The Intracanal Diffusion of 2% Aqueous Parachlorophenol in Endodontics: An Autoradiographic Study

Warren Y. Avny

Loyola University Chicago

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THE INTRACANAL DIFFUSION OF 2% AQUEOUS PARACHLOROPHENOL IN ENDOДONTICS:
AN AUTORADIOGRAPHIC STUDY

BY
WARREN Y. AVNY, D.D.S.

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

JUNE
1970
DEDICATION

I dedicate this thesis to my devoted wife Marilyn, whose love, devotion, patience and encouragement have never waivered.
ACKNOWLEDGMENTS

My sincere gratitude and appreciation to the following:

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To Mrs. Mickevicius and Mrs. Tumosa for their help in the laboratory.

To Loyola University for making all of this realizeable.
AUTOBIOGRAPHY

Warren Y. Avny was born in Haifa, Israel, on November 29, 1941. He and his family emigrated to the United States in 1948 and settled in Chicago, Illinois.

He was graduated from Brandeis Day School and Lake View High School. He attended the University of Illinois and Roosevelt University before entering Loyola University, School of Dentistry in 1962. He graduated from there with the degree of Doctor of Dental Surgery in June, 1966.

After graduation from Dental School he entered the United States Air Force Dental Corps as a commissioned officer. After serving two years in North Dakota with the Air Force he returned to Loyola University, School of Dentistry, Chicago, Illinois, Department of Endodontia as a resident and to the Department of Oral Biology, Graduate Department, Loyola University, to work toward a degree of Master of Science.

The author is married and has two children, a son and daughter.
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CHAPTER 1

INTRODUCTION

The penetration and diffusibility of endodontic medications has not been adequately demonstrated, explained, or investigated. It is of prime importance to the endodontist to know exactly how far his medicament is traveling in the tooth or teeth he is treating. Does the medication remain where he places it or does it travel into the root canal and if it does travel does it diffuse through the dentin and how far? Is the bone affected by the drugs he uses or is the medicament confined to the tooth tissue?

Many of the medicaments that have been tested were investigated in vitro outside the root canal. We do not know whether the results are the same in the root canal because medications behave differently in vivo and in vitro (Nicholson, 1968).

The majority of research done in the field of endodontic medications has been done on its antiseptic capabilities and not its penetrability. All of the drugs commonly used in endodontics have been tested as to their antimicrobial spectrum, toxicity, and usage, but not as to their diffusibility.

It is the purpose of this research to determine the diffusibility and permeability of an endodontic medicament and to develop a technique for testing the same property of other intracanal medications. The antiseptic chosen is a modification of the oldest and most popular root
canal preparation, camphorated paramonochlorophenol (CPC). Two percent aqueous parachlorophenol (PCP) is being tested because it is far less toxic than the more commonly used 35% CPC and may one day replace the 35% preparation in popularity (Harrison, 1969).
Dentin permeability studies date back to 1891 and since that time have been actively investigated. This literature review will discuss the most commonly used methods of research.

A. DYE PENETRATION STUDIES

In 1891, Harlan was the first to recommend the use of diffusible medicaments in pulp canal therapy. He stated that phenol, zinc chloride, sulfuric acid, and creosote are self-limiting because they coagulated protein and therefore had questionable penetrability into and through the tooth.

Shortly after Harlan, York (1897) was the first to show that carbolic acid did diffuse through the dentin. He took extracted teeth and after removing the pulp sealed carbolic acid plus fuchsin in the teeth. He then sectioned the teeth and observed that the fuchsin coloring had diffused entirely through the dentin. His second experiment was to take these teeth with carbolic acid in them and submerge them in water and to test the water for the presence of carbolic acid. The water was always found to contain carbolic acid which proved to York that carbolic acid did in fact diffuse entirely through the tooth.

Thirty years passed before Fish (1927) began his study of dentin permeability with dyes. He used India ink to demonstrate that dentinal
tubules are lymph channels in which a stream of lymph flows. He sawed off the crowns of dogs teeth and injected Reeves' waterproof India ink half-way into the root, careful not to exert pressure. Cement was then flowed and a seal was obtained. After intervals of from one to twenty-four hours the dogs were sacrificed and the teeth extracted, decalcified, sectioned and mounted. His results showed that the India ink suspension was carried up the tubules as far as the middle third of the dentin within one hour after their introduction into the pulp.

Coolidge (1929) like Harlan (1891), reported that drugs that coagulate protein only have a shallow penetration into the root dentin, and that low surface tension is of value only if the compound used does not precipitate protein. Coolidge further stated that both diffusion and osmosis may be increased by decreasing the surface tension of the medicament, for example, cresatin.

Buest (1931) used fuchsin staining followed by ground sections and examined the teeth under transmitted light. He showed that the penetration of a dye was greater in the dentin of young teeth than old teeth. Young unaffected dentin stained while sclerosed dentin did not.

In 1933, Fish again reported on dentin permeability with dyes. He used methylene blue dye as well as other diffusible dyes in the pulp chamber and examined ground sections. His observations revealed penetration in some areas of the tooth to the dentinoenamel and dentino-cemental junction.
Bodecker and Lefkowitz (1937) found that maturity, aging, abrasion, and caries greatly reduced dentin permeability. They also concluded that pulp removal temporarily increases permeability of dentin.

Van Huysen, Hodge, and Warren (1937) did roentgen ray absorption studies to determine the relative absorption of different parts of the tooth. Their experiment involved half-tone reproductions of X-ray images and contact prints of teeth. They concluded that root dentin is generally less radio-opaque than crown dentin.

Bodecker (1943) found that dyes do penetrate dentin and follow the paths of dental lymph which were demonstrated by Fish (1927). He believed that the newest studies using radioactive phosphorus had not given uniform results because the permeability of dental tissues is so variable from tooth to tooth due to many intrinsic and extrinsic factors. Bodecker used gentian violet and ground sections to prove his contentions.

In that same year, 1943, Lefkowitz also studied dental lymph flow using a silver dye. He made preparations into pulp chambers and deposited argyrol and examined ground sections. He also concluded that the dye followed a definite pattern through the dentin which is dependent upon the dentinal lymph flow.

One of the last dye studies conducted was by Yander and Smith (1945). They tried to determine whether silver nitrate precipitant was carried into the pulp by dental lymph or whether silver nitrate followed
physical laws governing the progress of fluid through capillary channels. Cavities were prepared in dogs teeth and silver nitrate solution was applied followed by cement. After the dogs were sacrificed the teeth were extracted, decalcified, and mounted. Their findings disagreed with those of Lefkowitz, Bodecker, and Fish, and therefore they concluded from their study that silver nitrate penetration is not necessarily associated with the dentinal tissue flow.

B. RADIOACTIVE ISOTOPE PENETRATION STUDIES

Hevesy, Holst, and Krogh (1937) published the first paper on experimental work with radioactive isotopes in teeth. They believed that the circumpulpal region of the tooth shows the highest degree of $\text{P}^{32}$ absorption from blood. Their experiment closely resembled the experiment of Manly and Bale (1939) who measured the amount of radioactive phosphorus, $\text{P}^{32}$, absorbed by the glycol extracted dentin of a rat incisor. They fed rats 120 mgm. of radioactive phosphorus daily and also a single daily dose via a stomach tube. At various times after feedings the animals were sacrificed. The rat incisors were divided into three parts, 4 tips, 4 middle, and 4 roots, and all were pooled. These samples were dissolved in dilute hydrochloric acid to a volume of 2 cc. and their radioactivity determined with a Geiger-Müller counter. Their results showed that teeth without pulps take up $\text{P}^{32}$ faster than intact teeth and that the tips of roots show high activity measurements.
Sognnaes and Volker (1941) used $P^{32}$ that was injected via a stomach tube and after sacrificing the animal the jaws and teeth were cleaned, dried, and separated. Only the surface enamel was ground off under a microscope and examined with a Geiger-Müller scale-of-four counter. They also separated dentin into apical and coronal sections and showed that the dentin permeability of $P^{32}$ varies with different morphological and pathological varieties of dentin. They further concluded that "The radiophosphorus metabolism in enamel of fully erupted teeth is of a considerably smaller magnitude than that found in the dentin." Volker and his researchers (1941) also explored the possibility of adsorption of radiofluorine, $F^{18}$, on hard surfaces. They used powdered enamel and dentin and measured radiation on a Geiger-Müller scale-of-four counter. The milligrams of fluoride obtained from calcium phosphate was calculated using the Freundlich adsorption isotherm equation. Volker, et al., demonstrated that enamel, bone, dentin and hydroxyapatite adsorb fluorine.

In the experiment conducted by Wasserman, Blayney, and Groezenger (1941) they used sodium phosphate containing radioactive phosphate (1.5 - 2.0 curies) in a mouth rinse. The patients rinsed with the solution and then the teeth were extracted. Using burs they separated enamel and dentin from the teeth and measured the radioactivity level. They concluded that in human teeth $P^{32}$ is absorbed less rapidly in pulpless teeth than in intact teeth. This conclusion is contrary to that expressed by Bodecker (1937) and Manly and Bale (1939).
McCauley (1942) observed, as did Hevesy and Holst (1937), that circumpulpal dentin had a greater absorption of radioactive isotopes than other parts. He also listed some of the artificial radioactive elements that are useful in dental research, the most promising of which were sodium radiofluoride$^{18}$, sodium$^{24}$, calcium$^{45}$, and strontium$^{89}$. This list was later updated by Bartelstone in 1950.

Gilda and McCauley (1943) rendered the teeth of opposing quadrants in dogs pulpless by extirpation and then filled the canals with zinc oxide and eugenol paste. The dogs then received intravenous injections of an aqueous solution of sodium phosphate containing $P^{32}$ and they were sacrificed thirty-six hours later. The teeth were sectioned and placed on X-ray films for forty-six hours. The developed films revealed that devitalized or pulpless teeth had a lower uptake of $P^{32}$ than vital teeth.

Amler and Bevelander (1945) showed that all topographical areas and all morphological varieties of dentin are permeable to $P^{32}$. However, the circumpulpal zone absorbed a much greater amount of $P^{32}$ than any other part of the tooth. This confirmed the studies of Hevesy and Holst (1937) and McCauley (1942). Therefore, it was concluded that the penetration of $P^{32}$ was inversely related to the density of dentin.

Bartelstone (1947) used radioactive iodine and concluded that "The results obtained demonstrate the efficiency of the use of radioautography with radioactive iodine as a method for studying the physiology of the fluid in teeth in communication with systemic circulation."
Amler (1948) again studied the penetration of $\text{P}^{32}$ into the dentin both in the presence of oxyphosphate cement and in its absence. He prepared cavities in dog teeth; applied phenol, phenol followed by alcohol, fluorine, silver nitrate followed by eugenol, cavity varnish, and zinc oxyphosphate to the cavities along with equal amounts of $\text{P}^{32}$ ($\text{Na}_2(\text{HPO}_4)_4$) and sealed them with silver amalgam. The animals were sacrificed and the teeth sectioned (100-150 microns) and autoradiographed. Amler reported that phenol, alcohol, fluorine, silver nitrate, eugenol, and cavity varnish increased dentin permeability while oxyphosphate cement was impervious to $\text{P}^{32}$.

Bevelander and Amler in 1951 continued their investigation of dentin permeability using radioactive phosphate ($\text{P}^{32}$) in dog teeth. This study showed that alcohol, silver nitrate, and phenol cavity varnish increased the permeability of dentin. This duplicated their results of 1948. This study, however, also revealed a proportionate increase in the permeability of dentin in the unmedicated control teeth.

Martin (1951) used the direct tissue radioautography technique to study the permeability of root dentin in human teeth. He reported an increased permeability of the dentin following treatment with all medications used except zinc phosphate cement and a precipitated layer of calcium fluoride. His results were similar to Amler's (1948) in that these two compounds rendered the dentin impermeable to $\text{P}^{32}$. 
Wainwright (1952) studied the penetration of dental root structure using $^{131}$I. $^{131}$I was applied on teeth that were still present in the mouth but were to be extracted. He applied the radioactive iodine directly over whole teeth and dried them, sectioned them under oil, and radioautographed them. He reported that penetration was greatest at the cervical dentin and that exposed dentin was more permeable than unexposed dentin.

In 1953, Wainwright duplicated his results of 1952. This time using freshly extracted central incisors and suspending them in a solution of radioactive iodine. These were then radioautographed. This study revealed that exposed cementum was more permeable than unexposed cementum.

Marshall, Massler and Dute (1960) reported that little had been written or done concerning the permeability of the dentin in the root canal or the permeability of certain medicaments during endodontic procedures. They used $^{32}$P, $^{22}$Na, $^{131}$I and $^{35}$S as the radioactive tracers in pulpless extracted teeth and autoradiographed the ground sections (50-100 microns). Silver nitrate, formalin, E.D.T.A. and eugenol were found to reduce dentin permeability whereas hypochlorite and peroxide solutions increased dentin permeability. They also concluded that $^{35}$S was the most effective tracer.
C. MEDICAMENT PENETRATION STUDIES

1. Dye Non-Radioactive Medicament Studies

In 1950, Shutte1worth studied the diffusion of pencillin from the dental root canal into the dentin. He took recently extracted teeth and divided his experiment into four groups: Group I used whole teeth; Group II were teeth that had their crowns amputated; Group III had middle root amputations; and Group IV had the apices removed. All of the teeth had their pulps removed by extirpation and paper points containing pencillin were sealed into them. The teeth were sealed at both ends and placed on blood-agar culture plates containing Staphylococcus aureus. All of the groups showed that the pencillin diffused not only through the dentin but proceeded beyond the cementum into the surrounding media, the blood-agar.

Coolidge and Kesel (1956) studied the usefulness of parachlorophenol, which had been recommended as a root canal antiseptic by Dr. Otto Wolkoff in 1891. They concluded that it should penetrate deeper than phenol because it does not coagulate albumin or cauterize tissue as does phenol.

In their textbook, Clinical Endodontics, Sommer, Ostrander and Crowley (1961) write that "Stamps (1953) demonstrated that camphorated parachlorophenol penetrated dentin well." Stamps, however, used PCP in his experiment, not CPC, and stated that phenol, "presented moderate ability" to penetrate 2 mm. into the dentin.
Patterson (1963) conducted an in vivo and in vitro study of the effect of the disodium salt of ethylenediamine tetra-acetate on human dentin. His experiment basically involved exposing root surfaces to a 10% solution of E.D.T.A. and then photographing them. He used hardness tests with the Knoop indenter, as well as scratch tests, and concluded that E.D.T.A. is not self-limiting. He could not prove whether the "liquid" is absorbed by the dentin or leaks through the foramen of accessory canals.

Von der Fehr and Ostby (1963) studied the affect of E.D.T.A. and sulfuric acid on root canal dentin. After extracting premolar teeth of children they removed part of the crowns and all of the pulp tissue. They enlarged the root canals to two to four times the normal size with a Davies bur. The canals were then filled with E.D.T.A. and sulfuric acid and sealed with wax. Transverse serial sections (150-200 microns) were examined with microradiograms as well as polarized light. Von der Fehr and Ostby could not substantiate Patterson's (1963) findings that E.D.T.A. was not self-limiting. Sulfuric acid, however, did penetrate deeply into the root as demonstrated under the light microscope. No calculations were made as to what depth the penetration reached.

Stewart, Kapsimalas, and Rappaport, (1969) investigated the effect of E.D.T.A. and urea peroxide on root canal dentin. They observed that when E.D.T.A.-urea peroxide combination was used for the preparation of
the root canal the 2% aniline dye penetrated the dentin completely. If
the urea peroxide was omitted the penetration was not complete. Their
study agreed with the earlier work done by Marshall, Massler and Dute
(1960) using hydrogen peroxide.

2. Radiolabeled Medicament Studies

Wainwright and Lemoine (1950) did radioautographic studies of the
penetration of $^{14}$C labeled urea in human teeth. They applied the
radioactive urea directly on to the crowns of extracted teeth. The
teeth were then dried and sectioned. The autoradiographs revealed that
more penetration occurred into the dentin near the gingival line and
near occlusal fissures than anywhere else on the tooth surface.

In 1955, Wach, Hauptfuehrer and Kesel used extracted human teeth
and divided their experiment into four groups: Group I had $^{35}$S labeled
penicillin added to a carious lesion with a cotton pellet; Group II were
pulpless teeth with radiolabeled paper points; Group III had their
cavity openings sealed with a filling material; and Group IV had the
isotope incorporated into the pulp canal filling material. These teeth
were subjected to radioautographic and chromatographic evaluations.
They concluded that $^{35}$S labeled penicillin had a varied permeability in
dentin and that there was no penetration of $^{35}$S into intact enamel or
cementum.

Hampson and Atkinson (1964) studied the relationship between drugs
used in root canal therapy and the permeability of dentin. The major
drugs tested were centrimides, chlorhexidine solutions and hypochlorite solutions. They incorporated radioactive sulfur and iodine into the solutions tested. The drugs were injected into the root canal with an Agla micrometer syringe and the apices sealed with red sealing wax. They were then longitudinally sectioned and radioautographed. The apical dentin appeared to be impermeable to the tracers used, whereas, the mid-root dentin was not. It was noted that chloramine increased the permeability of the dentin.

Nicholson, Stark, Nguyen and Scott (1968) experimented with autoradiographic tracings of Ca$^{45}$ labeled E.D.T.A. They used pulpless monkey teeth and inserted the labeled E.D.T.A. in the canals. After ground sections were made they were subjected to autoradiography. Nicholson, et al., found that the self-limiting properties and the progress in the root canal was questionable because it varied greatly. They concluded that medicaments behave differently in the root canal than they do in a test tube.

D. TRITIUM LABELED RADIOAUTOGRAPHY

Radioactive isotopes can be localized to a given area or to individual cells in an histologic section. The radiolabeled tissue is fixed, embedded in paraffin, sectioned, and placed on a slide (Preece, 1965). The slide is then covered with a photographic emulsion. Recorded on this emulsion is the radioactivity present in the sectioned tissue. This appears as accumulations of black dots or granules which
are superimposed over the areas in the tissue which contain the radioisotope. It is the ionization of the silver bromide crystals in the photographic emulsion that enables us to observe the presence of the radioisotope (Fitzgerald, 1963).

Stahl, Weiss, and Tonna (1969) observed the periapical response of the periodontal ligament following pulpal injury with thymidine labeled with tritium. They used 0.5 micro-curies of tritiated thymidine by intraperitoneal injections. They noted that a significant rise in labeled fibroblasts, osteoblasts, and cementoblasts was seen in the periapical regions.

Tritium would be the radioisotope of choice (Stahl, 1969) because "The low beta-ray energy of tritium localizes the label closely on the radiosensitive emulsion and prevents excessive scatter to and false labeling of tissue components distant... from actual labeled area (Zach, 1969)." A beta-ray will travel a maximum distance of six microns in tissue and half of the particles will travel less than one micron. Consequently, the majority of the activated silver grains in an autoradiogram should lie within one micron of their source (Hughes, 1958).

Tritium labeling services fill an important gap in satisfying requirements of researchers (Evans, 1968). "This is often the isotope of choice because of the relative ease of labeling compounds and the great versatility of this isotope" (Feinendfgen, 1967).
CHAPTER III

MATERIALS AND METHODS

A study of the penetrability of radiolabeled aqueous parachlorophenol was conducted by using microscopic and radioautographic techniques.

Tritium labeled parachlorophenol with a specific activity of 77 microcurie/millimole was made by a commercial laboratory using the catalytic exchange method. This solution was then diluted to make a two percent aqueous solution.

Sixteen freshly extracted human maxillary anterior teeth were obtained from the Loyola University School of Dentistry Oral Surgery Department. These teeth were divided into four equal groups with Group 1 as the control.

Group 1: Control Group

Access cavities were made with a high speed #701 steel bur and a #4 slow speed steel round bur. The pulp tissue was then removed with a fine-fine barbed broach. Stainless steel standardized style "B" files were used to completely cleanse and shape the root canal to within one millimeter of the apex. Instrumentation was considered

2. Union Broach; Long Island, New York.
complete when white dentinal shavings were evident on the instruments. Sodium hypochlorite\textsuperscript{1} and hydrogen peroxide\textsuperscript{2} were used as irrigating solutions. The root canal and pulp chamber were dried using sterile medium paper points and \#2 cotton pellets, respectively.\textsuperscript{3}

A \#2 dry cotton pellet was then placed in the pulp chamber. The coronal access cavity was sealed with cavit\textsuperscript{4} and the apex was sealed with impression compound.\textsuperscript{5} The teeth were then separately stored in 100\% humidity for forty-eight hours.

**Group II:**

The teeth in Group II were prepared in exactly the same manner as those in control Group I except that a medium paper point containing radioactive PCP was sealed in the pulp chamber. No cotton pellet was left in the pulp chamber.

**Group III:**

In Group III preparation of the teeth differed in that the cleansing and shaping was confined to lingual surfaces in order to leave pulpal tissue on the buccal wall. A \#2 cotton pellet saturated with radio-labeled PCP was sealed into the tooth.

---

Group IV:

The teeth in Group IV were prepared in exactly the same manner as those in control Group I except that the #2 cotton pellet sealed in the pulp chamber contained radioactive PCP.

All of the teeth were handled individually with rubber gloves to prevent cross-contamination. Care was taken to prevent excess PCP from flowing on to the external surface of the teeth and all instruments were thoroughly washed and scrubbed before entering each tooth.

After forty-eight hours in 100% humidity all of the teeth were placed in separate decalcifying solutions containing 50% of 88% formic acid and 50% of 20% sodium citrate (Preece, 1965). The teeth were kept in this solution for twelve days. All four groups of teeth were bisected bucco-lingually using razor blades. A one-blade per tooth procedure was followed to prevent contamination.

The teeth were then frozen and later sealed in paraffin, and sections with a thickness of 6-8 microns were made and autoradiographed. After five days the sections were stained with nuclear fast red, Indigo-Carmine dye and picric acid. The specimens were then examined microscopically and grain counts were taken using an automatic hand counter. A 50 micron square grid was superimposed over the grains to facilitate counting (Figure I).

Twenty-five random grain counts were taken on each of the control sections in order to establish the normal or background radiation level.
The sections in Groups II, III, and IV, were then divided into apical 1/3, middle 1/3, and coronal 1/3 areas. The grid was superimposed over the cemento-dentinal junction (Figure 1). The counts began at the periphery of the tooth (C-D Junction) and moved inward toward the root canal until a significant level of radiation above background was obtained (Figures II - IX).

The individual counts were pooled and statistically compared with the control values, using "t" test for comparing means.
CHAPTER IV

RESULTS

The counting of grains began at the cemento-dentinal junction and proceeded pulpally toward the root canal until a significant level of radiation, compared with the control was reached. This significant level of radiation was reached by all groups of teeth at the cemento-dentinal junction.

Group II

The average grain counts per square micron for Group II (teeth containing radioactive PCP paper point) were:

- Apical 1/3 - 162.7 grains
- Middle 1/3 - 124.5 grains
- Coronal 1/3 - 169.7 grains

This was compared with mean grain count for the control group which was 46.6. The standard deviation for Group II teeth was also determined and compared with control Group I. The statistics for the hypothesis concerning two means, the "t" Test, as well as probability and mean grain count per 50 micron square were also calculated and appear in Table I. The proportionate distribution of label with Group II was computed and appears in Table II.

1. Based on 100 counts.
TABLE I

PENETRATION OF PCP TO CEMENTO-DENTINAL JUNCTION IN GROUP II
(TEETH CONTAINING RADIOACTIVE PCP PAPER POINT)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Apical 1/3</th>
<th>Middle 1/3</th>
<th>Coronal 1/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Grain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count/50 Micron Sq.</td>
<td>18.64</td>
<td>65.07</td>
<td>49.80</td>
<td>67.89</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>19.28</td>
<td>26.60</td>
<td>20.36</td>
<td>11.81</td>
</tr>
</tbody>
</table>

| "t"                 | <.001   | <.001      | <.001      |
| P                   |         |            |            |

TABLE II

PROPORTIONATE DISTRIBUTION OF LABEL
AT CEMENTO-DENTINAL JUNCTION WITHIN GROUP II
(TEETH CONTAINING RADIOACTIVE PCP PAPER POINT)
USING "t" TEST

<table>
<thead>
<tr>
<th></th>
<th>Apical 1/3</th>
<th>Middle 1/3</th>
<th>Coronal 1/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical 1/3 t</td>
<td>---</td>
<td>4.56</td>
<td>0.9686</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;.001</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Middle 1/3 t</td>
<td>4.56</td>
<td>---</td>
<td>7.68</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.001</td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Coronal 1/3 t</td>
<td>0.9686</td>
<td>7.68</td>
<td>---</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.1</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>
Group III

The average grain counts per square micron for Group III (teeth containing radioactive PCP were the cotton pellet cleansing and shaping limited to lingual wall) were the highest of all the groups:

- Apical 1/3 - 337.5 grains
- Middle 1/3 - 350.3 grains
- Coronal 1/3 - 472.6 grains

The mean grain count per 50 micron square standard deviation, "t" Test and probability were determined and compared with control Group I and appear in Table III. The proportionate distribution of label within Group III teeth is demonstrated in Table IV.

---

**TABLE III**

PENETRATION OF PCP TO CEMENTO-DENTAL JUNCTION IN GROUP III

(TEETH CONTAINING RADIOACTIVE PCP COTTON PELLET-CLEANSING AND SHAPING LIMITED TO LINGUAL WALL)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Apical 1/3</th>
<th>Middle 1/3</th>
<th>Coronal 1/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Grain</td>
<td>18.64</td>
<td>135.00</td>
<td>150.13</td>
<td>189.06</td>
</tr>
<tr>
<td>Count/50 Micron Sq/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>19.28</td>
<td>61.07</td>
<td>53.92</td>
<td>29.65</td>
</tr>
<tr>
<td>&quot;t&quot;</td>
<td>33.24</td>
<td>39.00</td>
<td>103.49</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

1. Based on 100 counts.
TABLE IV

PROPORTIONATE DISTRIBUTION OF LABEL AT CEMENTO-DENTINAL JUNCTION WITHIN GROUP III

(TEETH CONTAINING RADIOACTIVE PCP COTTON PELLET-CLEANSING AND SHAPING LIMITED TO LINGUAL WALL)

USING "t" TEST

<table>
<thead>
<tr>
<th></th>
<th>Apical 1/3</th>
<th>Middle 1/3</th>
<th>Coronal 1/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical 1/3 t</td>
<td>---</td>
<td>0.6297</td>
<td>10.12</td>
</tr>
<tr>
<td>P</td>
<td>&gt;.05</td>
<td>---</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Middle 1/3 t</td>
<td>0.6297</td>
<td>---</td>
<td>10.86</td>
</tr>
<tr>
<td>P</td>
<td>&gt;.05</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Coronal 1/3 t</td>
<td>10.12</td>
<td>10.86</td>
<td>---</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>---</td>
</tr>
</tbody>
</table>

Group IV

The teeth in Group IV (teeth containing radioactive PCP cotton pellet) had the following average grain counts per 50 micron square:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical 1/3</td>
<td>212.6 grains</td>
</tr>
<tr>
<td>Middle 1/3</td>
<td>196.3 grains</td>
</tr>
<tr>
<td>Coronal 1/3</td>
<td>217.1 grains</td>
</tr>
</tbody>
</table>

Tables V and VI demonstrate the mean grain count per 50 micron square, standard deviation, "t" Test, probability, and proportionate distribution of label within the group, respectively.

1. Based on 100 counts.
TABLE V

PENETRATION OF PCP TO CEMENTO-DENTINAL JUNCTION IN GROUP IV
(TEETH CONTAINING RADIOACTIVE PCP COTTON PELLET)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Apical 1/3</th>
<th>Middle 1/3</th>
<th>Coronal 1/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Grain Count/50 Micron Sq/</td>
<td>18.64</td>
<td>85.03</td>
<td>78.45</td>
<td>86.85</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>19.28</td>
<td>36.41</td>
<td>30.53</td>
<td>13.46</td>
</tr>
<tr>
<td>&quot;t&quot;</td>
<td>33.67</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

TABLE VI

PROPORTIONATE DISTRIBUTION OF LABEL
AT CEMENTO-DENTINAL JUNCTION WITHIN GROUP IV
(TEETH CONTAINING RADIOACTIVE PCP COTTON PELLET)
USING "t" TEST

<table>
<thead>
<tr>
<th></th>
<th>Apical 1/3</th>
<th>Middle 1/3</th>
<th>Coronal 1/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical 1/3</td>
<td>---</td>
<td>1.37</td>
<td>0.4688</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.05</td>
<td>&lt;.05</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Middle 1/3</td>
<td>1.37</td>
<td>---</td>
<td>2.50</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.05</td>
<td>&lt;.05</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Coronal 1/3</td>
<td>0.4688</td>
<td>2.50</td>
<td>---</td>
</tr>
<tr>
<td>P</td>
<td>&gt;.05</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

A comparison of label between equivalent areas in Groups II, III, and IV are shown in Tables VII and VIII.
TABLE VII

COMPARISON OF LABEL BETWEEN GROUP II (TEETH CONTAINING RADIOACTIVE PCP PAPER POINT) AND GROUP III (TEETH CONTAINING RADIOACTIVE PCP COTTON PELLET AND CLEANSING AND SHAPING LIMITED TO LINGUAL WALL) AT CEMENTO-DENTINAL JUNCTION

<table>
<thead>
<tr>
<th>GROUP II</th>
<th>GROUP III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical 1/3 t</td>
<td>10.49</td>
</tr>
<tr>
<td>Middle 1/3 t</td>
<td>---</td>
</tr>
<tr>
<td>Coronal 1/3 t</td>
<td>---</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

TABLE VIII

COMPARISON OF LABEL BETWEEN GROUP IV (TEETH CONTAINING RADIOACTIVE PCP COTTON PELLET) AND GROUPS II AND III AT CEMENTO-DENTINAL JUNCTION

<table>
<thead>
<tr>
<th>GROUP IV</th>
<th>GROUP II</th>
<th>GROUP III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical 1/3 t</td>
<td>4.42</td>
<td>---</td>
</tr>
<tr>
<td>Middle 1/3 t</td>
<td>---</td>
<td>7.87</td>
</tr>
<tr>
<td>Coronal 1/3 t</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Apical 1/3 t</td>
<td>7.02</td>
<td>---</td>
</tr>
<tr>
<td>Middle 1/3 t</td>
<td>---</td>
<td>9.95</td>
</tr>
<tr>
<td>Coronal 1/3 t</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>
CHAPTER V
DISCUSSION

The natural defense mechanism inherent in the human body cannot function to combat the disease process which is present in the infected root canal. To resolve this problem extrinsic factors must be introduced.

Thorough biomechanical cleansing and the use of intracanal antimicrobial medications, eliminates the infection in the root canal. It is the ability of these medications to penetrate into the tooth structure that concerns us.

The minimum requirements of an ideal root canal medication are: (1) that it be germicidal to most organisms; (2) that it has deep penetration; (3) that it exhibits rapid effectiveness; and (4) that it is effective in the presence of organic matter (Sommer, Ostrander and Crowley, 1962).

Drugs that do not penetrate deeply into the dentin may not reach all of the organisms present. Root canal antiseptics should be capable of penetrating the tooth structure since microorganisms may be found in the dentinal tubules, in the irregularities of the root canal, in secondary or accessory canals and in periapical areas.

The most commonly used root canal disinfectant, camphorated parachlorophenol (Uchin, 1951), is considered clinically as a highly effective antimicrobial agent (Ostrander, 1958). The antimicrobial
activity is due to the parachlorophenol and the camphor serves merely as a vehicle. Camphor possesses little or no antiseptic value but has some anodyne qualities (Sommer, Ostrander and Crowley, 1961).

PCP is believed not to exert marked antiseptic action in the presence of solvents with low dielectric constants such as acetone, alcohol, chloroform, ether and xylene. However, it does exhibit marked antimicrobial activity in solvents of high dielectric constants, such as glycerin, nitrobenzene, and water (Harrison, 1969).

CPC was first introduced to the dental profession in 1891 by Dr. Otto Wolkoff. CPC is defined by the National Formulary, 1965, as containing between thirty-three and thirty-seven percent PCP and between sixty-three and sixty-seven percent camphor.

The first valid microbiological investigation into the antimicrobial activity of CPC was conducted by Dr. John Harrison in 1969. Until then the effective concentration of PCP was unknown. The results of Harrison's experiments in toxicity, antimicrobial activity, and effective concentrations indicated that:

"...2) A reduced PCP concentration in a vehicle of water is far less toxic than CPC, and 3) PCP is an effective antimicrobial agent in extremely small concentrations in aqueous solution against a variety of microorganisms commonly found in the root canal."

If, in fact, aqueous PCP is effective antimicrobially then we must determine its ability to penetrate into the tooth structure if we intend to use it clinically.
Penetration of aqueous PCP in Groups II (teeth containing radioactive PCP paper point), III (teeth containing radioactive PCP cotton pellet and cleansing and shaping limited to lingual wall), and IV (teeth containing radioactive PCP cotton pellet) examined in this study showed significantly higher values of radiation at the cemento-dentinal junction than in Group I, the control group. The label appeared to follow the dentinal tubules or the so-called "paths of dental lymph" described by Fish, 1927, and Bodecker, 1943. The results appear to indicate that the PCP penetrated the tooth well.

There was a significantly higher amount of label present in Group III teeth (teeth containing radioactive PCP cotton pellet and cleansing and shaping limited to lingual wall) than in Groups II and IV (teeth containing radioactive PCP paper point and cotton pellet, respectively). Due to the increased level of radiation present in Group III it appears that the aqueous PCP had a definite affinity for the protein in the remaining pulpal tissue. The pulpal tissue remaining in the teeth may well have been the reason for the increased amount of label found at the cemento-dentinal junction of Group III teeth.

The proportionate distribution of label within Groups II and IV was not as significant as the distribution within Group III (Tables VII and VIII). In Group III the label total was extremely high in the coronal one-third of the teeth, whereas in Groups II and IV there was
a tendency for the label to be only slightly higher in the coronal portion than in other areas. The high level of radioactivity in coronal portions is due to the fact that the medication was initially placed in the pulp chamber. In Group III the pulpal tissue left in the coronal one-third was probably responsible for the high radioactive level.

The radioisotope tritium was selected for this study because of its low beta ray energy. Beta rays travel no more than six microns in tissue and more than half of the particles will travel less than one micron. The label is closely localized on the radiosensitive emulsion thus preventing false labeling of the tissue (Zach, 1969). We can therefore assume that the majority of the grains present in and autoradiogram lie within one micron of their source (Hughes, 1958).

Decalcified sections were used because very thin sections without distortions were needed in order to accurately measure the exact depth of penetration through the dentinal tubules. It is very difficult to obtain very thin ground sections of teeth. There is also the inherent danger in preparing ground sections that during the cutting procedure the blade may force or scatter radioactive material all over the tooth thus giving an inaccurate and incorrect result.

It is essential in endodontic therapy for the chemotherapeutic agent used to reach all areas of the tooth. The results of this study indicate that PCP in a vehicle of water has deep penetration capability and that the use of either a paper point or a cotton pellet to carry
the medication into the tooth will prove effective in root canal therapy. Due to its low toxicity and high antimicrobial effectiveness, aqueous PCP is to be preferred over the presently used 35% camphorated solution. The 35% solution, while also having a high degree of antimicrobial effectiveness, has such a high level of toxicity that perhaps its use should be re-evaluated.

Further in vivo investigation is now needed to determine whether the diffusion of aqueous parachlorophenol terminates at the cemento-dentinal junction, as in this study, or extends into the bone and periapical tissues, and if so how far?
A study of the intracanal diffusion of aqueous parachlorophenol in endodontics was conducted in order to develop a technique for testing the permeability and diffusibility of intracanal medicaments. It was determined by the radioautographic investigation of tritium-labeled 2% aqueous PCP, that the medicament does indeed penetrate the dentin from the pulp chamber and root canal and travels at least to the cemento-dentinal junction and also diffuses to the apex.

In the light of results of this investigation, it appears that this technique of studying penetration has merit and should be further investigated using other root canal medications. Aqueous PCP should also be studied further in vitro and in vivo for possible future clinical use.
BIBLIOGRAPHY


Amler, M.H., and Bevelander, G.: Dentin permeability to radioactive phosphorus after specific time intervals following the application of various drugs. New York J. Dent. 21:295-300, 1951.


Figure 1: Appearance of decalcified sections (without presence of enamel)
Figure II: Original Magnification 1000x
Control Group I
Figure III: Original Magnification 250x
Radioactive Paper Point Group II - Pulp Canal
Figure IV: Original Magnification 450x
Radioactive Paper Point Group II - Pulp Canal
Figure V: Original Magnification 1000x
Radioactive Paper Point Group II - Circumpulpal Dentin
Figure VI: Original Magnification 1000x
Radioactive Cotton Pellet Group III - Circumpulpal Dentin
Figure VII: Original Magnification 1000x
Radioactive Cotton Pellet Group III - Circumpulpal Dentin
Figure VIII: Original Magnification 1000x
Radioactive Cotton Pellet Group IV - Circumpulpal Dentin
Figure IX: Original Magnification 1000x
Radioactive Cotton Pellet Group IV - Cemento-Dentinal Junction
APPROVAL SHEET

The thesis submitted by Dr. Warren Y. Avny has been read and approved by three members of the Graduate School faculty.

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 references to content, form and mechanical accuracy.

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

May 1, 1970
Date

Signature of Advisor