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The Effectiveness of Para-Chlorophenol as a Possible Antimicrobial Agent in Endodontia: An in Vitro Study

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THE EFFECTIVENESS OF PARA-CHLOROPHENOL AS A POSSIBLE ANTIMICROBIAL AGENT IN ENDODONTIA: AN IN VITRO STUDY

by

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AUTOBIOGRAPHY

Christian Vikari was born and raised in Munich, Bavaria, Germany on June 9, 1935. There he attended grade school, "Oberrealschule", and Engineering School. He graduated from there with the degree of E.T.Z. and subsequently passed a German Board examination.

After emigrating to the United States in 1953, he served as an enlisted man in the United States Army, Corps of Engineers, from 1954 to 1956.

From 1956 to 1958 he served on a mission to Austria and Switzerland for the Church of Jesus Christ of Latter Day Saints.

In the fall of 1958 he entered Brigham Young University, Provo, Utah, to continue studies in electrical engineering.

During the period from 1958 to 1960, his interests began to lean toward dentistry and medicine. With a strong desire to study dentistry, he entered the University of Oregon Dental School, Portland, Oregon in September of 1960. He graduated from there with the degree of Doctor of Dental Medicine in June of 1964.

After graduation from dental school, he entered the Dental Corps of the United States Army as a commissioned officer. In August of 1967, after serving for three years in Germany, he was assigned, by the Office of the Surgeon General, Dental Branch, to Loyola Dental School, 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 presently has a daughter and two sons. He holds the rank of Major, United States Army.

DEDICATION

To my dearest wife, Karma, whose love and loyalty, angelic patience, untiring devotion and continual encouragement were beyond words, I dedicate this thesis.

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INTRODUCTION AND STATEMENT OF THE PROBLEM

Introductory Remarks.

Since its inception by Dr. Otto Walkoff in 1891, 30% para-Chlorophenol (pCP) in a Camphor vehicle has been used in dentistry for Root Canal Treatment almost without change or great variation, to the present day with the advice, that if used wisely it could be a fine adjunct to Endodontic Therapy. It is used without a real foundation of major modern scientific evaluation as to its antimicrobial effectiveness at varied concentrations or in combination with different vehicles. Many answers are needed in such areas as toxicity, tissue tolerance or shelflife, for example.

Statement of the Problem.

It is the purpose of this research to determine the minimal concentration of pCP capable of killing a commonly found spectrum of microorganisms found in infected root canals in combination with various vehicles other than Camphor, which may effectively combine with smaller amounts of pCP while increasing its antimicrobial activity and decreasing its toxicity to host tissues. It is also important than after employment of the drug within the root canal no residue remain which may cause damaging changes to root canal cements or impair culturing techniques resulting in false negatives. The drug should be able to easily penetrate dentinal structures, while remain-

ing bacteriocidal for at least 48 to 72 hours. It should be non-allergenic to the host. The drug must not be inactivated by ions, components of necrotic or putrescent tissue or bacterial toxins.

Although it is not the scope of this investigation to directly pursue and find answers to all of the above areas mentioned, they have been considered carefully in the selection of the drugs, bacteria and test procedures.

Another important aspect of the objective of this study is to show if any relationship exists between the minimal bacteriocidal concentrations of the various drugs tested and the size of their zones inhibition with or without pCP on agar plates.

CHAPTER II

REVIEW OF THE LITERATURE

Walkoff (1891) suggested the use of camphorated para-Chlorophenol, (CpCP). He thought it to be a most serviceable therapeutic agent at a 30% concentration, as quoted by Prinz (1937).

Paul and Kronig (1897) gave evidence to substantiate that halogenation of phenols increases their bacteriocidal activity. However, they were unable to explain the mechanism of this phenomenon. They point out that halogens in the presence of organic substances enter into inactive combinations since they become oxidized easily and quickly by these materials. Therefore, they become unavailable to attack any bacteria present, whereas the phenols are not inactivated in the presence of organic matter. They also found that phenols in combination with glycerin will show a decreased bacteriocidal effect.

Peck (1898) tested essential oils for their antiseptic properties against samples of bacteria from the oral flora of students. He determined that eugenol was of no antibacterial value, since in a eugenol saturated broth bacteria continued to grow. His definition of an antiseptic states that it is a poison to vegetable cells and most animal cells.

Frei and Krupski (1915) define a good antiseptic as one which is capable of damaging protoplasm and its constituents, as well as altering the permeability of the cell. They found that two poisons can develop additive antibacterial effects if combined, because they pro-

bably are capable of influencing each other chemically or physically. This means that the properties of the solvent change in respect to solubility, surface tension and viscosity. The addition of glycerin and ethyl alcohol to meta-Cresol, for example, diminished the bacteriocidal activity of this normally good antiseptic agent. The explanation given is that the solubility of this poison in the vehicle increased and that a shift of the surface tension coefficient occurred; thus the drug did not permeate across the cell membrane any longer. These investigators also used ferric chloride ($FeCl_3$) in the quantitative colorimetric evaluation of Cresol concentrations.

Myers and Thienes (1925) found in their studies of fungicidal agents that camphor in a saturated aqueous solution did not inhibit the growth of pathogenic yeasts.

Myers (1927) determined the fungicidal activity of certain volatile oils. Eugenol and camphor were not active or fungicidal against pathogenic yeast or actinomyces isolated from the oral cavity and other upper respiratory areas. One per cent(1%) phenol solution was determined to be bacteriocidal in 50 minutes.

Engelhardt (1927) in his extensive research concludes that drugs in vehicles with high dielectric constants appear to have a higher antiseptic action. He quotes the dielectric points for water as being 80 and that of glycerin as being 56. His studies indicate that phenol, regardless of the dielectric constants of vehicles, maintains its antiseptic qualities. A 2% aqueous pCP solution

showed good antiseptic qualities upon spores of "Milzbrand." Similar results were obtained with a 2% glycerinated pCP solution. He further explains that pCP dissociates approximately 6 times as much as phenol and the same vehicular dielectric constant influence exists upon it. The difference between these two drugs becomes very apparent when the relatively strong dissociated pCP no longer shows any antiseptic effects in vehicles with low dielectric constants. Phenol, however, maintains its antiseptic powers rather well, with little change. Both pCP and phenol are lipid soluble. A 5% solution of pCP was effective in killing Staphylococcus aureus. He quotes Laplace (1888) as stating that the addition of acids increases the bacteriocidal activity of phenols.

Coolidge (1928) said that both laboratory and clinical tests must be made before satisfactory conclusions can be drawn as to the merits of a germocide. He found that diffusion and osmosis may be increased by decreasing the surface tension of a drug. He stated that cresatin had a low surface tension which readily wets surfaces penetrating deeply, while slowly soluble. It has prolonged effects, at least clinically, and showed no irritation of live tissues in full strength. It also has marked anodyne properties. He further explains that a germocide which lowers the surface tension of the medium tends to concentrate on the surface. It thus becomes more destructive to organisms because more molecules are attached to the surface by adsorption. Thus a more rapid diffusion through the cell membrane.

Coolidge (1929) described Cresatin as having a characteristic, persistent spiny odor and exhibited anodyne effects on living tissue. He observed it to be non-irritating on living tissue in full strength. He calls it a very satisfactory remedy in root canal treatment, although it has only mild antiseptic properties. Its effectiveness is attributed to its prolonged action. The drug is not readily exhausted due to its very low surface tension.

Grossman and Prinz (1932) suggested from their studies that electro-sterilization appears to be more effective than sealing a medicament into the root canal such as camphorated para-Chlorophenol.

Prinz (1937) describes pCP, ClC_6H_4 (OH), as a product of chlorine substitution, replacing the para hydrogen atom of phenol. The drug thus becomes more poisonous to microbes. The disinfectant powers are increased as well as the ability of penetration. Ordinary thermal or actinic influences do not affect its stability or potency. By trituration it combines with gum camphor to form a liquid. He also states that this solution does not cauterize soft tissue and proposes that its marked antiseptic qualities are due to the liberation of nascent chlorine in the presence of active phenol. Agreeing that the action of chlorine upon bacteria and their products seems to depend upon a process of chlorination, i.e. the amino group of the protein readily attack all substances containing active chlorine, in such a way that the hydrogen attached to the nitrogen atom is re-

placed by chlorine. He also believes that chlorine is a deodorizing agent which digests and removes necrotic tissue, as well as decomposing toxins. Absolute contact between the antiseptic agent the microorganisms, as well as the noxious substances to be acted upon is most essential. This contact must be maintained. Sufficient concentration of the antiseptic is important.

Suter (1941) agrees with Engelhardt that the germicidal effects of pCP are increased in a solvent with a high dielectric constant. He feels that this is not the case for phenol.

Pear (1942) established some criteria for the ideal root canal germicide in his studies of the germicidal activity of the vapors of various drugs. The drug must retain its germicidal effect for 48 to 60 hours. It must not irritate but should sterilize without surface contact with the organism. The germicidal vapors should be released slowly to not only establish but also maintain sterility. The drugs should vaporize at room temperature. Among the drugs tested were CpCP and Eugenol. He conducted his investigation with sealed agar petri dishes. Above the agar surface he suspended large filter paper discs containing the drugs to be tested. It was found that eugenol was capable only of retarding bacterial growth, whereas the vapors of camphorated para-Chlorophenol were bacteriocidal. He concluded that large amounts of CpCP are, therefore, not necessary

and if properly used, it would give the desired result. Thus it would be non-irritating.

Grossman (1944) in a "blind" study found that CpCP and Cresatin were without significant irritant effects when applied to the shaved skin of the human arm for 48 hours.

Lawrence (1945) tested the effects of antimicrobial compounds by increasing the dilutions of the compound in proper media. In this manner the minimal bacteriocidal drug concentration was determined. He subcultured if no growth was apparent in 72 hours. If the tubes were also negative at 24 hours, it was interpreted that the action of the drug was bacteriostatic; if the subcultures were negative, he assumed that the drug had been bacteriocidal.

Ostrander and Crowley (1948) in a study to find the relative efficiency of some root canal antiseptics found that eugenol, while relatively non-irritating, but anesthetic, displayed only minor antiseptic powers, while CpCP showed a very high antiseptic value with practically no irritating qualities. On a one hundred percent evaluation basis, eugenol was only 68.9% as effective as compared with a 74.4% value for CpCP.

Grossman and Christian (1952) determined in a serial dilution endpoint study the bacteriocidal effects of certain antibiotics. The dilutions ranged from 10^{-1} to 10^{-7} of the stock solution. In

a series of test tubes containing 4.5 ml of the required concentration of the drug. 0.5 ml of a 24 hour culture of micro-organisms were added. Some of the bacterial strains included Streptococcus faecalis and mitis, Staphylococcus aureus and Candida albicans. In this fashion the least or minimal bacteriocidal concentration of each drug against a spectrum of microbes was determined.

Hare (1953) points out that the mechanical preparation of root canals must be improved, since it essentially is the preparatory method readying the root canal for the reception of the filling materials. The use of bacteriocidal drugs is only an adjunct.

Grossman (1955) found that cresatin is an effective antimicrobial agent in Root Canal Therapy.

Coolidge (1956) describes the usefulness of pCP as recommended by Walkoff and states that it is a more germicidal agent than phenol and that it can penetrate deeper because it does not coagulate albumin or cauterize soft tissues in any way. He gives the prescription of camphorated para-Chlorophenol as follows:

para-Chlorophenol (crystals)..... 1 ounce (30 gm)

gum camphor.....2.5 ounces(70 gm)

These components are to be triturated until completely liquified. The camphor acts only as a vehicle without much therapeutic value.

Eklof (1956) found that micro-organisms can be resistant to camphorated para-Chlorophenol.

Sommer, Ostrander and Crowley (1956) gave their criteria for a good root canal antiseptic. It should be non-injurious to periapical tissues, effective and non-interfering with accurate culture technique after having been sealed into the root canal for 48 hours. Although several drugs may fit these requirements, CpCP was preferred since it was more effective than older, more caustic drugs.

Dietz (1957) advocated a combination cresatin and CpCP. The drug was named XP-7. He followed the U.S. Department of Agriculture Circular 198 with his test procedures. Zones of inhibition of the drugs individually and as XP-7 were almost equal and all drugs were found to be bacteriocidal. When tested on human skin by subcutaneous injection, CpCP and XP-7 caused severe ulcerations with permanent scar formation. Cresatin was far less irritating and after 9 months, the injection sites were almost completely invisible. No sensitization of the subject occurred as is possible with antibiotics. Endodontically used XP-7 has been effective and non-irritating. The antibacterial effect lasted for 6 months. Cresatin was reported as clinically effective because of its low surface tension.

Stewart (1957) reported the clinical effectiveness of Cresatin.

Ingle and Zeldow (1958) found that mechanical instrumentation of the root canal does not sterilize the canal, but temporarily reduces the number of organisms. By intra-canal medication, the bacterial flora was further reduced, giving the host an opportunity to fight off the infection. They also learned that a negative culture does not necessarily imply a sterile canal.

Engstrom (1958) discovered that both chlorine and iodine are of short duration in the root canal in aqueous solutions. These compounds decompose rapidly as they interact quickly with organic matter.

Wolfsohn (1958) sought to determine the effectiveness of certain therapeutic agents used in Endodontia. A part of his interest was devoted to water-in-oil suspensions as vehicular bases (hydrophillic absorption ointment bases), for some medicaments. He feels that a bacteriocidal agent or a group of them reduce the bacterial flora and their toxins enough to allow the repairative mechanism of the body to take over. He quotes Perrin and Halpern (1951) who reported a comparative study of these bases with commonly used dermatological agents stating the conclusion:

> "The water-in-oil emulsion is a desirable dosage form for topical therapy that may be used with all medicaments....This study demonstrates....the general desirability of the water-in-oil emulsion. Perhaps the reason for this lies in the mechanism of drug release from the base, whereby the active ingredient must traverse the continuous oil phase before it can be liberated to the treatment area. The thickness of this oil film would then be the determinant in the drug release and, therefore, would tend to regulate the concentration of the drug diffusing into the area. In this manner a slower, more steady drug release results and there is a minimum

diffusion of a large drug concentration which might be irritant to the skin."

The results of the study by Wolfsohn showed that CpCP without an ointment base had a zone of inhibition of 5 mm at 24 hours and 6 mm at 72 hours. When anhydrous lanolin was combined with CpCP, the zone was 4 mm at 24 hours and 72 hours. Camphorated para-Chlorophenol with a Xylocaine ointment equally showed 4 mm. The combination of CpCP with aquaphor caused a zone of inhibition of 2 mm at 24 hours and 4 mm at 72 hours. Stewart's pCP formula (mixture of hexachlorophene, thymol, CpCP and phenacaine HCl in a polyethylene base) elicited a 12.5 mm zone of inhibition at both 24 and 72 hours.

Astle and Shelton (1959) explain in their Textbook for Organic Chemistry that phenols are compounds in which the OH group is directly attached to the benzene ring. These compounds may form esters with acid anhydrides and acid chlorides and, in general, can behave much like the alcohols, although they are more acidic. Inasmuch as all bonds in benzene have some double-bone character, phenols are in fact enols, and like them form salts with strong bases, such as sodium hydroxide, because the R groups are strongly electronegative. Phenols for the most part are soluble in 5% NaOH. Should the hydroxyl group of the phenol be placed on a side chain, it becomes less acidic. The acidic nature of phenols may be explained on the basis of resonance. Since the oxygen has a slightly higher positive charge, it causes a repulsion of a proton and, thereby, increases the ease of proton removal. It follows that if any electronegative groups should be located

on the ring, the tendency for electrons to be attracted away from the oxygen would be further increased, thus ionization of the phenol would be increased. This is particularly the case if the electronegative group is located in the para position. In lieu of this information, the ionization constant for Phenol is 1.8×10^{-10} and for para-Chlorophenol, it is 7×10^{-10}. For this reason para-Chlorophenol is a stronger acid then phenol itself. A chlorine atom attached directly to the benzene ring is very inactive and cannot be removed by NaOH at reflux temperatures. However, this hydrolysis can be done at higher temperatures and high pressures. A chlorine atom may be activated by a strongly electronegative group in a para or ortho position. In this manner the halogen may be removed by aqueous NaOH at reflux temperatures.

Schilder and Amsterdam (1959) tested several drugs to determine their irritability to tissue. Rabbits were used for the project. The drugs, among which were cresatin, CpCP and eugenol, were used on skin by intradermal injection. They were also used to accomplish eye studies. The results showed that CpCP and eugenol were highly irritating. However, the authors note that it seems incorrect to speak of CpCP as non-irritating under conditions of good clinical use. Cresatin proved to show little or no inflammatory potential. The low surface tension may also permit the drug to penetrate oils and fats. As a result of this study cresatin has become the drug of choice in root canal dressings after vital pulp extirpation and after the first negative culture at Temple

University Dental School and Beth Israel Hospital.

Takigawa (1959) found that among several drugs tested CpCP and eugenol had the least sedative action with eugenol being less destructive to pulpal tissue than CpCP.

Healy (1960) describes camphor as only very mildly antiseptic. Its power is principally due to the pCP. In his review he shows that besides CpCP many other drugs and drug combinations have been suggested, tested in laboratories and clinics by many different investigators.

Grossman (1960) states that adequate mechanical preparation of the root canal cannot be stressed too strongly. Sterilization of the root canal can only follow after it has been thoroughly prepared. It is an axiomatic principle of surgery that before a wound is ready for chemotherapy, all necrotic material and debris must be removed.

Sommer, Ostrander and Crowley (1961) prefer CpCP as their studies indicated this drug to be more effective than other drugs. The exact mode of action, they feel, is subject to debate. They state that some authorities feel the major antiseptic activity of this agent is due to the splitting away of the chlorine atom in the presence of moisture, organic matter and warmth, with this nascent Cl atom actually producing the sterilizing action. They also state that the molecule as a whole possesses marked antiseptic properties. They conclude that the exact action is more of academic than clinical value. They report that soft tissue infections were treated successfully with a solution of 1 : 400 pCP in isotonic NaCl. The pCP is less caustic than phenol

and when combined with gum camphor in a 3:7 ratio, it becomes virtually non-irritating. Periapical tissue studies show only a mild degree of inflammation. It also has been demonstrated that CpCP penetrates dentin well. Cresatin has also been used to limited extent as a root canal antiseptic and has the advantage of causing a very low degree of tissue irritation. Some preliminary studies on the effectiveness of cresatin against micro-organisms commonly isolated from root canals suggested that it might be less effective against yeast than CpCP. It appeared to be an effective agent in the in vitro tests and thus deserves a place in root canal treatment, especially because of its high degree of tissue tolerance, as well as its apparent effectiveness against the most common pathogens found in root canals and periapical abscesses. Essential oils also have been widely used in Root Canal Therapy. Eugenol is the active principle of oil of cloves. It is thought to be an inferior antiseptic agent, quoting Ostrander and Kerr as having demonstrated that a drop or two of eugenol injected into rabbit muscle produced Zenker's necrosis. This irritant effect has also been confirmed by the authors with hamster experiments.

Mosteller (1962) advocated a combination of cresatin, CpCP and prednisolone for the prevention of hypersensitivity after operative procedures.

Stewart (1962) showed that the G.T.P. root canal dressing con-

sisting of hexachlorophene (G-11) 1%, thymol 5%, CpCP 6% and phenacaine HCl in a polyethylene base 1% to be of broad spectrum against gram positive and gram negative organisms, as well as fungi and enterococci.

LaSala (1963) states that CpCP is the most widely used antiseptic in Endodontia. It is well tolerated by tissues and combines readily with putrescent materials which cause the formation of gas. He states that CpCP has been combined with penicillin, but feels if applied with care and not beyond the periapex, it will cause no periapical problems. He quotes Coolidge and Kessel as using it in combination with benzol as an anodyne. Eugenol is labelled a poor antiseptic, but a good sedative.

Grossman (1965) writes that cresatin belongs to a group of phenol congeners. It is probably not as powerful a disinfectant as other members of this group. It is an acetyl acid ester of meta-Cresol. It is an antiseptic, fungicide and analgesic. Cresatin is stable, has low volatility and its low surface tension prolongs its antimicrobial activity. It is potentially non-irritating and will not precipitate protein. The different halogens vary in their disinfectant quality in that the disinfectant's power is inversely proportional to their atomic weight. Chlorine has the lowest, thus the highest disinfectant action. They dissolve necrotic tissue, but are highly unstable. Chlorine is a part of para-Chlorophenol, a substitute product of phenol.

Ingle (1965) considers CpCP a potent non-specific antimicrobial drug which is volatile and has a low surface tension. It is non-irritating under conditions of careful clinical use. Potentially, it is a highly inflammatory agent. Cresatin has a phenol coefficient of .75 and a surface tension of 35 dynes/cm^3. It is a mild antibacterial agent with marked antifungal properties. Animal studies indicate remarkably low tissue irritability. It has the added advantage of being an extremely effective pulp anodyne.

Mumford (1966) states that ideally a root canal medicament should kill all organisms <u>in vivo</u> and <u>in vitro</u>. Such a drug should not be disabled by surface tension, spoil in the presence of protein or harm the tissue. It should also be fast acting. It is Mumford's opinion that CpCP and polyantibiotic pastes best fit this criteria.

CHAPTER III

METHODS AND MATERIALS

A. Selection of the Media and the Micro-Organisms.

In this study the following media were used and prepared according to manufacturer's instructions*: Trypticase soy broth and agar, brain heart infusion broth and agar, nutrient broth and agar, Sabouraud's broth and agar, peptonized milk and lactobacillus selective agar. These media provide a rich nutritional base for good growth of the various micro-organisms used in the experiment.

The bacterial strains selected included:

Streptococcus faecalis, Microbiology Department, Northwestern University Medical School;

Staphylococcus aureus, Midwest Culture Collection;

Streptococcus mitis, Microbiology Department, Loyola University Dental School;

Streptococcus salivarius, Microbiology Department; Loyola University Dental School;

Neisseria catarrhalis, Midwest Culture Collection;

Lactobacillus caseii, Microbiology Department, Northwestern University Medical School;

Mycobacterium tuberculosis, Midwest Culture Collection;

*Baltimore Biological Laboratories, Cockeysville, Maryland

Corynebacterium diphtheriae, Midwest Culture Collection;

Bacillus subtilis, and its spores, American Type Culture Collection 6051;

Lactobacillus acidophilis, Microbiology Department, Northwestern University Medical School.

The choice of these particular micro-organisms was based upon two considerations:

1. They will provide as much as possible a replica of the bacterial flora commonly found in the oral cavity, which may become the possible cause of infection in root canals. (Burket, 1946; Ostrander and Crowley, 1948; Grossman, 1952; and Coolidge, 1956)
2. They provide a broad spectrum of pathogens, complete enough to test the drugs to be used against gram positive and gram negative micro-organisms, to include rods, cocci, acid fast, spores and yeast-like organisms. (Table 1)

B. Laboratory Equipment and Materials Used.

The usual microbiological laboratory armamentarium was used to perform the experiments. Broth and drugs, as well as bacterial cultures were transferred aseptically with sterile pipettes of proper size. Analytical balances* were used to accurately measure all drugs and media. All materials in the experiment were acquired new for every phase of the investigation to make certain that all components were

*Torbal Torsion Balance, Model EA-1, The Torsion Balance Co., Clifton, New Jersey.

TABLE 1

SELECTION OF THE MEDIA AND THE MICRO-ORGANISMS

Organisms	Media	
	Broth	Agar
1. Streptococcus faecalis	trypticase soy	same
2. Staphylococcus aureus	trypticase soy	same
3. Streptococcus mitis	trypticase soy	same
4. Streptococcus salivarius	trypticase soy	same
5. Neisseria catarrhalis	brain heart infusion	same
6. Lactobacillus caseii	peptonized milk	*
7. Mycobacterium tuberculosis	trypticase soy	same
8. Corynebacterium diphtheriae	trypticase soy	same
9. Bacillus subtilis	nutrient	same
10. Lactobacillus acidophilus	peptonized milk	*
11. Candida albicans	sabouraud's	same

*Lactobacillus selective

fresh and pure. The different drug mixtures and media were always prepared at the time of actual use, thus avoiding contamination or chemical changes. Bacterial cultures were used only after a new 24 hour broth incubation period, therefore, having assurance that the cultures were viable.

All of the equipment, media and drugs, wherever possible, were carefully sterilized before being used, in a steam autoclave for 20 minutes at 121° Centigrade temperature and 15 pounds/square inch pressure. Sterile indicator tape was employed to be sure of absolute sterility.

To accomplish the colorimetric phase of this project, a Coleman Spectrophotometer* was used.

C. Procedures.

The experiment was divided into four phases designed to give a spectrum of results which would, perhaps, substantiate and correlate all findings which would result in concrete conclusions.

Phase I:

Ten sterile test tubes filled with 8 ml of sterile trypticase soy broth were prepared. Two ml of 2.1% aqueous pCP was placed in the first tube, mixed thoroughly and serially diluted from 1×5^{-1} to 1×5^{-8}. 0.5 ml of a 24 hour culture of Streptococcus faecalis was placed into each dilution.

*Coleman, Junior II Spectrophotometer, Model 6/35, The Coleman Co., Maywood, Illinois.

Two other series of tubes were prepared by adding to 10 test tubes each filled with 10 ml of trypticase soy broth 0.05, 0.1, 0.15, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0 ml of 30% camphorated pCP respectively, while in the other series 30% glycerinated pCP was added in like fashion. All tubes were mixed thoroughly. To each tube 0.5 ml of Streptococcus faecalis was added.

In another study a 1% aqueous pCP solution was serially diluted in the same manner as the 2.1% para-Chlorophenol solution.

In all dilution series both positive controls, inoculated with the micro-organisms and not containing drug, and negative controls, consisting of drug but no micro-organism, were used.

The results made it possible to establish a standard aqueous pCP solution at a concentration of 1%. The dilutions ranged from 1:2 to 1:30. The following additional bacterial strains were used: Streptococcus faecalis, Staphylococcus aureus, Streptococcus mitis, Streptococcus salivarius, Neisseria catarrhalis, Corynebacterium diphtheriae and Candida albicans. The incubated dilution series were observed after 24, 48 and 72 hours, at which time they were visually compared with the control test tubes for growth. In cases where visual examination was inconclusive, samples were taken from these broths and plated on agar streak plates. These were allowed to incubate at 37° Centigrade, and examined after 72 hours. In this fashion growth or no growth of the bacteria was verified.

In order to examine the antimicrobial ability of each of the vehicles within the range of 1:2 to 1:30 of the 1% aqueous pCP, the following solvents were tested: H_2O, glycerin, diethylene glycol, eugenol, cresatin and camphor.

Phase II:

The study of the release of pCP into its surrounding media was accomplished by measuring the amounts of pCP in the supernate or water released by the vehicle at 24, 36, 48 and 72 hours, by making colorimetric measurements. By using this method the amount of pCP in the media was determined and correlated with the known amounts of the aqueous pCP standard.

In order to accomplish the actual colorimetric readings in the spectrophotometer, the point of maximum absorption had to be established for the 1% pCP. This value was 560 mμ.

In order to determine the concentration of pCP in unknown solutions, it was necessary to establish a standard curve of the effect of $FeCl_3$ on para-Chlorophenol. A 2% ferric chloride ($FeCl_3$) solution was chosen and added to the aqueous pCP dilutions at the previously determined serial dilution dose range. The color change in each test tube was measured with a spectrophotometer. The test described is known as the Ferric Chloride Test for Phenol. (English, 1961, English and Cassidy, 1961)

In order to determine the amount of pCP liberated by CpCP or

similar combinations, the pCP combinations were placed in the test solutions. The supernate was collected from the test solution after 24, 36, 48 and 72 hours of incubation at a constant temperature of 37° Centigrade, using only the drug combinations in serial dilution as outlined previously without bacteria or actual media. Since bacterial media is almost all water, it was thought that sterile distilled water would be an adequate substitute. Actual media would not allow proper colormetric readings. The amount of supernate collected was 7 ml, a quantity selected because it was easily handled by the spectrophotometer test well, to which 0.1 ml of 2% $FeCl_3$ was added. This was mixed thoroughly for 30 seconds, after which it was placed in the instrument to be read for its percent light transmission. Sterile, distilled water, 7 ml, with the addition of 0.1 ml of $FeCl_3$ served as the blank for the 100% adjustment.

The initial readings were accomplished with aqueous pCP solutions of 0.1, 0.2, 0.5, 0.7, 1.0, 1.2, 1.5, 1.8, and 2.1% strength. As in all subsequent tests, all determinations were made at least twice.

After establishing the basic criteria and the standard with a 1% aqueous pCP solution, the other drugs were tested: 1% pCP in cresatin, sterile glycerin, diethylene glycol, eugenol and camphorated para-Chlorophenol 30%. Since camphor will not mix with pCP 1% unless specially treated, it was used at the strength indicated. At this ratio it forms an eutectic which liquifies readily when tritu-

rated without forming a precipitate of camphor. In order to test this drug combination, which shows very little solubility in water, it was placed in 55 ml of water, an amount which was determined needed to derive at the proper supernate dilution level corresponding to the standard, in amounts graduated from 0.02 ml to 1.7 ml. Colorimetric readings were made as described earlier.

The amounts of light transmission was equated with the concentration or dilution of the para-Chlorophenol in the supernate. The concentration of pCP in the supernate was used as an estimation of the amount of pCP in the broth phase of the CpCP serial dilution bacterial tests.

Phase III:

The data of phases I and II were correlated and applied in the study of the minimal lethal dose concentration for all of the drug combinations used against the bacterial strains used in phase I, as well as the following additional strains of micro-organisms: Lactobacillus caseii, Mycobacterium tuberculosis, Bacillus subtilis and its spores, and Lactobacillus acidophilis. It was the purpose of this phase to determine the minimum lethal dose concentration which would, under the conditions given, kill the most resistant bacterial strain.

In this manner it was determined which of the drugs used under similar conditions is the most effective and the least effective, by comparison of the data obtained by the study above mentioned.

To test the effectiveness of the drug found to be most successful in killing the most resistant bacterial strains, the serial dilution method described in phase I was used with bacterial samples of root canals collected from patients in the Endodontic Clinic at Loyola Dental School. These root canal cultures apparently were resistant to other drugs by their repeated failure to eliminate a clinical infection. A comparative study series was made at the same time to check the results against the performance of the aqueous standard.

Phase IV:

A zone of inhibition study was conducted to test the possible correlation of the results of earlier phases with zones of inhibition tests. The agar media used corresponded to the broth media used in the serial dilution study. Also the same micro-organisms were used and placed in the corresponding media.

Two methods were employed to place the bacteria onto and into the agar media to see if any difference in the results would occur:

1. Streaking or "mopping" the agar surface with the bacterial cultures
2. Seeding the agar with the bacterial cultures.

In either case 0.5 ml of a 24 hour broth cultures was used to inoculate the media.

Sterile paper discs were impregnated with the appropriate drugs by employing a lambda micro-pippette. One drop of this pipette is equal to 0.001 ml. A high, medium and low concentration of drugs were

placed corresponding to 0.012 ml, 0.008 ml and 0.004 ml of the drug. Six discs were placed equidistant on the surface of the agar plate. All tests were done twice. After 48 hours of incubation at a constant temperature of 37° Centigrade, the plates were examined and the presence of a zone of inhibition was recorded. Organisms showing zones of inhibition at high, medium and low concentrations were considered very sensitive (vs), a zone at high and medium concentrations but not low indicated sensitive (s), a zone at high concentrations only was recorded as slightly sensitive (ss) and no zone on any of the discs indicated the organisms was resistant (r) to the drug.

Due to the fact that a zone of inhibition is influenced to a great extent by the solubility, the molecular weight, the surface tension and the diffusability of a drug placed upon agar plates, the actual measure of the diameter of the zone of inhibition is not an accurate method to determine the effectiveness of antimicrobial activity. Therefore, the relative estimation of antimicrobial activity from very sensitive to resistant was employed in this study.

This study was accomplished once with the vehicles without the pCP and once with the vehicles containing the pCP.

In order to place camphor in solution long enough to transfer it to the paper discs, it was dissolved in ether. The ether was allowed to evaporate before the discs were placed onto the agar surfaces.

CHAPTER IV

FINDINGS

Phase I

This part of the investigation was primarily designed to narrow the possible range of serial dilutions into an area close enough to finally determine the actual minimal dose concentration which is capable to destroy the most resistant test organism. It was noted that glycerin caused a milky cloudiness, which was thought to be due to possible protein precipitation. The results show that the minimal lethal dose range for Streptococcus faecalis in 1% aqueous pCP would be lower than 1:10 in the dilution series, since growth still occurred at this point.

With the results as indicated above, it was not possible to test other bacterial strains along with Streptococcus faecalis, at a dilution range most discriminating as to the minimal lethal dose endpoint for these micro-organisms. This study also gives a clue as to which bacteria is most resistant to the 1% pCP in H_2O. The findings show that the endpoint, or minimal concentration is a 1:9 dilution of 1% pCP for Streptococcus faecalis, making it the most resistant organism, and 1:25 for Candida albicans, placing it as the most sensitive organism (Table 2). These results were used as the minimal dose concentration standard to which all other results were compared.

In order to test the antimicrobial activity of the vehicles alone without the "active" pCP, the same serial dilutions were established

to correspond to that of the standard. Additional bacterial strains were used in this study to include all those previously employed, plus Lactobacillus caseii, Mycobacterium tuberculosis, Bacillus subtilis and its spores and Lactobacillus acidophilis. At the same time these bacterial strains were tested against the 1% aqueous pCP to determine the minimal lethal dose concentrations, for comparison to the standard, as well as their addition to the standard to include the total spectrum of micro-organisms used in this study. The results indicate that Streptococcus faecalis still is the most resistant organism.(Table 2) Of the vehicles tested cresatin proved most effective against the bacterial strains employed, while sterile distilled water, glycerin, diethylene glycol and camphor showed no bacteriocidal effects at all, and eugenol only very slight antimicrobial activity. Eugenol revealed what might have been interpreted as negative growth in the test tubes by visual examination, upon streak plating a loop full of the contents of the tubes excellent growth of the more resistant organisms after 72 hours of incubation. (Table 3)

A compilation was completed of all the aqueous minimal dose concentration endpoints. (Table 2)

Phase II

In order to quantitate the amounts of pCP released into the media accurately, the Ferric Chloride Test was employed. Ferric chloride when added to a solution containing phenol or a derivative of phenol,

TABLE 2

MINIMAL LETHAL DOSE ENDPOINT DILUTIONS
USING 1% AQUEOUS PARA-CHLOROPHENOL

STANDARD

Dilution Range 1:2 to 1:30

Organisms	Endpoint	mg pCP/ml
S. faecalis***	1:9*	1.11
S. aureus	1:23	0.43
S. mitis	1:20	0.50
S. salivarius	1:22	0.45
N. catarrhalis	1:16	0.63
L. caseii	1:20	0.50
M. tuberculosis	1:14	0.71
C. diphtheriae	1:23	0.43
C. albicans	1:25**	0.40
B. subtilis	1:20	0.50
spores	1:18	0.56
L. acidophilus	1:24	0.42

* highest concentration of pCP, Endpoint Dilution 1:9.
**Lowest concentration of pCP, Endpoint Dilution 1:25.
***Streptococcus faecalis, most resistant organism.

TABLE 3

THE ANTIMICROBIAL ACTIVITY OF POSSIBLE PARA-CHLOROPHENOL VEHICLES

	Minimal Lethal Dose Endpoint					
Organism	Water	Cresatin	Glycerin	Diethylene Glycol	Eugenol	Camphor
S. faecalis	< 1:2	1:8	< 1:2	< 1:2	< 1:2	< 1:2
S. aureus	< 1:2	1:14	< 1:2	< 1:2	1:6	< 1:2
S. mitis	< 1:2	1:12	< 1:2	< 1:2	1:5	< 1:2
S. salivarius	< 1:2	1:16	< 1:2	< 1:2	1:5	< 1:2
N. catarrhalis	< 1:2	1:8	< 1:2	< 1:2	< 1:2	< 1:2
L. caseii	< 1:2	1:10	< 1:2	< 1:2	1:4	< 1:2
M. tuberculosis	< 1:2	1:8	< 1:2	< 1:2	< 1:2	< 1:2
C. diphtheriae	< 1:2	1:13	< 1:2	< 1:2	1:6	< 1:2
C. albicans	< 1:2	1:18	< 1:2	< 1:2	1:5	< 1:2
B. subtilis	< 1:2	1:11	< 1:2	< 1:2	< 1:2	< 1:2
spores	< 1:2	1:10	< 1:2	< 1:2	< 1:2	< 1:2
L. acidophilus	< 1:2	1:12	< 1:2	< 1:2	1:4	< 1:2

will turn this normally colorless liquid into a blue colored compound. The intensity of the blue color increases with the amount of pCP in the solution.

The wave length for determining the optical density of the test solution was selected as the wave length showing maximum absorption of light. This maximum was found to be at 560 mμ. (Figure I) As part of this experiment, it was necessary to determine the relationship of pCP concentration in the test solution with the percent light transmission at the wave length of maximum absorption. It was thought that Beer's Law applies in this test. This law states that the log of the percent light transmission, if plotted against the color concentration, would result in a straight line. This would make possible the determination of actual amounts of pCP in a given test solution using such a straight line graph. The results of this exercise follow Beer's Law, in that a direct relationship between the color intensity and amounts of pCP in the test solution exist, making it possible to determine unknown concentrations of pCP in a solution by its percent light transmission, by comparison of such readings with those of a known color intensity and drug concentration. (Table 4, Figure II)

Having confirmed the basis of the colorimetric evaluation phase, a standard curve was prepared to correspond with the minimal lethal dose concentration dilution results (1:2 to 1:30 1% pCP solution) The same serial dilutions were established and their percent light transmission determined within the

FIGURE I

DETERMINATION OF THE MAXIMUM LIGHT ABSORPTION WAVE LENGTH FOR THE $FeCl_3$ Test for pCP

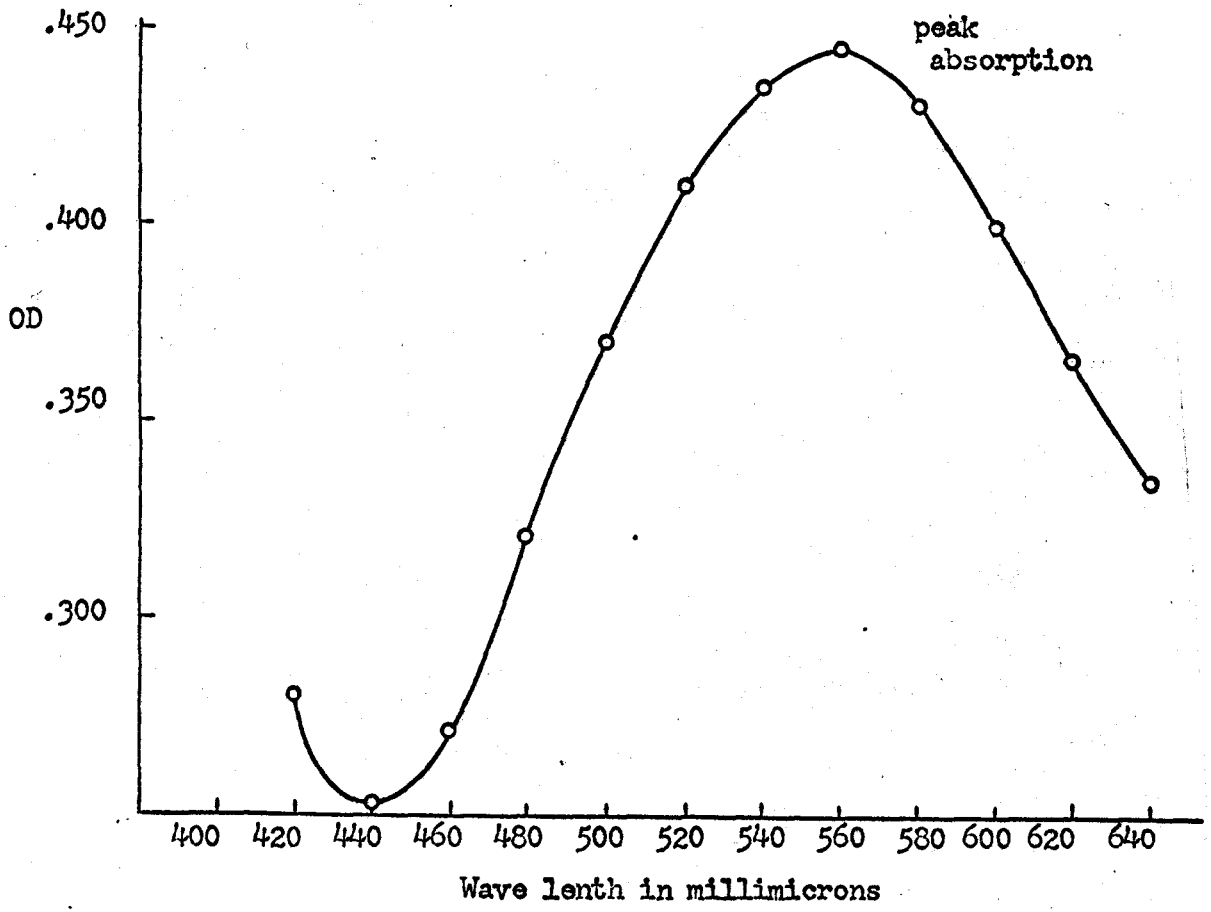


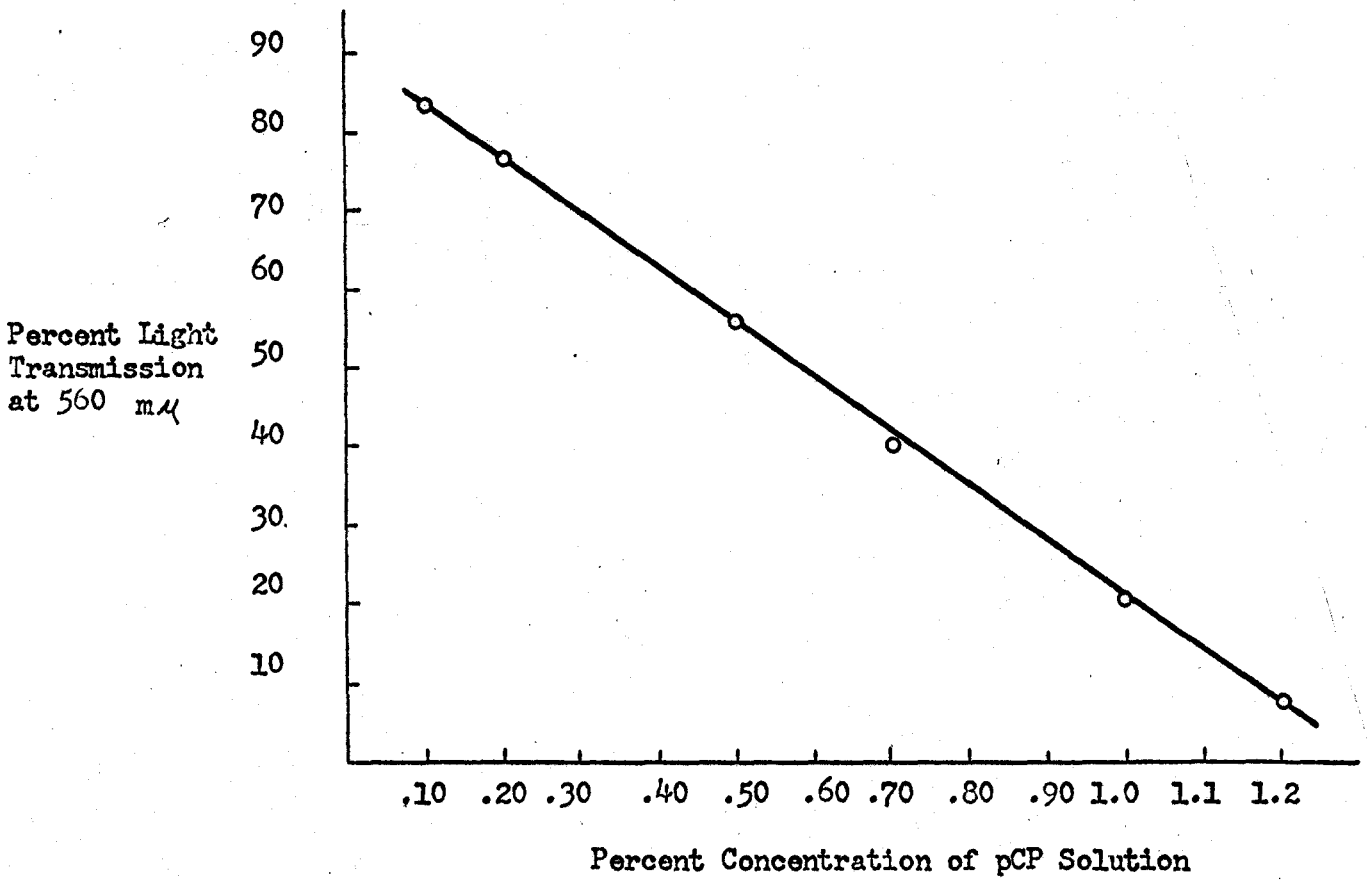
TABLE 4

PERCENT LIGHT TRANSMISSION OF AQUEOUS pCP SOLUTIONS IN $FeCl_3$ TEST FOR pCP

Concentration %	Light Transmission %
0.10	83.40
0.20	76.50
0.50	55.20
0.70	40.20
1.00	21.20
1.20	0.81
1.50	18.90
1.80	18.50
2.10	10.90

FIGURE II

LINEAR RELATIONSHIP OF PERCENT LIGHT TRANSMISSION AND pCP CONCENTRATIONS
$FeCl_3$ TEST FOR pCP



confines of this criteria. This made it possible that any test solution containing an unknown concentration of pCP could be evaluated as to its percent light transmission and its concentration determined by comparison of these values to the standard. (Table 5, Figure III)

With the establishment of the standard, it was now possible to evaluate the pCP in various vehicles. Since pCP is soluble in cresatin, glycerin, diethylene glycol and eugenol at a 1% concentration, it was possible to set up the same serial dilutions as used in the standard. The problem at hand was whether or not the vehicles would release all of the pCP into the media. Since glycerin and diethylene glycol are readily soluble in water, it was assumed that all the pCP would also be in the aqueous phase. Cresatin and eugenol display very little solubility in water and, therefore, it was doubtful that all the pCP would be released into aqueous phase of the media. The results (Table 6) of the colorimetric evaluation of the supernates of glycerin, diethylene glycol, cresatin and eugenol in a water medium shows that each vehicle behaves differently to the Ferric Chloride Test than does an aqueous pCP solution. In every case a different color complex formed which was due to the inherent light transmission properties of the vehicles per se. In the case of eugenol, a milky cloudiness resulted which was not capable of transmitting any light within the established limits of the standard. However, the light transmission values for the other drugs can be plotted on a graph resulting in a straight line indicating a parallel between the stan-

TABLE 5

PERCENT LIGHT TRANSMISSION FOR 1% AQUEOUS pCP
USING THE FERRIC CHLORIDE TEST
STANDARD at 560 mμ

mg/ 100cc Water	% Light Transmission
5.00	43.9
3.33	61.0
2.50	65.5
2.00	70.4
1.66	74.5
1.42	76.5
1.25	78.0
1.11	79.5
1.00	80.5
0.90	81.3
0.83	82.4
0.76	83.0
0.71	83.6
0.66	84.0
0.63	84.4
0.58	84.9
0.56	85.1
0.52	85.5
0.50	85.7
0.47	86.1
0.45	86.3
0.43	86.5
0.42	86.7
0.40	86.9
0.38	87.0
0.37	87.1
0.35	87.2
0.34	87.3
0.30	87.9

FIGURE III

RELATIONSHIP OF THE LIGHT TRANSMISSION, THE CONCENTRATION AND THE DILUTION FACTORS OF 1% AQUEOUS pCP at 560 mμ

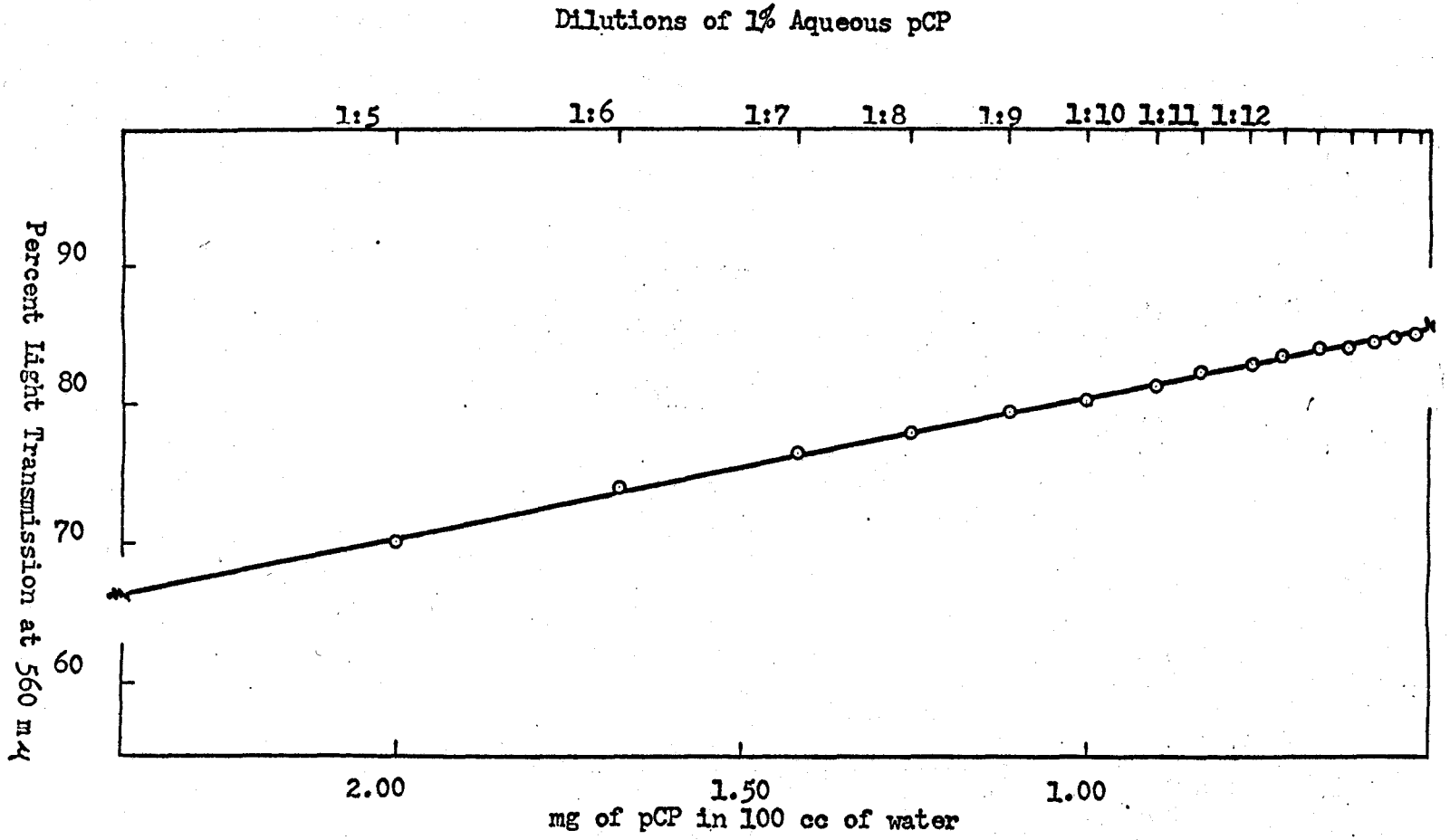


TABLE 6

PERCENT LIGHT TRANSMISSION OF VARIOUS 1% pCP AND VEHICLE COMBINATIONS*

% Light Transmission				mg pCP in the Vehicle	Dilution of Aqueous 1% pCP Equivalent
Glycerin	Diethylene Glycol	Cresatin	Eugenol		
77.5	66.5	93.5	ur	2.50	1:5
81.4	71.8	94.2	ur	2.00	1:6
83.7	74.9	95.3	ur	1.66	1:7
85.2	77.2	95.8	ur	1.44	1:8
87.0	79.5	96.3	ur	1.25	1:9
88.5	81.3	97.0	ur	1.11	1:10
greenish	faintly blue-greenish	yellow	milky	Immediate color	
yellow	colorless	yellow	milky	Color after 15 min.	

*Supernates collected at 72 hours of incubation

ur=unreadable

dard pCP solution and the release of pCP from glycerin, diethylene glycol and cresatin vehicles into the media. Therefore, it is possible to interpret the percent light transmission of these drugs as being quantitative for the pCP concentration, as in the aqueous standard. The results were plotted on a graph, the readings having been made at the same wave length as the one for the standard, showing a straight line in every case. (Figure IV)

A 30% CpCP solution is relatively insoluble if placed in water. Since the pCP in camphor does not completely go into solution, it is impossible to calculate the amount of pCP in the aqueous phase by dilution statistics. The Ferric Chloride Test was used to determine the amount of pCP in the aqueous phase of the CpCP-water mixture.

In a separate study (Table 7) selected concentrations and selected volumnes of CpCP were placed into water,the supernate collected and tested for its percent light transmission after 24,48 and 72 hours. It was found that the amount of pCP released from the vehicle into the media increased through 72 hours. The observation was made that larger volumes of CpCP released pCP much slower than smaller amounts. This would suggest that the exposed surface area of the CpCP to the media is important in the time factor of the quantitative release of pCP. On the basis of these findings, the quantity of CpCP added to the media for the minimal lethal dose endpoint studies was chosen so that the

FIGURE IV

THE RELEASE OF pCP FROM DIFFERENT SOLVENTS USING THE FERRIC CHLORIDE TEST

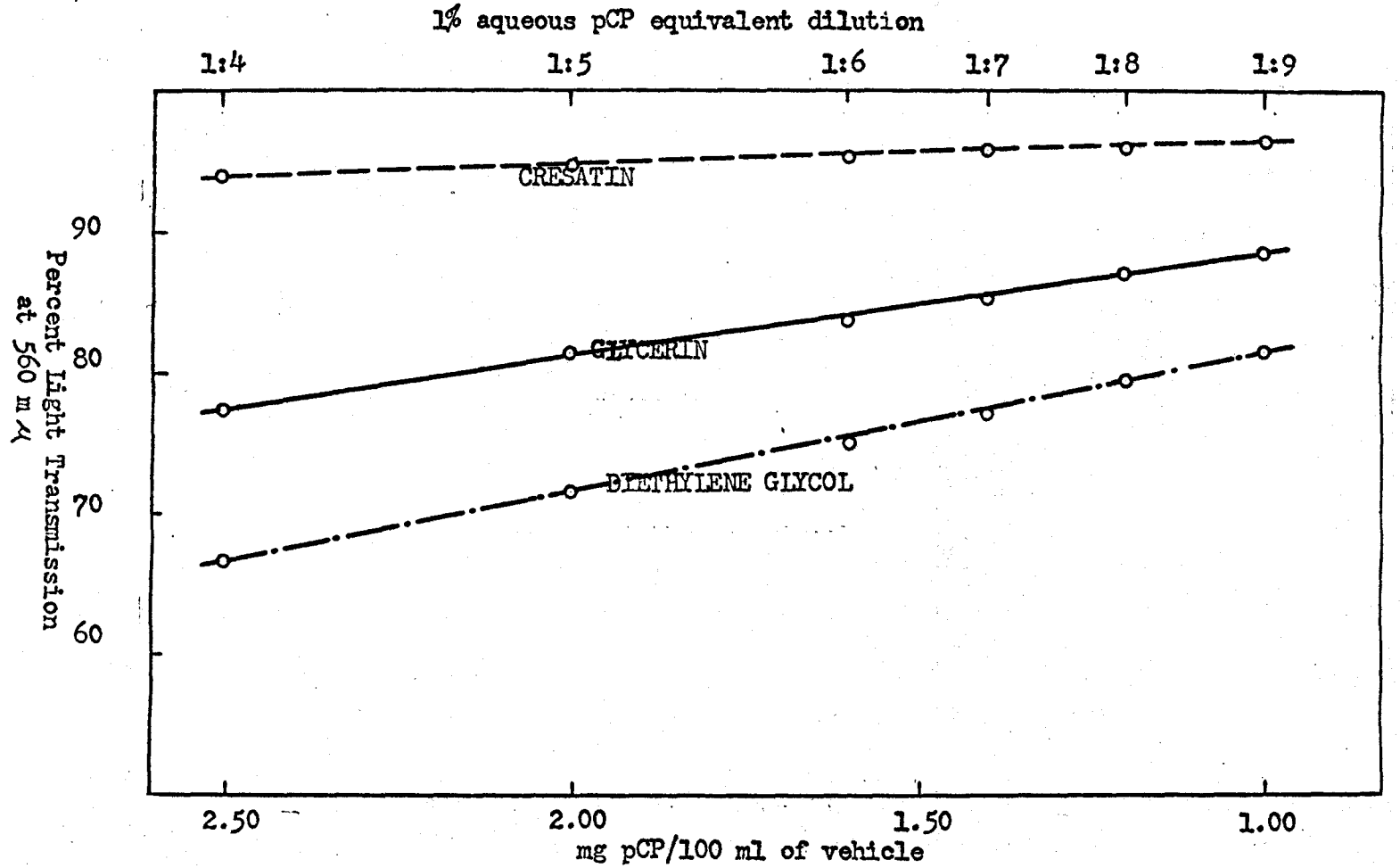


TABLE 7

PERCENT LIGHT TRANSMISSION OF 30% CpCP SUPERNATES AT VARIED TIME INTERVALS

ml of CpCP in 55 ml Media	Percent Light Transmission at 24 hrs.		48 hrs.		72 hrs.	
	%	mg/pCP*	%	mg/pCP*	%	mg/pCP*
1.20	69.5	2.10	68.5	2.20	67.7	2.28
1.10	73.2	1.72	72.5	1.80	71.9	1.88
1.00	75.8	1.48	75.3	1.52	74.9	1.58
0.80	79.2	1.14	78.6	1.20	78.0	1.25
0.70	80.9	1.00	80.2	1.06	79.5	1.11
0.60	81.5	0.98	80.9	0.99	80.5	1.00
0.40	82.3	0.94	81.3	0.98	80.9	0.98
0.35	82.8	0.88	81.7	0.89	81.3	0.90
0.30	83.5	0.73	82.9	0.78	82.4	0.83

*Supernates

aqueous phase would have a pCP concentration comparable to the range of 1% aqueous pCP used. (Table 8)

Phase III

With the information gained through experimental phases I and II, it was now possible to complete the minimal bacteriocidal dose concentration studies, using all of the vehicles and bacterial strains. It was the purpose of this phase to search out the minimal lethal dose concentration for each of the vehicles when combined with 1% pCP, capable of destroying the most resistant strain of micro-organism in the test flora, as compared to the aqueous standard. The results (Table 9) clearly indicate by comparative analysis that 1% pCP in cresatin is the most bacteriocidal combination. One percent (1%) pCP in glycerin, diethylene glycol and eugenol are less effective, with CpCP being approximately as powerful as an antimicrobial agent as the aqueous standard.

One percent (1%) pCP in cresatin and the aqueous standard were employed to test the bacteriocidal effects of these drugs upon actual bacterial samples from the root canals of patients. Both drugs were highly effective and bacteriocidal. Gram stains of the cultures revealed gram positive streptococci of two types, a very small coccus and a larger type. In all three cases the cultures were pure and of only one type. (Table 10) By no means do the results of the above study represent a complete clinical investigation, rather a sampling which may be indicative of what such a clinical study may reveal.

TABLE 8

PERCENT LIGHT TRANSMISSION OF 30% CpCP SUPERNATE COLLECTED AFTER 72 HOURS

ml of CpCP in 55 ml Water	mg pCP in Camphor	1% Aqueous pCP Equivalent**		Supernate pCP mg	%Light Transmission Supernate
		Dilution	mg pCP/ml		
1.15	6.90	1:4	2.50	2.50	65.5
0.90	5.40	1:7	1.42	1.42	76.5
0.80	4.80	1:8	1.25	1.25	78.0
0.70	4.20	1:9*	1.11	1.11	79.5
0.60	3.27	1:10	1.00	1.00	80.5
0.35	2.22	1:11	0.90	0.90	81.3
0.30	1.90	1:12	0.83	0.83	82.4

*Minimal Lethal Dose Endpoint for Streptococcus faecalis, 1% aqueous pCP.

**Concentration of aqueous pCP in an aqueous vehicle were used instead of camphor.

TABLE 9

THE ANTIMICROBIAL ACTIVITY OF PARA-CHLOROPHENOL IN VARIOUS VEHICLES

	Minimal Lethal Dose Endpoint					
Organism	Water 1% pCP	Cresatin 1% pCP	Glycerin 1% pCP	Diethylene Glycol 1% pCP	Eugenol 1% pCP	Camphor 30% pCP
S. faecalis	1:9	1:12	1:6	< 1:2	1:5	1:7
S. aureus	1:23	1:27	1:7	1:9	1:9	1:16
S. mitis	1:20	1:21	1:8	< 1:2	1:7	1:14
S. salivarius	1:22	1:23	1:9	< 1:2	1:7	1:14
N. catarrhalis	1:16	1:18	1:5	< 1:2	1:8	1:15
L. caseii	1:20	1:20	1:8	< 1:2	1:8	1:13
M. tuberculosis	1:14	1:18	1:7	< 1:2	1:6	1:11
C. diptheriae	1:23	1:25	1:7	1:9	1:8	1:12
C. albicans	1:25	1:35	1:9	< 1:2	1:9	1:16
B. subtilis	1:20	1:21	1:6	< 1:2	1:8	1:14
spores	1:18	1:20	1:6	< 1:2	1:6	1:12
L. acidophilis	1:24	1:27	1:8	< 1:2	1:8	1:13

TABLE 10

EFFECTIVENESS OF 1% pCP IN AN AQUEOUS AND IN A CRESATIN VEHICLE TESTED AGAINST RESISTANT BACTERIAL SAMPLES FROM ROOT CANALS

Dilution Range 1:2 to 1:20

Culture I*	Samples: II**	Endpoint III**	Drug	Media Broth
1:9	1:8	1:9	1% pCP in water	BHI
1:18	1:18	1:16	1% pCP in cresatin	BHI

*Vital Canal Sample
**Non-Vital Canal Sample

Phase IV

The zones of inhibition results for both the "mopped" and the seeded plates were so close that they were recorded as being equal. (Table 11) From the data obtained, there appears to be excellent correlation between the zone of inhibition study results and the performance of the various drug combinations in the minimal lethal dose dilution study.

The results show that sterile distilled water, camphor, glycerin and diethylene glycol showed no growth inhibition at all. Cresatin and eugenol showed zones of inhibition.

Several factors may account for these results when compared to the minimal lethal dose findings, due to the particular behavior of these drugs upon agar media. It may be suspected that CpCP and eugenol are very lyophobic which would account for their spreading capacity. Camphor without pCP, although not showing any anti-bacterial activity, showed a "halo" indicative that the drug does spread and diffuse into the agar media. It is also possible that the media is being altered by certain drugs; eugenol is known to be a chelating agent and glycerin has the strong capacity to precipitate protein from aqueous environments. This alteration of the media may change the required basic nutrient availability to the bacteria. It is also possible that the pCP-vehicle combinations chemically act differently, changing their bacteriocidal qualities by either enhancing them or decreasing them.

TABLE 11

ZONE OF INHIBITION PRODUCED BY VEHICLE
and VEHICLE-PARA-CHLOROPHENOL COMBINATION

Organism / pCP Concentration	Water		Cresatin		Diethylene Glycerin		Diethylene Glycol		Eugenol		Camphor	
	0	1%	0	1%	0	1%	0	1%	0	1%	0	30%
S. faecalis	r	ss	vs	vs	r	s	r	ss	vs	vs	r	vs
S. aureus	r	s	vs	vs	r	s	r	s	vs	vs	r	vs
S. mitis	r	s	vs	vs	r	s	r	vs	vs	vs	r	vs
S. salivarius	r	vs	vs	vs	r	vs	r	vs	vs	vs	r	vs
N. catarrhalis	r	vs	vs	vs	r	vs	r	vs	vs	vs	r	vs
L. caseii	r	s	vs	vs	r	s	r	s	vs	vs	r	vs
M. tuberculosis	r	ss	vs	vs	r	s	r	s	vs	vs	r	vs
O. diphtheriae	r	s	vs	vs	r	vs	r	vs	vs	vs	r	vs
B. subtilis	r	s	vs	vs	r	s	r	s	vs	vs	r	vs
spores	r	s	vs	vs	r	s	r	s	vs	vs	r	vs
L. acidophilis	r	s	vs	vs	r	vs	r	vs	vs	vs	r	vs
C. albicans	r	s	vs	vs	r	vs	r	vs	vs	vs	r	vs

CHAPTER V

DISCUSSION

Evaluation of the data obtained in this study revealed that *in vitro* a 1% pCP-cresatin solution was more bacteriocidal than CpCP suggested by Walkoff (1891). Although 30% CpCP is generally accepted and most widely used, as pointed out by LaSala (1963), it appears unnecessary since its toxicity at a 30% concentration is capable of seriously damaging tissue as apparent from studies by Dietz (1957), Schilder and Amsterdam (1959) and Sommer, Ostraander and Crowley (1961). At the same time it has been recognized for many years that cresatin, while not as effective as a bacteriocidal agent as CpCP, however adequate, is remarkably non-irritating to tissues. This fact has been substantiated by Coolidge (1928 and 1929), Grossman (1944 and 1955), Dietz (1957), Sommer, Ostrander and Crowley (1961), Grossman (1965) and Ingle (1965). In lieu of this information it would appear that a less irritating drug, with increased bacteriocidal qualities, such as 1% pCP-cresatin or 1% aqueous pCP, have the potential of becoming the drugs of choice in the treatment of root canals.

When the 1% pCP-cresatin solution was compared with 30% CpCP and 1% aqueous pCP as to its antimicrobial activity, it was determined by the results that the cresatin combination was far superior when compared to CpCP, which was less effective than the aqueous standard. Thus 1% pCP in water or cresatin, *in vitro*, are superior antimicrobial agents.

Although Engelhardt (1927) in his work found that a 2% aqueous pCP solution was as effective as a 2% glycerinated pCP against the spores of "Milzbrand", the findings in this project agree with Paul and Kronig (1897), that the addition of glycerin diminished the bacteriocidal activity of pCP. The same was found to be true when diethylene glycol was used as the vehicle in this study.

When eugenol was used as the vehicle, the results would indicate that it is an inferior bacteriocidal agent. Against the more resistant micro-organisms, even in combination with pCP, eugenol proved to be a very poor antiseptic agent. Its bacteriocidal activity was found to be far below that of cresatin, cresatin and water with pCP and CpCP. Peck (1898), Myers (1927) and Pear (1942) also have found that eugenol was an inadequate antiseptic agent, because it was bacteriostatic only.

Myers and Thienes (1925) and Myers (1927) in their studies found that camphor was completely inactive against pathogenic yeasts. The findings of this research show that this vehicle is totally inactive not only against yeast but all other bacterial strains used in this study as well.

Although much further clinical evaluation is needed, as implied by Coolidge (1928) when he said that both laboratory tests and clinical tests must be made before drawing conclusions as to the merits of any drug, a very small clinical sample of bacterial cultures,

which were unusually resistant, were promptly destroyed by a 1% aqueous and cresatin pCP solution within the range established by the minimal lethal dose standard. This perhaps would point the way to direct clinical research in the direction of further evaluating both of these drug combinations under clinical conditions. It is interesting to note that in the root canal cultures used in this investigation, two cases did not respond to repeated CpCP treatments and one to repeated treatments with microcide. In vitro 1% pCP in water and cresatin was effective. Eklof (1956) also found that micro-organisms may be resistant to CpCP.

In the colorimetric evaluation of the release of pCP from its vehicles, it was found that a direct correlation between the aqueous supernates, including the supernate of CpCP, and those of eugenol, glycerin and diethylene glycol were not possible. A different color complex for the latter two drugs appeared by the addition of $FeCl_3$. In the case of eugenol, it became a milky percipitate. However, the plotted results showed a straight line on a graph indicating some quantitative relationship between pCP release into the media and the color intensity, as was the case in the aqueous dilutions.

Comparison between the minimal lethal dose dilution results and those of the zone of inhibition study showed good correlation. When, however, the results of the minimal lethal dose study were compared to the zones of inhibition by measuring their diameter, no correlation existed. The zone of inhibition test cannot be employed to quantitate

the potency of a drug against micro-organisms by using zone diameters. This would have to be interpreted to mean that zones of inhibition are only a measure of the sensitivity of an organism to a specific drug.

From all of the data collected it becomes apparent that when certain drugs are placed in combination, the resultant compound may become more or less bacteriocidal than either of the two components separated. This has been found to be fact by many early investigators of antiseptics, as Paul and Kronig (1897), Frei and Krupski (1915) and Engelhardt (1927), and so many drug combinations have been advocated over the years, such as the addition of acid to phenol by Laplace (1888), the trituration of camphor with pCP by Walkoff (1891), the combination of water or glycerin with pCP by Engelhardt (1927), the mixture of cresatin with CpCP by Dietz (1957), the compound containing hexachlorophene, thymol, CpCP, and phenacaine HCl tested by Stewart (1962), to mention only several of them, and many others including those which combine antiseptics with antibiotics. Pear (1942) and Sommer, Ostrander and Crowley (1956) set forth their criteria for the ideal root canal dressing. It appears from all of the available experimental and clinical data that 1% pCP-cresatin fits these criteria exceptionally well: it is bacteriocidal <u>in vitro</u> and <u>in vivo</u>; it has an extremely low surface tension with the ability to wet surfaces and penetrate deep; it is volatile but only enough to remain

active for long periods of time; it does not interfere with proper culture technique; and it is non-injurious to tissues.

Although 1% aqueous pCP is almost as effective against microorganisms as 1% pCP in cresatin, the difference being only of academic value perhaps, it does not comply with the criteria of the "ideal" root canal antiseptic as mentioned in the previous paragraph in all categories. The volatility, surface tension and ability to penetrate deeply of the aqueous pCP has not been established, nor has it been evaluated clinically as extensively as cresatin. The antimicrobial activity of 1% aqueous pCP agrees with the findings of Engelhardt (1927), whose 2% aqueous pCP was bacteriocidal. His findings were thought to be due to the fact that pCP in vehicles with high dielectric constants would increase its antiseptic action. The dielectric constant of water is 80 and would fit this category very well. The solubility of pCP in lipid substances is higher than in water; therefore, pCP may be vitually "driven" into tissues and microbes which contain a high degree of these particles, thus becoming susceptible to the actions of this drug. In the face of this, such criteria as volatility and surface tension may lose their importance.

It has long been a problem to Endodontists to remove all of the oily vehicular substances from the root canal before culturing the contents. Such residues frequently result in a negative culture sample, leaving the operator with the impression that the canal is

sterile, when in reality it is not. Since aqueous para-Chlorophenol is readily removed and its concentration is very low, it would be well suited as a root canal antiseptic in this context.

In this study it was discovered that a vehicle such as camphor may act as a reservoir for the active pCP, releasing it slowly into the surrounding environment. (Table 7) The real value of this phenomenon is thought to be the constant and extended release of the bacteriocidal component during its deployment in the field of infection, thus avoiding further bacterial insult to the host. From the findings in this project, it appears clear, however, that time is an important factor in the release of the active portion from the vehicle. Thus, it is conceivable that bacteriocidal levels may not be reached for a period of time during which the infective process continues while the micro-organisms become resistant to the drug employed. Eklof (1956) determined that bacteria have the potential to become resistant to CpCP. In the case of CpCP the pCP was released after 72 hours of incubation. (Table 9) One percent (1%) aqueous or cresatin pCP were more effective against the test bacterial strains of this investigation because their activity may not depend upon the slow release of the active pCP portion. Cresatin alone appears to be a good antiseptic and pCP in water is already prepared in the phase necessary for it to act upon bacteria. There is no waiting period necessary for its release from an oily vehicle into the aqueous phase which will allow it to attack microbes.

Another possible explanation for the less effective bactericidal test results of CpCP as compared to the 1% pCP in an aqueous or a cresatin vehicle may be because the oily camphor coats the cell wall of micro-organisms prohibiting direct contact of the active pCP with the cell wall and its subsequent absorption. Frei and Krupski (1915) defined a good antiseptic as one which is capable of damaging protoplasm as well as altering the permeability of the cell.

It was also observed that large amounts of CpCP would not release the active pCP as readily as smaller amounts. This finding may indicate that the exposed surface area of the CpCP to the environment is important in the release of the active drug portion, pCP.

"Overmedication" of the root canal with subsequent clinical symptoms of discomfort have been reported with the use of CpCP; thus the advise is given to use this drug with the greatest care as it is a highly inflammatory agent (Ingle, 1965). Coolidge (1920) observes that cresatin is an anodyne, non-irritating to living tissue at full strength. This would suggest that cresatin or water in combination with pCP would reduce the potential hazard of "overmedication" while being potentially bacteriocidal at the time of employment.

CHAPTER VI

SUMMARY AND CONCLUSION

A. Summary.

Five chemotherapeutic drug combinations were investigated as to their antimicrobial activity in vitro when tested against a wide spectrum of bacterial agents. The principle of each combination was pCP.

As part of the study the vehicles or solvents were tested without the "active" part, against the same spectrum of microbes.

The minimal lethal concentration serial dilution method was used to determine the minimal lethal dose for each drug necessary to kill the most resistant bacterial strain.

Colorimetric studies were conducted to study the release of the active principle from the vehicles into the media.

A zone of inhibition study was performed to test the antibacterial effects and the resulting zones of inhibition using the vehicles without pCP and using the vehicles with pCP. The results of this study were compared to the minimal lethal dose serial dilution findings.

B. Conclusions.

1. 1% para-Chlorophenol in cresatin has been found to be the most bacteriocidal drug combination. Its minimal lethal dose endpoint for the most resistant organisms was found to be 1:12 as compared to the 1% aqueous minimal lethal dose dilution of 1:9.

2. The most resistant test strain of micro-organism was found to be Streptococcus faecalis.

3. Camphorated para-Chlorophenol was less effective than the equivalent aqueous pCP performance results, whereas 1% pCP in eugenol was even less effective. 1% pCP in glycerin or diethylene glycol showed minimal lethal dose values far below the aqueous standard and had to be rated least effective.

4. Eugenol was shown to be a poor bacteriocidal agent against the spectrum of bacterial strains of this study.

5. Camphor, diethylene glycol and glycerin without pCP showed no antimicrobial activity at all, whereas cresatin without its active principle performed almost as well as the aqueous standard dilutions.

6. There exists a definite relationship for correlative analysis between the results of the minimal lethal dose dilution study and the findings of the zone of inhibition sensitivity scores.

7. Large amounts of CpCP tend to release pCP slower into the media than do smaller amounts. Time is a factor in the release of pCP.

8. Colorimetric test results showed that in aqueous vehicles the concentration of para-Chlorophenol can be established by the addition of $FeCl_3$, which changes the colorless compounds to blue colored complexes, which when placed into the spectrophotometer will give readings of the percent light transmission. These readings, when plotted on a graph result in a straight line. It was determined that

the percent light transmission was directly related to the concentration of pCP in the test solution, i.e. the color intensity of the compound corresponded to the drug concentration.

9. The supernates of the other test vehicles in combination with pCP showed different color complexes as compared to the aqueous standard when the Ferric Chloride Test was applied. For this reason a direct correlation with the aqueous results was not possible, although in the case of cresatin, glycerin and diethylene glycol, a straight line graph resulted when the readings were plotted, indicating some relationship on the aqueous findings.

10. 1% para-Chlorophenol in water is an excellent bacteriocidal agent in vitro. In comparison with 1% pCP in cresatin, the difference in their activity is only academic. The aqueous pCP is easy to prepare and control. Clinical evaluation of this drug is indicated based upon the findings of this project.

CHAPTER VII

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APPROVAL SHEET

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

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

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

May 21, 1969
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