflammation around both the Kerr's and the Wach's sealer after six months; only a few lymphocytes were found. The sealer or sealer and gutta-percha combination was well encapsulated by fibrous connective tissue with remodelled bone in close proximity. There was evidence of cementum resorption being partially repaired with new cementum.

Monkey B was found to have a more intense inflammatory response but it was relatively the same for both sealers, except for one Wach's specimen being slightly milder. There was evidence of a fibrous connective tissue capsule around the overfills, but it was less mature than that seen in the other monkey. Around this loose capsule was granulation tissue and many lymphocytes and plasma cells. Cementum and bone resorption was present but there was evidence of bony repair, too. There was evidence of phagocytosis with particles of Kerr's and Wach's seen in macrophages in their respective specimens.

b. **Three Month Specimens**

The three month samples from Monkey A were very similar to those seen at six months. There was one exception, that being a Kerr's specimen. It had many chronic inflammatory type cells associated with its large, loose connective tissue
capsule and showed more cementum and bone resorption than had been seen in this animal thus far.

Monkey B had an inflammatory response consistent with what had been seen in its six month microscopic sections, again the inflammation was greater in this monkey than in the other. Granulation tissue and bone remodelling were associated with the overfills of both sealers.

c. One Month Specimens

A more intense cellular inflammatory response was now seen in Monkey A. There was evidence of bone and cementum resorption with many lymphocytes and plasma cells in the granulation tissue associated with the overfills. One Kerr's specimen from a tooth with a preoperative non-vital pulp and periapical radiolucency, showed a tremendous number of polymorphonuclear leukocytes, lymphocytes, and plasma cells in a granuloma. This was the same tooth that was previously mentioned that developed a sinus tract following endodontic treatment.

Monkey B specimens had essentially the same degree of inflammatory response after one month as was seen with the vital pulp teeth in Monkey A. A notable response here was associated with one incisor that according to sectioning had proved not to be overinstrumented nor overfilled, yet still showed an inflammatory response almost as intense as the other one month samples from Monkey B.
d. One Week Specimens

Monkey A had a notably more intense histopathologic response to Kerr's sealer than Wach's at the one week sacrifice. There were many more polymorphonuclear leukocytes and lymphocytes associated with the Kerr's specimen. One molar root was perforated during instrumentation and Wach's sealer was expressed and seen as small globules in the periodontal ligament space. There was no evidence of inflammation or other adverse reaction to these small particles of Wach's Paste.

Monkey B showed a more consistent response for the two sealers at the one week sacrifice. Considerable inflammation around the sealer overfill and dentinal debris was seen for both Kerr's and Wach's. There was evidence of bone and a small degree of cementum resorption. One of the Kerr's specimens had an acute periapical abscess with many polymorphonuclear leukocytes.

The relative histopathologic responses are illustrated in Tables V and VI.
CHAPTER V

DISCUSSION

A. PART ONE

Several investigators (9)(25)(31)(35)(41)(42)(46)(50)(56)(58)(59)(77)(83) have suggested and, or used rodent subcutaneous connective tissue for the initial screening or even total evaluation of various materials used in root canal procedures. In agreement with these authors, it was felt that this study should use the dorsal subcutaneous connective tissue of white rats, but only for the initial screening of Kerr's and Wach's sealers. Diaket was included because in previous studies it was found to cause a great deal of irritation (11)(59) and to be toxic to tissue culture cells (59). An implant of chrome-cobalt metal was also included because it was felt that it would be well tolerated (4)(23)(25). The thought was that Diaket and Durallium might show opposite responses, with Kerr's and Wach's somewhere between them.

Although the results of this screening portion of the study showed Diaket was not as well tolerated as the other implants, there was little inflammatory response difference at the fourteen and thirty day comparisons. At four days, Diaket did have necrosis and much more inflammation around it than the other materials.
On the other hand, Durallium gave an unexpectedly more severe response than Wach's or Kerr's at the four day sacrifice. In the following time periods, Durallium was better tolerated than the other implants.

The histologic picture of the response to Kerr's and Wach's was indeed similar for all three observation periods.

An interesting speculation as to the disappearance of the sutures from the rats might be that the animals recognized them as something that didn't belong on their companions, and removed the sutures from each other.

There is little doubt that different results might have been obtained if more animals had been used. With so small of sample size, the application of statistical analysis would have been of dubious significance.

In any event, the similarity of histopathologic response of Kerr's and Wach's indicated the need for additional and expanded investigation.

B. PART TWO

Primate research to study histopathologic response to root canal filling materials has been suggested and, or performed by many investigators (10)(41)(58)(62)(63)(69). Other authors (66)(70) have reported on the histopathologic response of primate periapical areas to root canal instrumentation. The
fact that humans and monkeys are both primates is, in itself, an indication for the use of monkeys for biological research that will be pertinent to humans.

The similarity to humans of monkeys' teeth and periapical anatomy, although somewhat smaller, makes them highly desirable as experimental animals for histopathologic studies of endodontics. However, the reduced size of the teeth also meant smaller root canals, which in the cases of some molar and bicuspid teeth made instrumentation very difficult, particularly violation of the apical foramen. In addition, monkey teeth seemed to be more curved than experience with comparable human teeth had shown. These factors quickly made it obvious that it was impossible to use all teeth and canals for the study. On the other hand, monkey cuspids are proportionally much larger and would have been difficult to use in the experiment. Another problem was the proximity of some root apices to each other that might have made interpretation of response difficult. Therefore, a decision was made to limit experimental specimens to incisors and larger bicuspid and molar root canals.

Although dental caries were not seen in either monkey, trauma was seen involving one mandibular incisor. As a result of the trauma, the incisal edge was fractured and although there was no pulpal exposure, pulpal necrosis and resultant
periapical rarefaction and apical resorption occurred.

The results of this part of the study showed similarity of response to overfills of Kerr's and Wach's sealers. Generally, more inflammation was seen in Monkey B than Monkey A, but there was consistency within each animal at each time period. The difference between the two monkeys is interesting in that the older monkey did not react as much to the sealer extrusions as the younger monkey did.

As has been noted, there was no discernable difference in the health of either monkey, compared to each other, or compared pre-operatively and post-operatively. The younger monkey was more active in his cage at times. From a pharmacologic standpoint, more dosage of the drugs used was required to obtain adequate effect in the older monkey. Perhaps there was a relationship of the lesser inflammatory reaction to the lesser response to drugs.

There seemed to be a definite increase in the degree of inflammation with an increased amount of sealer extruded. A comparison of the two sealers, having taken the amount of the overfill into account, seemed to show that the size of the overfill was a more important factor, than which sealer was used.
Davis, et al (15) found their best result with regard to how far to instrument and how far to fill the canal was instrumentation beyond the apex but filling three mm. short of the apex. A difference between their study and this one was that here it was found to be impossible to not express sealer through the apex once it had been violated with other than the smallest instruments.

Sinai, et al (70) have reported inflammation in the periapical area due to instrumentation alone, whether or not the instrument passed through the apical foramen. The partially instrumented and incompletely filled canals of some molars in this experiment did not confirm their findings. However, one maxillary incisor that was neither overinstrumented nor overfilled did have mild inflammation periapically and slightly more inflammation in the remaining pulp tissue.

The disappearance of sealer as seen in the periodic radiographs and the presence of sealer particles in macrophages in the histologic sections indicated that the tissue was actively dealing with the overfill. Resorption of both sealers was noted even though inflammation diminished and almost completely disappeared. The fact remained that the overfill was a foreign body and the body continued to act to remove it. The resorbability of Wach's Paste has been shown in clinical observations (49) with which this study agrees.
There was slightly noticable discoloration of the crowns of teeth that had been treated with either sealer. The matter of dentin staining by sealers has been mentioned by other authors (31)(48)(75). The belief is that Kerr's has the ability to stain dentin, probably due to the silver that it contains. In this study, extreme care was taken to remove excess filling materials from the coronal aspect with a bur, and then a deep amalgam filling was placed. Due to the thorough cleansing of the access and the large size of the silver amalgam filling, any discoloration was probably due to the amalgam and not the root canal sealer. It does seem reasonable that excess sealer left in the coronal portion of an endodontically treated tooth could cause discoloration, especially if the sealer contained silver.

Although twenty-six experimental root canals were done on the two monkeys, no statistical evaluation was carried out on the results. The results would seem to indicate that further studies are indicated and that statistical analysis of a larger sample might prove interesting, if not essential.
CHAPTER VI

CONCLUSIONS

1. The rat is a convenient animal for screening endodontic materials for biologic compatibility.
2. The monkey is a valuable research animal for histopathologic study of endodontic filling materials and procedures.
3. The biologic compatibility of Wach's Paste and Kerr's Sealer is very similar.
4. Both Wach's Paste and Kerr's Sealer are resorbable.
5. The amount of sealer extruded through the apical foramen is more significant than whether it is Kerr's or Wach's.
6. It seems biologically better to confine root canal instrumentation and filling materials within tooth structure.
7. Further research with Wach's formula root canal sealer is indicated.
Kerr's, Wach's, and Diaket sealer and chrome-cobalt pellets were implanted in the dorsal subcutaneous tissue of three white rats. The purpose of this portion of the experiment was initial screening for biologic compatibility. The animals were sacrificed at four days, fourteen days, and thirty days. After fixation of the tissues and preparation of histologic sections, the results were interpreted. Of the four samples in the first sacrifice period, Diaket showed the most severe reaction. The same was found at the fourteen day sacrifice. At the thirty day sacrifice, the four materials all showed similar biocompatibility. There was little degree of difference between the Kerr's and Wach's at any of the sacrifice times.

Two rhesus monkeys were used for the second part of the research. Conventional endodontic therapy involving hand instrumentation and obturation with gutta-percha and sealer combination was performed on twenty six teeth. Canals were instrumented slightly beyond the apical foramen and sealer extruded into the periapical area. Quadrants of teeth were operated so that observation periods of six months, three
months, one month, and one week would be obtained from each animal. After sacrifice the jaws were removed and fixed in formalin solution. The teeth and jaws were decalcified and histologic sections prepared. The results showed a similar degree of biologic acceptance for both Wach's and Kerr's sealers.
CHAPTER VIII

REFERENCES


CHAPTER VIII

APPENDIX

A. TABLES - B. FIGURES

Symbols used in TABLES:
UR = maxillary right
UL = maxillary left
LR = mandibular right
LL = mandibular left
M = mesial
D = distal
P = palatal
MB = mesiobuccal
DB = distobuccal
ML = mesiolingual
DL = distolingual
+ = inflammatory cells present
0 = no inflammatory cells
PMN = polymorphonuclear leukocytes
PDL = periodontal ligament
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<th>Sealer</th>
<th>Powder</th>
<th>Liquid</th>
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<td>Precipitated silver 30 gm.</td>
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<td>Zinc oxide 41.21 gm.</td>
<td>Canada Balsam 22 cc.</td>
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<td>Thymol iodide 12.79 gm.</td>
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</tr>
<tr>
<td></td>
<td>White rosin 16 gm.</td>
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<td>Calcium phosphate 2 gm.</td>
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<td>Bismuth subnitrate 3.5 gm.</td>
<td>Eucalyptol 0.5 cc.</td>
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<td>Bismuth subiodide 0.3 gm.</td>
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<td>Heavy magnesium oxide 0.5 gm.</td>
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<td><strong>Diaket</strong></td>
<td>Zinc oxide</td>
<td>2, 2 dihydroxy 5, 5 dichlorodiphenylmethane co-polymer of vinyl acetate</td>
</tr>
<tr>
<td></td>
<td>Bismuth phosphate 2%</td>
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<td>Vinyl isobutyl ether</td>
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### TABLE II

#### HISTOLOGIC RESULTS - PART ONE

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<td>Extensive necrosis of muscle.</td>
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<tr>
<td>Diaket</td>
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<td>++++</td>
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<td>Durallium</td>
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<td>+</td>
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<td><strong>Fourteen Day Observations</strong></td>
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<td></td>
</tr>
<tr>
<td>Kerr's sealer</td>
<td>+</td>
<td>+++</td>
<td>Epithelium lined communication through skin.</td>
</tr>
<tr>
<td>Wach's sealer</td>
<td>++</td>
<td>+++</td>
<td>Numerous capillaries.</td>
</tr>
<tr>
<td>Diaket</td>
<td>+++</td>
<td>++++</td>
<td>Necrotic debris.</td>
</tr>
<tr>
<td>Durallium</td>
<td>+</td>
<td>++</td>
<td>Less vascular.</td>
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<td><strong>Thirty Day Observations</strong></td>
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<td></td>
</tr>
<tr>
<td>Kerr's sealer</td>
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<td>Most of pellet missing, many capillaries and extra-vascular blood cells and pigment, phagocytosis.</td>
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<tr>
<td>Wach's sealer</td>
<td>+</td>
<td>++</td>
<td>Less mature capsule.</td>
</tr>
<tr>
<td>Diaket</td>
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<td>Fibrous connective tissue capsule.</td>
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<td>Durallium</td>
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# TABLE III

**TREATMENT - MONKEY A**

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TABLE IV

TREATMENT - MONKEY B

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### TABLE V

**HISTOLOGIC RESULTS - PART TWO**

**MONKEY A**

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<td>+</td>
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</tr>
<tr>
<td></td>
<td>UR2</td>
<td>Kerr's</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UR6</td>
<td>Wach's</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>three months</td>
<td>UL1</td>
<td>Kerr's</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UL2</td>
<td>Wach's</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UL6</td>
<td>Kerr's</td>
<td>+++</td>
<td>large overfill</td>
</tr>
<tr>
<td>one month</td>
<td>LR1</td>
<td>Wach's</td>
<td>++</td>
<td>poor histologic section, difficult to interpret</td>
</tr>
<tr>
<td></td>
<td>LR2</td>
<td>Kerr's</td>
<td>++++</td>
<td>large overfill, preoperative rarefaction, many PMN</td>
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<tr>
<td></td>
<td>LR7</td>
<td>Kerr's</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>one week</td>
<td>LL1</td>
<td>Kerr's</td>
<td>++++</td>
<td>very large overfill</td>
</tr>
<tr>
<td></td>
<td>LL2</td>
<td>Wach's</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LL7</td>
<td>Wach's</td>
<td>0</td>
<td>perforation, sealer in PDL only</td>
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### TABLE VI

**HISTOLOGIC RESULTS - PART TWO**

**MONKEY B**

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<tr>
<th>Time</th>
<th>Tooth</th>
<th>Sealer</th>
<th>Inflammation</th>
<th>Additional Observations</th>
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<td>six months</td>
<td>LL1</td>
<td>Wach's</td>
<td>+++</td>
<td>large overfill</td>
</tr>
<tr>
<td></td>
<td>LL2</td>
<td>Kerr's</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LL5</td>
<td>Wach's</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LL7</td>
<td>Kerr's</td>
<td>+++</td>
<td>small area of granuloma, otherwise</td>
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<td>fibrous encapsulation</td>
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<td>LR1</td>
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</tr>
<tr>
<td></td>
<td>LR2</td>
<td>Wach's</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LR5</td>
<td>Kerr's</td>
<td>+++</td>
<td></td>
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<tr>
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<td>UL1</td>
<td>Wach's</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UL2</td>
<td>Wach's</td>
<td>++</td>
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<tr>
<td></td>
<td>UL5</td>
<td>Kerr's</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UL6</td>
<td>Wach's</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>one week</td>
<td>UR1</td>
<td>Kerr's</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UR2</td>
<td>Kerr's</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UR6</td>
<td>Wach's</td>
<td>+++</td>
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Figure 1: Rat subcutaneous connective tissue in contact with Kerr's sealer pellet (K). (Pellet removed to facilitate sectioning.) Minimal number of inflammatory cells present. (Fourteen-day specimen, hematoxylin and eosin stain. Original magnification, X40.)

Figure 2: Rat subcutaneous connective tissue in contact with Wach's sealer pellet (W). (Pellet removed to facilitate sectioning.) Minimal number of inflammatory cells present. (Fourteen-day specimen, hematoxylin and eosin stain. Original magnification, X40.)
Figure 3: Rat subcutaneous connective tissue surrounding pellet of Wach's Paste (W). (Pellet removed to facilitate sectioning.) Note fibrous connective tissue capsule (arrows). (Thirty-day specimen, Masson's trichrome stain. Original magnification, X25.)

Figure 4: Rat subcutaneous connective tissue adjacent to Wach's Paste pellet (W). (Pellet removed to facilitate sectioning.) Deeper serial section from the specimen in Figure 3, vicinity of black arrow. Minimal inflammatory cells present. (Thirty-day specimen, hematoxylin and eosin stain. Original magnification, X250.)
Figure 5: Immediate post-sacrifice radiograph of Monkey A anterior maxilla. Sacrifice time periods labelled. Note overfills. (Magnification, X3.)

Figure 6: Immediate post-sacrifice radiograph of Monkey B anterior maxilla. Sacrifice time periods labelled. Note overfills. (Magnification, X3.)
Figure 7: Extruded Kerr's sealer (K) in monkey periapical area. Apical cementum (Ce) normal. Minimal inflammation present. (Three-month specimen, hematoxylin and eosin stain. Original magnification, X100.)

Figure 8: Extruded Wach's sealer (W) in monkey periapical area. Apical cementum (Ce) and bone (B) normal. Minimal inflammation present. (Three-month specimen, hematoxylin and eosin stain. Original magnification, X100.)
Figure 9: Overfill of palatal root of monkey maxillary molar. Gutta-percha and Wach’s sealer (W) extruded. Note fibrous connective tissue capsule (large white arrow), cementum resorption repairing (small white arrows), normal bone (B) and periodontal ligament (pdl). (Six-month specimen, Masson's trichrome stain. Original magnification, X40.)

Figure 10: Fibrous connective tissue capsule (C) adjacent to Wach's sealer (W). Deeper serial section from the specimen in Figure 9, vicinity of large white arrow. Normal bone (B), no inflammatory cells present. (Six-month specimen, hematoxylin and eosin stain. Original magnification, X250.)
This thesis, submitted by Henry B. Feinberg, has been read and approved by three members of the faculty of the Department of Oral Biology.

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

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

F. S. Weine, D.D.S., M.S.D.

Signature of Advisor