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Acqueous Parachlorophenol: Its Toxicity and Antimicrobial Effectiveness

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ACQUEOUS PARACHLOROPHENOL:
ITS TOXICITY AND ANTIMICROBIAL EFFECTIVENESS

by

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A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science
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1969

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sense of the word, a teacher.

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Curriculum Vitae

John W. Harrison was born in Cedartown, Georgia on November 29, 1934. He graduated from Tuskegee (Alabama) High School in June 1953 and entered the University of Alabama the following September.

Upon receiving a Batchelor of Science degree in 1957, he entered the University of Alabama School of Dentistry and graduated in May 1961 with the degree of Doctor of Dental Medicine.

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CHAPTER I
INTRODUCTION

Parachlorophenol, N.F., is commonly used in endodontic therapy in a thirty-five percent concentration in a vehicle of camphor. This mixture is known as camphorated parachlorophenol\textsuperscript{161}, and is a highly toxic\textsuperscript{120}, though effective\textsuperscript{109,121}, intracanal antimicrobial agent.

Camphorated parachlorophenol enjoys probably the highest popularity\textsuperscript{112} of all the root canal medications with its prime advantages of good clinical effectiveness and ease of handling. The question arises: Is it possible to reduce the toxicity of this drug without greatly diminishing its disinfecting power and effectiveness in root canal therapy?

Two factors are of prime importance in answering this question, the vehicle and the concentration of parachlorophenol.

The antimicrobial activity of camphorated parachlorophenol is due to the parachlorophenol\textsuperscript{162} and the camphor serves merely as a vehicle. The bacterial action of parachlorophenol varies greatly with the solvent. In solvents of low dielectric constant, such as alcohol, acetone, ether, xylene, chloroform, etc., parachlorophenol is alleged to exhibit little antiseptic action; while in solvents of high
dielectric constant, such as glycerin, nitrobenzene and water, it reportedly exhibits marked antimicrobial activity.(163)

In addition to the above consideration, it has never been established that thirty-five percent is the optimum antimicrobial concentration of parachlorophenol. There is for every disinfectant a maximum concentration beyond which, according to the law of diminishing returns, a proportionate increase in antimicrobial effectiveness is not evidenced. The irritating potential of these agents does, however, normally increase with the concentration.

Therefore, as the vehicle camphor possesses toxic qualities(164), it would also seem logical to change the vehicle in an attempt to reduce toxicity. Also, as there is no valid scientific evidence that a thirty-five percent concentration of parachlorophenol is necessary for disinfecting purposes, it would seem logical to test microbiologically a reduced concentration.

An aqueous vehicle presents the opportunity to test a non-toxic vehicle and a greatly reduced concentration of parachlorophenol.

The purposes of this investigation are: (I) To study the toxicity of aqueous parachlorophenol and compare with that of other endodontic antimicrobial agents and (II)
To study the antimicrobial effectiveness of aqueous para-chlorophenol.
CHAPTER II
REVIEW OF THE LITERATURE

The number of root canal medicaments suggested, tested and used in the past century are legion. Soon after the inauguration of the antiseptic era in 1867, by Lister,\(^1\) dentistry adopted his methods for the antimicrobial treatment of root canals in an empiric manner and with little regard to the protection of the periapical tissues. The history of root canal antiseptics and disinfectants is closely related to that of the evolution of the specialty of endodontics and even of dentistry itself.

1830 – 1887

During the early years, arsenic trioxide, phenol and creosote were commonly used in canals as caustics, obtundents and, unwittingly, as antiseptics.\(^2\) Arsenic was the most popular agent used and had been suggested for the purpose of destroying the pulp by Spooner in 1836.\(^3\)

The first recorded root canal filling took place "about 1830"\(^4\) when Dr. Edward Hudson of Philadelphia filled the canals of two maxillary incisors. Other records indicate that he had been filling canals prior to 1830, perhaps as early as 1809.\(^5\) But, as a rule, no effort was made during this period to remove the pulps or to fill
the canals, and restorations were placed over necrotic pulps with little regard to the consequences.

In addition to devitalization of the pulp with caustic drugs, some pulps were "knocked out" by driving wooden points into the root canals. A single blow crushed the pulp tissue and one can only speculate at the number of fractured roots which must have resulted from this practice.

Also in vogue during this period was the practice of stuffing the root canals with cotton saturated with creosote following pulp devitalization. The purpose of the creosote was to serve as a preservative. White\(^6\) in 1864 stated that this method has been used for "at least twenty-five years" and the creosoted cotton was packed in the canal and left as a root filling.

In 1857, Watt\(^7\) recommended filling root canals with gutta percha, and more and more practitioners began to condemn the practice of leaving the pulp in the canal to "disintegrate and rot, proving a source of much offense."\(^8\)

The first warning of possible ill-effects which may be caused by caustic drugs in the canal was issued by McMurtrie\(^9\) in 1861. Six years later, McLain\(^10\) criticized the use of creosoted cotton for filling canals and
was the first to suggest the possibility of using antiseptics in the canal, stating that "should they do no good, their presence could not possibly exert any bad influence." He recommended a grain of iodine dissolved in half an ounce of chloroform, to which is added a drachm of creosote. This is the first mention in the literature of the use of an antiseptic for antiseptic purposes in the canal.

After 1870, attempts to save teeth with necrotic pulps became common practice. Many operators used gold wires or gold foil for filling the canal but, as the years passed, gutta percha came more and more into general use,\(^{(11)}\) as did the rubber dam which was introduced in 1864 by Barnum.\(^{(12)}\)

The period from 1870 to 1887 was a period of trial and error and, except for the work of McQuillen\(^{(13)}\), Rich\(^{(14)}\) and Raymond\(^{(15)}\) in anesthesia, produced little of interest in endodontics.

\[1888\text{ - }1909\]

The most exciting and stimulating period in the history of root canal therapy, and likewise dentistry per se, began with the publication of an article by W. D. Miller in 1888.\(^{(16)}\) This epoch-making paper was the first to call...
attention to the presence and importance of microorganisms in root canals. Long before William Hunter aroused the laity and the medical and dental professions, Miller stated: "...each gangrenous tooth pulp may be in itself a center of infection, or it may serve as a channel through which pathogenic bacteria from the oral cavity may invade the tissue surrounding the point of the root, or even obtain entrance into the circulation."

In 1890, Miller presented a second paper(17) which stressed the importance of the decomposition of the pulp as an irritating factor in the treatment of pulpless teeth.

These two papers were followed by a third important treatise in 1891 by G. V. Black(18) in which he pointed out the difference between "infectious matter" and "septic matter." He emphasized that the products of pulpal putrefaction or decomposition, even though containing no microorganisms, may be poisonous to the periapical tissues.

These three papers initiated the slow change in dentistry from a technical to a scientific profession, and provided a biological foundation for the treatment of root canals. The importance of removing microorganisms and pulpal remnants from the canal was clearly stated, but, as is the case with most changes, almost a quarter of a
century passed before the profession as a whole recognized and accepted these facts.\(^{(19)}\)

The papers of Miller and Black initiated the use of antiseptics in root canals. It was the beginning of a parade of intracanal medicaments which has lasted some eighty years and continues today.

In 1889, Black\(^{(20)}\) tested the effectiveness of certain antiseptics and found many of the essential oils to be worthless against salivary microorganisms. He also demonstrated the greatly diminished antimicrobial activity of these antiseptics in the presence of albumen. Oil of cassia and eucalyptol were recommended for root canal therapy.

Dr. A. W. Harlan\(^{(21)}\) in 1891 argued against the current practice of disinfecting canals with phenol, zinc chloride, sulfuric acid or cresote. He reasoned that these agents coagulate protein and are therefore self-limiting. Harlan thus was the first to advocate the use of a diffusible root canal antiseptic and suggested the possible use of potassium permanganate, chlorinated soda, sodium fluosilicate and certain essential oils.

During this period, little or no thought was given to the toxic effect on the periapical tissues of agents sealed
in the canals. In 1892, a mixture of iodoform, oil of cinnamon and finely powdered, ground coffee was suggested and, a year later, Dr. Emil Schreier\(^{(23)}\) of Vienna proposed the use of metallic potassium and sodium "to convert the septic contents of the canal into an aseptic condition ..." The pryotechnic display following the insertion of metallic sodium and potassium into the moist canal undoubtedly created some anxious moments for those subscribing to Schreier's therapy.

Cassidy\(^{(24)}\) suggested the intracanal use of formalin in 1894 and Wooley\(^{(25)}\) in 1898 advocated "the most powerful antiseptic agents we can find." Pfeifer\(^{(26)}\) also in 1898, re-emphasized the importance of thorough debridement, of protection of the tooth with a rubber dam and of the use of a diffusible antiseptic such as Blacks 1-2-3 (oil of cassia, phenol and oil of gaultheria), eucalyptol or oil of cassia. If these drugs failed, phenol or zinc chloride were recommended.

It was in 1898 that Dr. A. H. Peck\(^{(28,29)}\) reported on the first scientific series of experiments on the irritating potential of antiseptics. Cotton pellets, saturated with the test solution, were applied to the skin of the forearm with a rubber cup, held by adhesive tape, to
prevent evaporation. On the basis of his experiments, Peck recommended for canal disinfection the use of beechwood creosote, oil of cloves, oil of bay, Blacks 1-2-3, and eucalyptol. He rejected the intracanal use of oil of cassia (too toxic), oil of peppermint (odor objectionable), 95% phenol (not a "permanent" antiseptic), oil of myrtle (too toxic), oil of gaultheria (not antiseptic), eugenol (not antiseptic), formalin (too toxic) and oil of cajeput (not antiseptic.)

Following Peck's report, G. V. Black(30) pleaded with the profession to choose their antiseptics "...not with relation to their power as a poison to microbes entirely, but...(with) especial reference to their action upon the animal tissue to which they are applied". The importance of Peck's work lies in its initiation of the profession's concern with the toxic properties of root canal antiseptics. Two decades later, these tests were confirmed by Willard at Northwestern University.(31)

At the turn of the century, other events occurred which were to have profound effects on the future of root canal therapy.

Speaking before the New York Institute of Stomatology in 1901, Dr. T. W. Onderdonk(32) proposed "having a culture
made from the cotton dressing...and with the aid of the bacteriologist, know when we have secured an aseptic root." This was the first suggestion that root canals be cultured.

The secrets hidden below the gums were revealed for the first time when Dr. Otto Walkoff of Munich first used the X-ray for dental purposes two weeks after its discovery in 1895. In the United States, the roentgen ray was employed in root canal therapy in 1896 by C. Edmund Kells and others soon followed his example. This new and important diagnostic aid came into general use shortly before 1910.

The first paper in English on the use of Novocaine as a local anesthetic was published by Part in 1906, a year after its discovery. Like the X-ray, it also came into general use about 1910.

And so it was that in the year 1910, dentistry was slowly progressing, blissfully unaware of the explosion which was shortly to take place. Caustic drugs were being sealed in root canals despite the warnings of Peck, Black and others. The heavy reliance placed on the use of powerful and highly toxic intracanal medications, combined with the fear with which many dentists approached instrumentation of the canals, lead to countless failures and prolonged, painful treatments. Because the canals were not properly debrided and because the increasing use of
X-rays began to reveal the existence of periapical radiolucencies, the use of more powerful and penetrating antisepsics seemed logical. This overreliance on powerful drugs complicated an already complicated therapy. It is surprising, when one considers the handicaps under which the early practitioners worked, that endodontics was successful in maintaining any degree of popularity. Anesthesia and the roentgen ray were still in their infancy; root canal instruments were not readily available; pulpal and periapical pathoses were poorly understood and the intricate anatomy of the root canals had not been investigated.

Unfortunately, with the advent of the roentgen ray and the local anesthetic came the theory of focal infection.

1910-1939

The event which rocked the dental profession to its very foundations, and placed the field of endodontics in near suspended animation for almost thirty years, took place in Montreal, Canada, on October 3, 1910.

On that date, Dr. William Hunter, a physician, blamed oral foci of infections as the etiological factor in many systemic disorders. This was soon followed by extensive laboratory and clinical studies; Frank Billings of Chicago, who investigated the relationship between the
diseases of rheumatism and arthritis and the infections of the teeth and tonsils. An associate of Billings, Dr. E. C. Rosenow, gave further impetus to the theory by his observations. (37)

Almost immediately, the theory was grasped by the medical profession in the hope that many obscure conditions confronting them might be relieved by removal of localized areas of oral infection. The dental profession, being anxious to cooperate in combating disease, joined the crusade, (38) and the clinical application of the theory of focal infection began with the wholesale extraction of "infected" teeth.

With few exceptions, (39-45) the dental and medical practitioners insisted upon the immediate extraction of all pulpless teeth. Oral infections were routinely condemned in the literature as the cause of almost every systemic disorder of doubtful origin. (46-51)

Nodine (52) in 1919, listed those systemic disorders which could be caused by oral infections. They included anemia, hysteria, epilepsy, leukemia, nephritis, lichen planus, arthritis, herpes zoster, melancholia and scores of others.
It remained for research in histology and pathology\(^{(53-56)}\) and in microbiology\(^{(57,58)}\) to place the focal infection theory in its proper perspective; an event which Ingle\(^{(59)}\) terms one of the three greatest improvements in the history of endodontics.

Along with the improvement of the X-ray and local anesthesia, came the first comprehensive studies of the anatomy of root canals by Guido Fischer,\(^{(60)}\) Callahan,\(^{(61)}\) and Hess.\(^{(62)}\)

Abraham,\(^{(63)}\) in 1915, introduced Rhizophor, the fibers of the Piassa Palm impregnated with zinc chloride, creosote, thymol and formaldehyde, to be used as both a filling material and "in pellet form to sterilize the roots of difficult access." Bauchwitz,\(^{(64)}\) in the same year, recommended that a mixture of benzoic acid, boric acid and iodoform be heated over an alcohol flame and the ascending vapor guided into the root canal.

Electrosterilization, using a one percent sodium chloride solution, was introduced in 1917 as a method for obtaining canal sterility.\(^{(65)}\)

Cullen and Taylor\(^{(66)}\) demonstrated the irritating potential of Dakins Solution, but found two other commonly used root canal antiseptics, dichloramine-T and chloramine-T, to be less toxic.
Prinz, (27) in 1919, listed those drugs and drug compounds which had been recommended and used in root canal therapy. They included: creosote, parachlorophenol, camphorated phenol, lysol cresol, creolin, beta-naphthol, salicylic acid, hydrogen peroxide, zinc chloride, thymol, eugenol, eucalyptol, Black's 1-2-3, sodium dioxide, formaldehyde and sodium chloride in conjunction with electro sterilization. He also discussed in detail the merits and deficiencies of the more commonly used intracanal antisepsics such as phenol, silver nitrate, mercuric chloride, iodoform, iodine solutions, the essential oils and dichloramine-T.

The "sight and smell" method of determining when to fill a root canal continued to be used until 1919, when LaRoche (67) and Coolidge (68) elevated the treatment to a scientific level by employing microbiological tests, as suggested by Onderdonk, (32) to determine the sterility of pulpless teeth.

Price, (69) in 1923, reported on the results of antimicrobial and toxicity studies of certain root canal medications and stated that only phenol, an iodine-creosote solution, formalin, formocresol and dichloramine-T consistently gave negative cultures, with the latter being the
most effective, but too toxic for use in the canal. Among the drugs tested, but failing to give satisfactory results, were parachlorophenol, iodine and sulfuric acid.

In 1924, the L.D. Caulk Company introduced Osogen\textsuperscript(R)(70). The liquid component of this powder-liquid mixture was used separately as an antiseptic and contained potassium iodohydrargyrate which was hailed as the "most powerful inorganic germicide known." It is interesting to note, however, that the instructions for its use recommended flooding the canal for three or four minutes with phenol prior to placing the Osogen liquid in the canal.

Dr. J. R. Blayney in 1924,\textsuperscript(71) and again in 1926, and 1928,\textsuperscript(72) admonished the dental profession not to ask nature to resist chemical necrosis in addition to infection. He emphasized the dangers of using caustic drugs in canals, of forcing these drugs beyond the apex and of failing to completely remove all organic debris and softened dentin.

In 1929, Coolidge\textsuperscript(73) stated that drugs which coagulate protein have only a shallow penetration and are not qualified for the treatment of infected canals, and that low surface tension is of value only if the germicide doesn't precipitate protein. He also warned of the consequences of using caustic drugs in the canals and used
laboratory animals to test for tissue toxicity.\(^{(74)}\) Eugenol, eucalyptol, phenol, formalin, formocresol and chloramine-T were found to be particularly toxic.


The proponents of electrosterilization received support from a detailed and meticulous study by Grossman and Appleton,\(^{(76)}\) which presented evidence that more than twice the antibacterial effect was obtained from an electrolyzed chemical solution than from the same solution without electrolysis. A zinc-iodide-iodine solution, the best of some fifty solutions tested, was recommended with a thirty milliampere-minute dosage for canal sterilization.

Werther\(^{(77)}\) and Grossman and Prinz,\(^{(78)}\) in 1932, compared the clinical effectiveness of camphorated para-chlorophenol and electrosterilization. The former ranked a poor second, requiring twice as many appointments to
obtain a negative culture.

Prinz' and Rickert's text, (79) published in 1938, discussed in great detail the antimicrobial and toxic properties of the intracanal medicaments.

Despite all the warnings, a large segment of the dental profession in 1939, still did not consider it necessary to treat the periapex with the care and delicacy recommended. Evidence of this is seen in the first article suggesting the use of a sulfa drug for endodontic therapy. (80) Instructions called for dissolving sulfanilamide in hot water, and when the solution begins boiling it is drawn into a syringe and forced into the canal with sufficient force so that it emerges through the fistula. The dentist was to place a finger over the fistula for the first few seconds to allow pressure to build up. If the affected tooth had no fistulous tract, it was suggested that one be surgically created. The author did not, however, have a total lack of concern for tissue irritation as evidenced by his warning to those dentists with sensitive hands:

"If the hand is particularly sensitive to heat, a rubber glove can be worn...and when (the solution) emerges from the fistula, it will be hot enough to cause the patient discomfort and even mild burns could be produced except
for the fact that the assistant will keep a stream of cold water flowing over the opening of the fistula."

There is little argument that the period between 1910 and 1939 produced tremendous progress in most facets of dentistry, including root canal therapy. Putting this progress into practice was still many years in the future, as the dominant factor in the treatment of pulpless teeth remained the theory of focal infection.

1940 - 1963

As opposed to its quick inception following Hunter's address, the theory of focal infection died a gradual death over many years. Endodontics likewise developed gradually as it threw off the shackles of this theory. The knowledge gained by those who continued through the years to practice and do research in the treatment of pulpless teeth began to be used by more and more of the profession.

During the period from 1940 to 1963, when so many advances were made in endodontic instruments, equipment, diagnostic procedures and therapeutic techniques, it is surprising that no new non-specific root canal medication was introduced, although new combinations of old germicides were suggested. Indeed, Ingle states that very
little true progress has been made in sterilization of the infected root canal since Walkoff recommended parachlorophenol in 1891.

Following Adam's\(^{(80)}\) suggestion of the use of sulfa drugs in root canals, the next two decades found much of the endodontic literature devoted to the use of sulfa drugs\(^{(85-91)}\) and antibiotics.\(^{(91-102)}\)

In 1942, Pear\(^{(103)}\) at the University of Detroit experimented with the antimicrobial effectiveness of the vapors of certain root canal antiseptics and concluded that only Methiolate, formocresol and camphorated parachlorophenol possessed effective vapors.

Morse and Yates\(^{(104)}\) reported the isolation of a significant number of anaerobic microorganisms from teeth undergoing root canal therapy. The importance of the existence of anaerobes was later emphasized by Cran\(^{(105)}\) and Blechman.\(^{(106)}\)

Grossman\(^{(107)}\) in 1944, used Peck's\(^{(28)}\) method of forearm skin testing and found beechwood creosote and formocresol to be far more irritating than camphorated parachlorophenol and cresatin. The following year, the same author clinically tested camphorated parachlorophenol, azochloramide, beechwood creosote and a 15% sulfadiazine solution.
The latter solution required a greater number of treatments to obtain two consecutive negative cultures.\(^{(86)}\)

In 1947, Casey, Gurney and Rapp\(^{(88)}\) introduced a sulfonamide, para-aminotoluene sulfondiethylamide, for use as an intracanal medication. Gurney and co-workers\(^{(89)}\) later claimed that the parent compound, para-aminotoluene sulfonamide (Benzylog) performed even better than its derivative and, unlike other sulfonamides, was highly active in presence of para-aminobenzoic acid. They reported a wide antimicrobial spectrum and extremely low tissue toxicity. A paper point impregnated with the para-aminotoluene sulfonamide was inserted into the canals and moistened with an isotonic solution of the same drug. Gurney and Best\(^{(90)}\) reported on the addition of an antifungal agent to Benzylog and called the preparation Endo-cide.\(^{(R)}\) This was eventually to be known as Microcide\(^{(R)}\) and is one of the most non-toxic agents used in current endodontic therapy.

Following World War II, Penicillin became available for dental research and in early 1947, Bender\(^{(101)}\) reported that radiographic healing appears to occur faster with penicillin therapy because of its non-irritating effect, but warned against looking upon the antibiotic as a panacea for all endodontic problems. Buchbinder\(^{(100)}\) noted a lack of
irritation even when freely pumping penicillin past the apex, and reported no irritation with eugenol or penicillin using a modified forearm skin test.

In 1948, Stewart (108) determined the approximate volumes of medication that can be sealed into prepared canals. This was of particular interest in determining the number of units of an antibiotic which could be sealed in a canal.

After extensive testing of penicillin, Ostrander, Crowley and Dawson (109) concluded it was not an adequate root canal antiseptic, and studies from the University of Michigan (110) and the University of Pennsylvania (94) demonstrate the isolation of resistant strains of microorganisms, particularly streptococci, from the root canals.

Realizing that the intracanal use of penicillin alone was inadequate, Seltzer and Bender (102) tested antifungal agents; Grossman and Stewart (95) tested a penicillin-streptomycin suspension and in 1951 a combination of antibiotics (Penicillin, Streptomycin and bacitracin with the antifungicide sodium caprylate) was proposed by Grossman. A clinical study (99) endorsed the antimicrobial effectiveness of this polyantibiotic paste which was given the name PBSC. Although many other antibiotic combinations were suggested (113, 119, 122, 143) PBSC was destined to become
of the two most popular intracanal medications of modern times.\(^{(112)}\)

Hedman's\(^{(53)}\) classic investigation into residual periapical infection following root canal therapy proved that elimination of demonstrable microorganisms from the canal, as evidenced by two successive negative cultures, leads to resolution of the infection of the periapical tissues. Auerbach\(^{(114)}\) reported in 1953 that seventy-eight percent of infected root canals would yield negative cultures following mechanical debridement and irrigation with sodium hypochlorite and warned the profession against overreliance on antibiotic therapy. Two years later, this study was confirmed by Stewart\(^{(115)}\) who used hydrogen peroxide and sodium hypochlorite as irrigants. Zeldow and Ingle,\(^{(116)}\) however, obtained only 4.6 negative cultures after mechanical instrumentation and sterile water irrigation and attributed the results of Auerbach and of Stewart to the antibacterial action of the irrigating solutions.

The reports of resistant strains of microorganisms\(^{(94, 109, 110)}\) were only the first of the problems encountered by the advocates of antibiotics and in 1954, it was demonstrated that the frequency of false negative cultures with intracanal antibiotics is as high as thirty-one percent unless incubated for a full week.\(^{(117)}\) This anti-
biotic interference with culturing had been described in two earlier studies.\textsuperscript{(108, 118)}

In 1957, Victor Dietz\textsuperscript{(81)} suggested a combination of parachlorophenol (25 grams), camphor (50 grams) and meta-cresylacetate (25 grams) which he hailed as the "universal" endodontic medicament and originally labeled XP-7. He failed to show any antimicrobial advantage to this compound but did report the remarkable irritational qualities of XP-7 and camphorated parachlorophenol, both causing forearm ulcerations lasting one month and residual scars visible after two years. This agent is simply a combination of camphorated parachlorophenol and cresatin\textsuperscript{(R)}, and is marketed under the trade name Cresanol\textsuperscript{(R)}.

In 1958, Rubbo, Reich and Dixson\textsuperscript{(119)} noted that camphorated parachlorophenol, beechwood creosote and formocresol produced severe necrosis and ulceration following subcutaneous injection into laboratory animals. This was confirmed by Schilder and Amsterdam\textsuperscript{(120)} who also showed a high inflammatory potential following the intradermal injection of polyantibiotic paste (PBSC).

Studies comparing the clinical effectiveness of PBSC\textsuperscript{(121)} and other polyantibiotic combinations\textsuperscript{(122)} with camphorated parachlorophenol indicate no statistically significant differences.
Sargenti and Richter in 1959 suggested to the American dental profession the use of N2 as a filling material and as a root canal disinfectant. N2 Medical, the disinfectant, was claimed to be so powerful that neither cultures nor rubber dam protection was necessary during treatment. Though enjoying great popularity in Europe, it has not found favor in the United States. Ehrmann,(123) Guttuso(124) and others,(125) including the American Dental Association's Council on Dental Therapeutics,(126) warned of the dangers of using this highly toxic paraformaldehyde preparation.

Healy(127) sanctioned the use of a drug rotation program during endodontic therapy employing cresol, followed by beechwood creosote and, finally, camphorated parachlorophenol.

In 1959, Winkler and van Amerongen(128) reported the results of the most extensive study to date on the frequency of specific microorganisms infecting vital and necrotic pulp tissue. Streptococci were found to compose some sixty-three percent of the microorganisms in infected canals, with Streptococcus faecalis being the most frequently isolated microorganism in pure culture. This predominance of streptococci had been earlier reported by Ostrander and Crowley.(111)
Sciackny and Sultzenu(129) pointed out the presence of anaerobic microorganisms in ten percent of the infected canals and agreed with those who reported the high frequency of streptococci present. Crawford and Shankle(130) were able to demonstrate the presence of intracanal microorganisms histologically which could not be detected by culturing. A comparison of oral flora with root canal flora revealed a marked restriction of some oral forms. Beta hemolytic streptococci and staphylococcus aureus were conspicuous by their absence from infected canals. The absence of these common pathogens in canals had been reported earlier by Hobson. (131)

Grossman(132) clinically tested an antibiotic, Kanamycin sulfate, in combination with an antifungal agent, Nifuroxine, and suggested that further studies were warranted.

Calvin Torneck(133) surgically inserted polyethylene tubing containing various root canal medications into the connective tissue of hamsters. He observed histologically the presence of necrosis associated with formocresol, phenol, beechwood creosote, eugenol and PBSC. A less severe reaction resulted with camphorated parachlorophenol and cresatin.
1963 - 1968

It was in 1963 that endodontics reached the long-sought plateau of specialty status, as conferred by the American Dental Association. This destroyed the last vestiges of resistance by the few who still clung to the theory of focal infection and was the crowning achievement of twenty years of organizational efforts by the American Association of Endodontists.

It is perhaps paradoxical that in this same year, there appeared the first significant report questioning the time-honored, scientific principle of culturing root canals. Seltzer, Bender and Turkenkopf (134) at the University of Pennsylvania reported no significant difference in successful repair between teeth presenting a negative culture and those presenting a positive culture just prior to filling the canals. The following year, the same authors (135) bluntly challenged the accepted principles of never filling a canal which demonstrates the presence of microorganisms. Repudiating the conclusions of Buchbinder, (136) Zeldow and Ingle, (137) Oliet (138) and other protagonists of the culture concept, they raised the question of a change in bacteriologic status of the canal between the culture appointment and the filling appointment.
They observed that sixteen percent of the teeth which yielded a negative culture on the previous visit, yielded positive cultures immediately prior to filling the canal. Seltzer, et al. (139) confirmed this clinical study with a histologic evaluation of the repair of the periapical tissues of laboratory animals.

If one accepts these findings, and considers the high incidence of false negative cultures, serious doubts are indeed raised as to the validity of the conclusions drawn from other studies showing the importance of the negative root canal culture.

Also in 1963, a combination of penicillin and streptomycin with iontophoresis was suggested (140) for canal sterilization and, in Sweden, the effectiveness of these same antibiotics with the addition of a quarternary ammonium compound was tested. (141)

Uchin and Parris (142) tested the antimicrobial activity of four commonly used canal medications after varying time intervals sealed in the canal and concluded that PBSC maintained a greater effectiveness over a longer period than cresatin, camphorated parachlorophenol or microcide.

In 1964, Theilade and Schiott (143) of Denmark found a five percent incidence of yeast in two thousand cultures. In sixty-seven teeth treated with a combination of neomycin
and bacitracin, yeast occurred in thirty-seven percent of the cultures. They concluded, therefore, that intracanal treatment with certain antibiotic combinations greatly increase the incidence of yeast.

Shovelton, (144) investigating the occurrence and distribution of bacteria in infected, nonvital teeth, reported their presence in the dentinal tubules of seventy-nine of ninety-seven teeth studied by serial sectioning. The deepest penetration was halfway through the thickness of the dentin and in no section were bacteria seen to reach the cementum.

A clinical study at Tufts University (145) found no significant advantage in sealing camphorated parachlorophenol in the canals of asymptomatic nonvital teeth.

Chlorhexidine and Cetrimide were recommended for root canal therapy by Hampson and Atkinson (146) who hailed their antiseptic and detergent properties as well as lack of toxicity.

In 1965, Gurney, et al, (147) reported on the screening of endodontic antifungal agents and the selection of 5-nitro-2-methyl-furfuryl ether as the best antifungal agent for intracanal use with para-aminotoluene sulfonamide (Microcide(R)). For necrotic pulps, Vasilesucu and
Ionescu (148) proposed a combination of neomycin, erythromycin and griseofulvin.

Grossman (149, 150) demonstrated the residual antimicrobial effect of camphorated parachlorophenol after ten days in the canal. He concluded that an inactivator must be used to prevent a false negative culture.

Engstrom and Lundeberg (151) in 1966, disagreed with Seltzer, Bender and coworkers (134, 135) and reported a significantly higher success rate after four years with teeth which yielded negative cultures at the time of obturation. In a second study, these authors reported that sixteen percent of two hundred thirty-six teeth tested gave a positive test for microorganisms following two consecutive negative tests, thus agreeing in fact, if not in principle with Bender, et al. They supported the view of Amerongen who in 1957 stated that the risk of a positive test in endodontic therapy is the same whether proceeded by one or two negative tests. There is, they conclude, no necessity for two negative tests prior to filling the canals (152).

Reviewing the status of microbiological knowledge as applied in current endodontic therapy, Hobson (153) and Butler (154) were appalled by the lack of utilization of available information and termed the presently used procedures primitive, inadequate and, worse, misleading.
In 1966, benzylkonium chloride\(^{(155)}\) and hot chloramine-T\(^{(156)}\) were suggested for canal sterilization. The following year Spanberg and Engstrom\(^{(157)}\) tested the cytotoxic effect of four intracanal medications on HeLa cells and reported all (tricresol-formalin, zinc iodide-iodine, Biosept\((R)\) and eugenol) were more toxic than phenol. Grossman\(^{(158)}\) also reported a severe inflammatory response with necrosis associated with the subcutaneous injection of camphorated parachlorophenol in hamsters.

In 1967, Fox and Isenberg\(^{(159)}\) found there was no single antibiotic of the twelve tested to which one or more microorganisms isolated from root canals were not resistant to one or more of the antibiotics.

Frank and co-workers\(^{(160)}\) reported a significant interappointment reduction in pain by using a sulfathiazole paste in the canals following opening and biomechanical cleansing. Although the purpose of this paste is only to reduce discomfort, its inherent antimicrobial properties require its mention in this review.

1969

The present status of root canal chemotherapy is not unlike that which existed at the turn of the century. The only significant difference which emerges from an objective review of root canal chemotherapy since
Joseph Lister's time is that most toxic drugs which were not good antiseptics have been reluctantly discarded while those toxic drugs which are good antiseptics remain in general use.

The oldest and perhaps most popular\(^{\text{(112)}}\) of the root canal antiseptic agents is camphorated parachlorophenol. Its chief advantages are in its clinical effectiveness and ease of use, while its major disadvantage is its high degree of toxicity. It is surprising to note that not one study could be found in the dental literature which reports the antimicrobial spectrum of camphorated parachlorophenol. Likewise, the effective concentration of this drug has not been determined against the various microorganisms most frequently inhabiting the infected root canal. It is the combination of these factors, the known and the unknown, which prompted the present study.
CHAPTER III
METHODS AND MATERIALS

A. Toxicity

A comparison of the toxic properties of aqueous para-chlorophenol and currently used endodontic intracanal medications was studied by the conjunctival inflammatory test and by intradermal injection into the abdomen of rabbits.

1. Conjunctival Inflammatory Response

Eighteen male, white rabbits weighing two to three kilograms each were obtained from a commercial laboratory and housed in individual wire cages.

The test solutions selected were Microcide A*; Cresatin**; 1½% aqueous parachlorophenol (PCP); 2½% PCP; camphorated parachlorophenol, N.F.; and eugenol ***. Physiologic saline was used as a control.

At the time of experimentation, each animal was placed in a hooded box which allowed only the head to protrude. 0.15 ml. of physiologic saline was drawn into a tuberculin syringe, and with the head of the animal turned to the appropriate side, the saline was carefully

*Novol Chemical Mfg. Co., Inc.; Brooklyn, N.Y.
**Acros, Sharp and Dohme; West Point, Pa.
dropped from about one inch into the cornea of the left eye. The head of the animal was then turned to the opposite side and 0.15 ml. of one of the six test solutions was similarly dropped into the right eye. Both the left and right eye were gently held shut for approximately one minute after exposure to the solution to insure its retention.

The left eye of each animal was used as a control and the right eye received the test solution. Each of the six solutions were tested on three animals.

Gross observations and, in most instances color photographs, were made prior to the application of the solutions, and after five minutes, five hours, twenty-four hours and ninety-six hours. The presence and degree of inflammatory response was recorded using the following criteria: hyperemia of the conjunctiva, exudation, edematous swelling of the conjunctiva, clouding of the cornea with loss of clarity of the anatomic striations of the iris and external swelling of the eye.

Results were recorded using the following descriptive terminology:

Very mild - very slight hyperemia (only).
Mild - mild to moderate hyperemia, slight exudation.
Moderate - moderate to severe hyperemia, mild to moderate edematosus swelling, cloudiness of cornea with some loss of clarity of iris striations, exudation.

Moderate to Severe - severe hyperemia, moderate swelling of conjunctiva, marked corneal clouding, exudation.

Severe - all criteria severe, external swelling.

2. Dermal Connective Tissue Inflammatory Response

Fourteen male rabbits of the type described in the previous study were used in this experiment. In addition to the six test solutions listed above, a five percent sodium hypochlorite solution was tested. Physiologic saline was used as a control.

Each animal was anesthetized by injecting fifty mg of sodium pentobarbital into an ear vein. The animals were then shaved with an electric razor along two three-inch wide strips, one on each side of the midline of the abdomen and extending from foreleg to hindleg. A depilatory was used for removal of hair remnants.

*Zonite; Chemway Corp.; Wayne, N.J.
**Nembutal; Abbot Laboratories; North Chicago, Ill.
***Nair; Carter Products; New York, N.Y.
Six injection sites, three on each side, were selected on each animal and marked with ink. One of the six sites on each animal was used as a control site. In the five test sites on each animal, 0.1 ml of a different test solution was injected intradermally using sterile tuberculin syringes with 27 gauge needles. In the control site, 0.1 ml of physiologic saline was injected intradermally. The presence of a wheal at the injection site indicated intradermal deposition of the solutions.

The animals were sacrificed by intravenous injection of an overdose of sodium pentobarbital after twenty-four or seventy-two hour intervals. Biopsies of the eighty-two injection sites (two sites were not used) were immediately placed into separate coded containers, each with a minimum of twenty volumes of 10% formalin. Care was taken to include normal tissue as well as the ink-marked injection site in each biopsy.

A minimum of five biopsies, each from a different animal, were obtained for each test solution in the twenty-four hour interval. A minimum of three biopsies, each from a different animal, were obtained for each test solution in the seventy-two hour interval.
Following a fixation period of approximately seven days, the biopsies were dehydrated and cleared with alcohol and xylol, embedded in paraffin, sectioned and stained with hematoxylin and eosin. A minimum of ten sections were taken from varying levels of each biopsy to insure obtaining the area of maximum tissue response.

In a blind study conducted by the Department of Oral Pathology, Loyola University School of Dentistry, each coded section was graded on a scale from zero to five according to the severity and extent of tissue response to the injected test solution. Criteria used for rating the degree of inflammatory response were: vasodilatation, margination, vascular engorgement, fluid exudation, inflammatory cell infiltration and extravasation of red blood cells. The presence and degree of tissue necrosis were also recorded. The ratings for each of the biopsies of a given test solution were totaled, and the mean response determined. This mean response was used as the final rating for the solution.

A rating between 0.5 and 1.4 was termed very mild; between 1.5 and 2.4 was termed mild; between 2.5 and 3.4 was moderate; and above 3.5 was considered a severe reaction.
B. Antimicrobial Effectiveness of Aqueous Parachlorophenol

To determine the effective antimicrobial concentration of parachlorophenol for microorganisms commonly found in infected root canals, serial tube dilution studies were conducted.

1. Specific Microorganisms

In order to find the concentration at which parachlorophenol exerts antimicrobial activity against a specific microorganism, a minimum of three series of experiments, each with a narrowed range, were conducted. Each series consisted of two identical experiments to insure accuracy. When results from the identical experiments did not agree, the entire series was repeated.

A twenty-four hour, turbid growth of the selected test microorganism was used as an inoculum in each experiment and was prepared by needle inoculation of 10 ml of sterile broth media from a stock culture maintained by the Department of Microbiology, Loyola University School of Dentistry. The twenty-four hour growth was always cultured in the same media to be used in the experiment. Gram stains were used before and after each experiment to insure the presence of the test microorganism alone.
The selected test microorganisms included:

1. **Streptococcus faecalis** (Microbiology Department; Northwestern University School of Dentistry)
2. **Streptococcus mitis** (Microbiology Department; Loyola University School of Dentistry)
3. **Streptococcus salivarius** (Microbiology Department; Loyola University School of Dentistry)
4. **Staphylococcus aureus** (American Type Culture Collection (ATCC) #12600)
5. **Staphylococcus epidermidis** (ATCC #155)
6. **Neisseria catarrhalis** (Midwest Culture (MC) #56)
7. **Candida albicans** (MC#95)
8. **Sarcina lutea** (ATCC #272)
9. **Corynebacterium diphtheriae** (MC#27)
10. **Escherichia coli** (ATCC #11755)

Freshly prepared 1% PCP (1.0 gm parachlorophenol in 100 ml of water) was used for each experimental series and strict adherence to sterile microbiological techniques was observed during all procedures.

The tube dilution tests consisted of a series of media dilutions of 1% PCP, initially involving a broad range of concentrations and subsequently being narrowed...
to a very small range until the endpoint determination could be made to the nearest \(1 \times 10^{-4}\) gm parachlorophenol per ml. Each succeeding test series therefore confirmed the preceding series. For example, the initial dilution series with each test microorganism involved a logarithmic progression from a dilution of \(1:5(2 \times 10^{-3}\) gm parachlorophenol per ml) to \(1:5^4(1.6 \times 10^{-5}\) gm parachlorophenol per ml). The subsequent series continued to narrow the endpoint and finally a series with 1 ml dilution increments determined the final endpoint.

Endpoint determination for each test microorganism was based on the smallest concentration of parachlorophenol inhibiting that microorganism (i.e., the smallest bactericidal or fungicidal concentration).

0.4 ml of a twenty-four hour growth of the test microorganism was inoculated into each of the dilution tubes.

Four control tubes, each containing 8 ml of media, were used for each experiment. Two of the tubes received only the 1% PCP (1 ml) to test for any precipitation or contamination. The remaining two tubes received only the test microorganism to insure its capability of growth under normal test conditions.
The tubes were incubated at 37°C for seventy-two hours, after which the presence or absence of turbidity was noted. Final results, however, were based only on the results of subculturing each tube onto a pour plate of similar media. The pour plates were then incubated for seventy-two hours. The presence of colonies of the test microorganism, as confirmed by gram stains, was considered positive evidence of growth in the corresponding tube.

Tryplicase Soy* media was used for all experiments except those with Candida albicans, for which Sabouraud’s* media was used.

2. Positive Root Canal Cultures

In order to determine the antimicrobial effectiveness of parachlorophenol against clinically virulent microorganisms, five positive cultures were selected from the endodontic clinic of Loyola University School of Dentistry. Each was either the second or third consecutive positive culture from teeth which were relatively intact, making salivary contamination less likely.

*Baltimore Biological Laboratories; Cockeysville, Md.
Gram stains of each revealed that cultures #1, #2 and #5 were pure cultures and were gram positive cocci in short chains. Cultures #3 and #4 were mixed, with the former showing large gram positive diplococci and smaller gram positive cocci in short chains. Culture #4 revealed gram positive cocci in clusters and gram negative cocci appearing singly. No attempt was made to identify the microorganisms.

A twenty-four hour growth was prepared by placing one loopful of the positive culture in 10 ml of sterile Trypticase Soy broth.

An experimental range of 1:6(1.6x10^{-3} gm PCP/ml) to 1:20(5x10^{-4} gm PCP/ml) was selected on the basis of results from the previous study with specific microorganisms. The initial series was conducted as previously described using increments of 4 ml (i.e., 1:6, 1:10, 1:14, etc.).

A second series was then conducted using 1 ml increments to further narrow the endpoint. Each series consisted of two identical experiments. All procedures, including incubation and subculturing, were conducted as described with the specific microorganisms.
C. Neutralization of Aqueous Parachlorophenol

The effect on the antimicrobial activity of 1% PCP exerted by certain test solutions, suspensions, necrotic tissue and environmental conditions were tested.

1. Test Solutions and Suspensions

A sterile dentin suspension was prepared by placing one gram of finely powdered dentin in 100 ml of distilled water. The suspension was autoclaved at 120°C, fifteen pounds pressure, for twenty minutes and was agitated daily for three days.

A sterile physiologic saline solution was prepared by dissolving 0.85 g of sodium chloride in 100 ml of distilled water and autoclaving as above.

100 ml of human saliva was collected and sterilized with a membrane filter apparatus* using a filter of 0.45 micron pore size.

Two commercial preparations were also tested, EDTAC** and Bacto-Ascitic Fluid***.

Fresh, whole, citrated human blood was obtained from a hospital blood bank and used experimentally six days after being drawn.

*Millipore Filter; Bedford, Mass.
***Difco Laboratories, Inc.; Detroit, Mich.
Each of the above test preparations were selected because they represent fluids which may be present in a root canal and could possibly affect the activity of 1% PCP.

With the exception of the blood, each of the above test solutions and suspensions were checked for sterility by transferring a loopful into separate test tubes containing Trypticase Soy broth and incubating for seventy-two hours at 37°C. Each tube was then subcultured onto pour plates which were incubated for an additional seventy-two hours: No growth occurred in any tube.

*Streptococcus faecalis* was selected as the test microorganism because previous studies had proved it to be the most resistant of the microorganisms tested to parachlorophenol, a concentration of 0.00125 gm/ml (1:8 dilution) being required for bactericidal activity. 0.4 ml of a turbid twenty-four hour growth was used as an inoculum for each experiment.

A dilution range from 1:6(1.6x10^{-3} gm PCP/ml) to 1:11(9x10^{-4}) was selected for all except blood for which dilutions of 1:4.5(2.2x10^{-3} gm PCP/ml) and 1:3.5(2.6x10^{-3}) were added.
Sterile techniques were used during all experimental procedures and gram stains were made prior to and following each experiment. The experimental procedures were the same as used in the previously described experiments. Subculturing and incubating procedures were also identical.

With each of the six preparations, four experimental tube dilution series were conducted, with each series being repeated at least once for confirmation of results.

Series 1 was a control series using 1% PCP prepared twenty-four hours prior and not subjected to contact with one of the test preparations.

Series 2 was conducted with 1% PCP which had been placed with one-half its volume of the test preparation (1 PCP:0.5 test preparation) and allowed to stand together twenty-four hours. For example, 20 ml of 1% PCP was pipetted into a bottle containing 10 ml of test preparation, thoroughly agitated, and allowed to stand. This mixture, already a 1:1.5 dilution of 1% PCP, was used in the experimental series and the desired dilutions established.

Series 3 was the same as above except that equal volumes of 1% PCP and each test preparation were allowed
together for twenty-four hours.

Series 4 was again the same with the exception that one volume of 1% PCP was placed with two volumes of test preparation (a 1:2 ratio).

Of the test preparations, only blood required an extra procedure. After twenty-four hours, the PCP-blood mixture was centrifuged for fifteen minutes to remove the denatured blood. The supernatent was then used as described to obtain the desired dilutions of parachlorophenol.

An additional study was added for the EDTAC preparation because it contains a powerful bactericidal agent, cetyl trimethyl ammonium bromide. An identical series of tests as described in series 1, 2, 3 and 4 were conducted at three additional dilutions (1:15, 1:20 and 1:25).

2. Necrotic Tissue

The neutralizing effect of necrotic tissue on 1% PCP was tested, using Streptococcus faecalis as a test microorganism.

A frog was pithed, the legs removed at the hip joint, and the feet, skin and bones removed. The remaining tissue was suspended by thread in a capped glass container
above 5 ml of water to prevent drying. After twenty-four hours, the necrotic tissue was cut into small sections of about 3X3 mm to increase the exposed surface area. Three grams of this tissue was placed in a sterile bottle to which was added 30 ml of 1% PCP. Following thorough agitation, this was allowed to stand for twenty-four hours.

At the end of this period, 1 ml of the liquid was pipetted under sterile conditions into each of a series of tubes to obtain dilutions from 1:2(5X10^{-3} gm parachlorophenol per ml) to 1:10(1X10^{-3} gm/ml) in 1 ml increments. SF media* was used in this experiment to insure that only the test microorganism, *Streptococcus faecalis*, would survive. Contamination from the necrotic tissue was thus eliminated so that the results could be properly interpreted. This media contains sodium azide and is a selective media for the test microorganism.

All test procedures, controls, incubation and sub-culturing were conducted as previously described. A control series, using SF media and 1% PCP which had been prepared twenty-four hours earlier were conducted in the manner described under Series 1 above.

* Baltimore Biological Laboratories; Cockeysville, Md.
3. Thermal Extremes

The neutralizing effect of thermal extremes on 1% PCP was tested, using Streptococcus faecalis as the test microorganism in Trypticase Soy media. Sterile techniques were employed in all procedures. Test procedures, controls and subculturing were conducted as previously described.

Twenty ml of freshly prepared 1% PCP was placed in each of five numbered sterile screw-cap bottles. Container #1 was placed in an incubator at 37°C, #2 was placed in a refrigerator at 6°C and #3 was refrigerated at approximately -8°C. All were allowed to remain under these environmental conditions for twenty-four hours. Container #4 was autoclaved at 120°C, fifteen pounds pressure, for twenty minutes and allowed to cool and remain at room temperature for twenty-four hours. The contents of Container #5 was used for a control and remained at room temperature for twenty-four hours. Each of the 1% PCP solutions were used in a separate experimental tube dilution series as described previously. A dilution range of 1:5(2X10⁻³ gm PCP/ml) to 1:10(1X10⁻³) in one ml increments was selected for these tests. Each series was conducted twice and gram stains were employed to confirm
the test microorganism before and after each experiment.

4. Shelflife

The neutralizing effect of prolonged shelflife in dark and clear containers were tested, using *Streptococcus faecalis* as a test microorganism. Trypticase Soy was used as media and sterile techniques were used in all procedures. Gram stains were used before and after each experimental series to confirm the presence only of the test microorganism.

**Control Series:** Freshly prepared 1% PCP was tested in dilutions of 1:5 and 1:10 exactly as described above.

**Light Exposure Series:** 200 ml of the same 1% PCP preparation used in the Control Series was pipetted into a sterile, clear-glass, screw-cap bottle and placed on a shelf receiving full light exposure, both artificial and indirect sun light. Portions of this solution were tested after six months and twelve months using the same procedures as the Control Series.

**Dark Series:** 200 ml of the same 1% PCP preparation as used in the Control Series was pipetted into a sterile, dark-stained, glass, screw-cap container and placed on a shelf receiving minimal amounts of artificial light.
This solution was tested after six months and twelve months using the same procedures as the Control Series.
CHAPTER IV
RESULTS

A. Toxicity

1. Conjunctival Inflammatory Response

The reactions ranged from no response with Microcide to severe responses with camphorated parachlorophenol (CPC) and eugenol. The maximum responses to the irritating test solutions were noted at the five hour observation period. (Fig. 1)

At the end of ninety-six hours, only those eyes which were tested with CPC and eugenol continued to be inflamed. In no case did a response occur due to the saline controls. (Fig. 2)

Cresatin and 1½ PCP caused only very slight hyperemia, while 2½ PCP caused a moderate inflammatory response after five hours. CPC caused severe inflammation after five hours and continued to produce a moderate response after twenty-four and ninety-six hours. Eugenol exhibited a moderate response after only five minutes and resulted in a severe response at the five hour observation with no improvement after ninety-six hours. Typical responses are seen in Fig. 3 - 9.
The inflammatory responses at each observation period are presented in tabular form in Table 1.

Table 1 - Conjunctival Inflammatory Response

<table>
<thead>
<tr>
<th>Solution No.</th>
<th>Animal No.</th>
<th>5 Minutes</th>
<th>5 Hours</th>
<th>24 Hours</th>
<th>96 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cresatin</td>
<td>1</td>
<td>Normal</td>
<td>Very mild</td>
<td>Very mild</td>
<td>Very mild</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Normal</td>
<td>Very mild</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Normal</td>
<td>Very mild</td>
<td>Mild</td>
<td>Normal</td>
</tr>
<tr>
<td>2. 35% CPC</td>
<td>4</td>
<td>Mild</td>
<td>Severe</td>
<td>Moderate</td>
<td>Mild to  Moderate</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Mild</td>
<td>Severe</td>
<td>Severe</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Mild</td>
<td>Moderate</td>
<td>to Severe</td>
<td>Moderate</td>
</tr>
<tr>
<td>3. Microcide</td>
<td>7</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>4. Eugenol</td>
<td>10</td>
<td>Moderate</td>
<td>Severe</td>
<td>Severe</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Moderate</td>
<td>Severe</td>
<td>Severe</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Mild to</td>
<td>Severe</td>
<td>Severe</td>
<td>Severe</td>
</tr>
<tr>
<td>5. 2% PCP</td>
<td>13</td>
<td>Mild</td>
<td>Moderate</td>
<td>Mild</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Normal</td>
<td>Mild to</td>
<td>Moderate</td>
<td>Mild</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Mild to</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Mild</td>
</tr>
<tr>
<td>6. 1% PCP</td>
<td>16</td>
<td>Very mild</td>
<td>Very mild</td>
<td>Very mild</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Very mild</td>
<td>Mild</td>
<td>Very mild</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Very mild</td>
<td>Very mild</td>
<td>Very mild</td>
<td>Normal</td>
</tr>
</tbody>
</table>

2. Dermal Connective Tissue Inflammatory Response

The saline control sites presented a broad range of responses from 0.04 in two biopsies to 3.04 (moderate) in three biopsies, with a mean response of 1.54 (mild)
in the twenty-four test period and 1.0+ (very mild) in the seventy-two hour period.

The mean response of the tissues to the test solutions revealed a mild reaction in the twenty-four hour group to 1% PCP, 2% PCP, microcide and cresatin. Sodium hypochlorite provoked a moderate response, whereas eugenol and camphorated parachlorophenol caused severe inflammatory responses. (Fig. 10)

In the seventy-two hour group, microcide caused the mildest response with a mean rating of 1.0+ (very mild). 1% PCP and cresatin were rated as mild (2.0+) and 2% PCP and sodium hypochlorite caused moderate reactions (2.5+ and 2.8+ respectively).

Eugenol and CPC both received a severe rating of 4.0+. (Fig. 11)

Fig. 12 - 18 represent typical tissue reactions as observed microscopically.

Mean responses as well as the rating given individual biopsy responses are presented in tabular form for the twenty-four hour group in Table 2 and the seventy-two hour group in Table 3.
### Table 2 - Twenty-four Hour Connective Tissue Inflammatory Response

<table>
<thead>
<tr>
<th>Solution</th>
<th>Response</th>
<th>Biopsy Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cresatin</td>
<td>2.3↑</td>
<td>2.0↑</td>
<td>2.0↑</td>
<td>3.0↑</td>
<td>3.0↑</td>
<td>1.0↑</td>
<td>3.0↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35% CPC</td>
<td>3.8↑</td>
<td>2.5↑</td>
<td>3.5↑</td>
<td>4.5↑</td>
<td>4.5↑</td>
<td>4.5↑</td>
<td>3.5↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% PCP</td>
<td>1.9↑</td>
<td>2.0↑</td>
<td>3.0↑</td>
<td>2.0↑</td>
<td>1.0↑</td>
<td>1.0↑</td>
<td>2.0↑</td>
<td>2.0↑</td>
<td>2.0↑</td>
<td>2.0↑</td>
</tr>
<tr>
<td>2% PCP</td>
<td>2.2↑</td>
<td>4.0↑</td>
<td>3.0↑</td>
<td>1.0↑</td>
<td>2.0↑</td>
<td>1.0↑</td>
<td>3.0↑</td>
<td>2.0↑</td>
<td>2.0↑</td>
<td>2.0↑</td>
</tr>
<tr>
<td>Microcide</td>
<td>2.3↑</td>
<td>2.0↑</td>
<td>3.0↑</td>
<td>3.0↑</td>
<td>2.0↑</td>
<td>3.0↑</td>
<td>1.0↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eugenol</td>
<td>3.6↑</td>
<td>4.0↑</td>
<td>4.5↑</td>
<td>2.0↑</td>
<td>4.5↑</td>
<td>3.0↑</td>
<td></td>
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<td></td>
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<tr>
<td>NaOCl</td>
<td>3.1↑</td>
<td>3.5↑</td>
<td>1.5↑</td>
<td>3.0↑</td>
<td>3.5↑</td>
<td>4.0↑</td>
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<tr>
<td>Saline</td>
<td>1.5↑</td>
<td>3.0↑</td>
<td>1.0↑</td>
<td>0.0↑</td>
<td>1.0↑</td>
<td>0.0↑</td>
<td>3.0↑</td>
<td>3.0↑</td>
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Table 3 - Seventy-two Hour Connective Tissue Inflammatory Response

<table>
<thead>
<tr>
<th>Solution Response</th>
<th>Mean Response</th>
<th>Biopsy Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Cresatin</td>
<td>2.0†</td>
<td>1.0†</td>
</tr>
<tr>
<td>35% CPC</td>
<td>4.0†</td>
<td>4.5†</td>
</tr>
<tr>
<td>1% PCP</td>
<td>2.0†</td>
<td>2.0†</td>
</tr>
<tr>
<td>2% PCP</td>
<td>2.5†</td>
<td>2.0†</td>
</tr>
<tr>
<td>Microcide</td>
<td>1.0†</td>
<td>1.0†</td>
</tr>
<tr>
<td>Eugenol</td>
<td>4.0†</td>
<td>4.5†</td>
</tr>
<tr>
<td>NaOCl</td>
<td>2.8†</td>
<td>2.5†</td>
</tr>
<tr>
<td>Saline</td>
<td>1.0†</td>
<td>1.0†</td>
</tr>
</tbody>
</table>

Ruined Section
B. Antimicrobial Effectiveness of Aqueous Parachlorophenol

1. Specific Microorganisms

Parachlorophenol was effective against all ten test microorganisms in a range varying from a $1.2 \times 10^{-3}$ gm per ml concentration to $4 \times 10^{-4}$ concentration. (Fig. 19-20)

Streptococcus faecalis was the most resistant of the test microorganisms whereas Candida albicans, Corynebacterium diphtheriae, Sarcina lutea and Escherichia coli exhibited much greater sensitivity. The latter three were destroyed in all experiments at a concentration of $4.1 \times 10^{-4}$ gm PCP per ml but in two experiments exhibited an even greater sensitivity.

The effective antimicrobial concentrations of parachlorophenol from the test microorganisms are presented in Table 4.
<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Effective Antimicrobial Dilution of 1% PCP</th>
<th>Antimicrobial PCP Concentration (gm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus faecalis</td>
<td>1:8</td>
<td>.00125</td>
</tr>
<tr>
<td>Streptococcus mitis</td>
<td>1:20</td>
<td>.00050</td>
</tr>
<tr>
<td>Streptococcus salivarius</td>
<td>1:22</td>
<td>.00045</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1:23</td>
<td>.00045</td>
</tr>
<tr>
<td>Neisseria catarrhalis</td>
<td>1:16</td>
<td>.00062</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td>1:12</td>
<td>.00033</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1:25</td>
<td>.00040</td>
</tr>
<tr>
<td>Corynebacterium diphtheriae</td>
<td>1:24</td>
<td>.00041</td>
</tr>
<tr>
<td>Sarcina lutea</td>
<td>1:24</td>
<td>.00041</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1:24</td>
<td>.00041</td>
</tr>
</tbody>
</table>
2. **Positive Root Canal Cultures**

For the five selected positive cultures, the effective range of parachlorophenol concentrations varied from $1.1 \times 10^{-3}$ gm/ml to $7.1 \times 10^{-4}$ (Fig. 21).

The antimicrobial concentrations for the test cultures are presented in Table 5.

**Table 5 - Endpoint Determination - Aqueous Parachlorophenol**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Effective Antimicrobial Dilution of 1% PCP</th>
<th>Antimicrobial PCP Concentration (gm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Canal Culture (I)</td>
<td>1:9</td>
<td>.00111</td>
</tr>
<tr>
<td>Positive Canal Culture (II)</td>
<td>1:10</td>
<td>.00100</td>
</tr>
<tr>
<td>Positive Canal Culture (III)</td>
<td>1:14</td>
<td>.00071</td>
</tr>
<tr>
<td>Positive Canal Culture (IV)</td>
<td>1:14</td>
<td>.00071</td>
</tr>
<tr>
<td>Positive Canal Culture (V)</td>
<td>1:12</td>
<td>.00083</td>
</tr>
</tbody>
</table>

C. **Neutralization of Aqueous Parachlorophenol**

1. **Test Solutions and Suspensions**

The control Series 1 experiments gave relatively
uniform results with either a $1.1 \times 10^{-3}$ or $1.2 \times 10^{-3}$ gm/ml parachlorophenol concentration being effective against the test microorganism.

Blood exerted the most profound inhibitory effect with bacterial growth occurring even at a concentration of $2.8 \times 10^{-3}$. In a ratio of two parts suspension to one part $1\%$ PCP, as tested in Series 4, dentin also inhibited parachlorophenol activity with growth occurring at a concentration of $1.6 \times 10^{-3}$ gm/ml.

No bacterial growth occurred at the low parachlorophenol concentration of $4 \times 10^{-4}$ gm/ml with the EDTAC test series 2, 3 and 4.

The results of test series 2, 3 and 4 with saline, saliva and ascites fluid showed a slight variation in effective antimicrobial parachlorophenol concentration from $1.4 \times 10^{-3}$ gm/ml (saline: to $1.6 \times 10^{-3}$ (ascites fluid).

The results of the control and test series of each test preparation is shown in Fig. 22.

2. Necrotic Tissue

Results of the control series showed parachlorophenol to be effective against the test microorganism at a concentration of $1.1 \times 10^{-3}$ gm/ml.

Following twenty-four hour exposure to necrotic
tissue a concentration of $3 \times 10^{-3}$ gm/ml was required. (Fig. 23)

3. **Thermal Extremes**

Thermal extremes appeared to have little effect on the antimicrobial activity of parachlorophenol against the test microorganism.

The control, incubator and autoclave series showed an effective parachlorophenol concentration of $1.1 \times 10^{-3}$ gm/ml. The 6°C series and -8°C series resulted in an effective concentration of $1.2 \times 10^{-3}$ gm/ml. (Fig. 23)

4. **Shelflife**

No change in the antimicrobial activity of parachlorophenol against the test microorganism resulted after prolonged shelflife in either a dark or light-transmitting container.

The control series and all test series at six-month and twelve-month intervals resulted in an effective parachlorophenol concentration of $1.2 \times 10^{-3}$ gm/ml. (Fig. 23)
CHAPTER V
DISCUSSION

The elimination of infection from human tissue is a necessary goal based on fundamental biological principles. It is an even more essential procedure in an environment in which the natural defense mechanisms of the body are unable to function. Such an environment is the root canal.

Elimination of infection from the root canal is accomplished by thorough biomechanical cleansing and the use of intracanal antimicrobial medications. The relative merits of each have been much discussed, but it is the latter which presently concerns us.

There is little disagreement as to the requirements of an ideal root canal medication$^{(27, 163, 169)}$ and there is likewise little disagreement that such a drug does not at present exist. As the choice of a root canal disinfectant cannot be based on the ideal, there are minimum requirements which should be met.$^{(170)}$ The disinfectant should: 1) be effective, 2) noninjurious to periapical tissues, and 3) not interfere with accurate culture techniques after forty-eight hours.
It is of interest to note that the most commonly used root canal disinfectant\textsuperscript{(112)}, camphorated parachlorophenol, N.F., meets only the first requirement, and even that is highly debatable.

The following objective review of the known and unknown factors of this drug, relative to these requirements, raises some interesting points of consideration.

1) **Effectiveness:** As defined by the National Formulary\textsuperscript{(161)}, camphorated parachlorophenol (CPC) contains not less than thirty-three percent or more than thirty-seven percent parachlorophenol and not less than sixty-three percent of more than sixty-seven percent camphor. It is considered clinically as a highly effective\textsuperscript{(109, 111, 121)} antimicrobial agent.

The antimicrobial activity of CPC is due to the parachlorophenol\textsuperscript{(162)} and the camphor serves merely as a vehicle. Camphor is an irritant which possesses some anodyne qualities\textsuperscript{(164)} and is of little or no antiseptic value. Parachlorophenol per se reportedly has a phenol coefficient of 4.0\textsuperscript{(155)}, but the bactericidal action varies greatly with the solvent. In solvents of low dielectric constant such as alcohol, acetone, ether, xylene, chloroform,
etc., parachlorophenol is believed to exert no marked antiseptic action. However, in solvents of high dielectric constant, such as glycerin, nitrobenzene and water, it reportedly exhibits marked antimicrobial activity. (163)

CPC was introduced to the dental profession in 1391 by Walkoff (166) and since that time there has been no valid microbiological investigation into the antimicrobial activity of this drug against the array of microorganisms commonly found in root canals. Most investigators seem concerned primarily with the economics of the number of applications, related to patient visits, required to obtain negative cultures. (121, 122)

A critical look at our current endodontic culturing procedures (153, 154) points out the fallacy in drawing the all too obvious conclusion that a negative culture indicates antimicrobial effectiveness of the intracanal medication. (154, 155) As presently employed, a negative culture indicates only that we have either: 1) reduced the number of microorganisms, 2) eliminated the microorganisms, or 3) used an inadequate culturing technique. As we cannot distinguish between these three, we cannot unequivocally state that our intracanal medication has eliminated any or all microorganisms from the canal.
In addition, Auerbach\(^{(114)}\) and Stewart\(^{(115)}\) have shown that over seventy-five percent of the infected root canals will present negative cultures after mechanical debridement and irrigation. This is a further indictment of drawing the conclusion that negative cultures are directly related to the effectiveness of the intracanal medication.

The use of CPC has been predicated on this assumption that clinical results, as evidenced by negative cultures, indicate antimicrobial effectiveness.

The effective parachlorophenol concentration against the microorganisms of the root canal is an unknown quantity. The question of the concentration of parachlorophenol in CPC becomes suspect, as there is no valid evidence that thirty-five percent is the optimum concentration. For every disinfectant there is a maximum concentration beyond which, according to the law of diminishing returns, a proportionate increase in antimicrobial activity is not evidenced. The toxicity, however, normally increases with the concentration.\(^{(133)}\)

2) **Toxicity:** Pain following endodontic therapy may result from infection, overinstrumentation, or the penetration of a toxic intracanal medication into the peri-
apex. (154, 171) This reason alone is sufficient to warrant the use of a non-toxic medication.

If, in addition, the intracanal medication is toxic to the degree of causing coagulation of protein, the disadvantage is compounded by a concomitant inhibition of antimicrobial activity. (20) Agents which coagulate protein have only a shallow penetration, despite low surface tension, and according to Coolidge (73) are not qualified for the treatment of infected canals containing organic material.

There is, surprisingly, some disagreement as to the toxic qualities of CPC. Parachlorophenol produces toxic manifestations in animals similar to those produced by phenol (165), and animal studies (119, 120) with CPC have demonstrated its high toxicity. Despite this, a leading endodontic text contains the following statement... "when combined in the proportion of three to seven with gum camphor, it becomes virtually non-irritating." (170)

The results of the present investigation demonstrate the severe toxic qualities of CPC as well as its ability to coagulate protein.

In the dermal toxicity test, severe coagulation necrosis was pointedly evident in the twenty-four and
seventy-two hour biopsies. Cellular detail as well as the general architectural tissue pattern was completely destroyed in a wide area adjacent to the injection site. (Fig. 17, 18) The area evidenced coagulation necrosis, displaying a large pale-staining area lacking organizational arrangement and with an absence of connective tissue or inflammatory cells. Cellular debris was observed in the necrotic area and inflammatory cells were evident in the viable tissues bordering the area.

Menkin(167) observed that with powerful irritants fixation is the initial phase in the development of severe inflammatory reactions, and that this precedes the migration of leucocytes.

3. Interference with Culturing: In 1965, it was convincingly demonstrated by Grossman(149) that CPC does, in fact, inhibit culturing and therefore requires an inhibitor for accurate culturing, as do the antibiotics. Such an inhibitor is not presently available for clinical use.

Considering all of the above factors, the present study sought to investigate the possibility of a more advantageous use of parachlorophenol. An aqueous vehicle was chosen because it presented the best opportunity to
test parachlorophenol without superimposing toxic or antimicrobial qualities. The results of all tests with aqueous parachlorophenol could therefore be interpreted as a manifestation of the solute.

**Toxicity of Aqueous Parachlorophenol**

In sharp contrast to the toxicity displayed by CPC, 1% aqueous parachlorophenol (1% PCP) produced only mild inflammatory responses and no evidence of necrosis was observed in any microscopic section. (Fig. 14, 15) Mild to moderate acute inflammatory cell infiltration with mild edema was noted in the twenty-four hour biopsies, whereas chronic inflammatory cell infiltration was evident after seventy-two hours.

The conjunctival inflammatory responses confirmed the microscopic evidence of the vast difference in toxicity potential between 1% aqueous and 35% camphor solutions of parachlorophenol. (Fig. 4, 6)

Both the dermal and conjunctival tests also indicated a slightly higher inflammatory potential for 2% aqueous parachlorophenol when compared to 1% PCP. (Fig. 4, 5) However, it must be noted that a wide range of responses were observable microscopically with all the test solutions,
as well as with the saline control. (Fig. 10) As a result of this, no conclusions should be drawn as to the relative toxicity of the test solutions within a given degree of response. One would not, therefore, be justified on the basis of the present dermal inflammatory tests to state that 2% PCP is slightly more toxic than 1% PCP.

It may be concluded, however, that contrary to the severe toxic reactions produced by CPC (Fig. 17, 18) and eugenol (Fig. 9) a mild inflammatory response results from 1% PCP (Fig. 4, 14, 15), Microcide (Fig. 8, 16) and Cresatin (Fig. 7). Microcide was particularly conspicuous in its lack of inflammatory potential in the conjunctival tests. (Fig. 1, 2)

Sodium hypochlorite (5%) produced small localized areas of necrosis in four of six biopsies in the twenty-four hour test period. This was accompanied by moderate to severe edema, extravasation of red blood cells, and moderately severe acute inflammatory cell infiltration. In seventy-two hours, a similar though less intense reaction was manifested with chronic inflammatory cell infiltration.
Only eugenol proved to be as irritating and caustic as CPC, presenting a microscopic picture of severe coagulation necrosis and a conjunctival response of even greater comparative intensity.

**Antimicrobial Effectiveness of Aqueous Parachlorophenol**

Far more surprising than the relative lack of toxicity displayed by 1% PCP was the antimicrobial effectiveness displayed by this preparation.

The most comprehensive and well-controlled microbiological study of the root canal flora was reported in 1959 by Winkler and van Amerongen\(^{128}\) and later confirmed by other investigators.\(^{129, 130}\) Their study involved the results from 1,141 positive root canal cultures. *Streptococcus faecalis* was found to be the most frequently isolated microorganism in pure culture, indicating its resistant nature. This microorganism also proved to be the most persistent, as evidenced by its high frequency in latur cultures.

*Streptococcus faecalis* proved to be the most resistant test microorganism to parachlorophenol. An effective bactericidal concentration was 1.2X10\(^{-3}\) gm/ml, which represented a 1:8 dilution of the 1% PCP. *Staphylococcus*
epidermidis and neisseria catarrhalis were effectively destroyed at parachlorophenol concentrations of $8.3 \times 10^{-4}$ gm/ml and $6.2 \times 10^{-4}$ gm/ml respectively. All other microorganisms tested were sensitive to concentrations of $5 \times 10^{-4}$ gm/ml or less, with Candida albicans being the most sensitive of those tested. (Fig. 19, 20)

The microorganisms tested were selected as representatives of the various groups of organisms isolated by Winkler and van Amerongen. (123) They included:

- Streptococcus (faecalis, mitis, salivarius)
- Staphylococcus (aureus, epidermidis)
- Gram positive rods (Corynebacterium diphtheriae)
- Gram negative rods (Escherichia coli)
- Gram negative cocci (Neisseria catarrhalis)
- Gram positive cocci (Sarcina lutea)
- Yeasts (Candida albicans)

These microorganisms represent groups of microorganisms which comprise about eighty-five percent of those groups isolated. The most important group not tested are anaerobes. (104, 105, 106)

The effectiveness of parachlorophenol against the test microorganisms isolated from positive root canal cultures agreed with the results obtained from the
specific microorganisms, relative to the effective range of concentrations. (Fig. 21) The least sensitive microorganism (pure culture #1) required a bactericidal parachlorophenol concentration of $1.1 \times 10^{-3}$ gm/ml whereas the most sensitive required a $7.1 \times 10^{-4}$ gm/ml concentration.

Neutralization of Aqueous Parachlorophenol

Marked inhibition of 1% PCP antimicrobial action was exhibited by blood and necrotic tissue, ostensibly due to protein binding. (20,73) The ratio of necrotic tissue tested (three grams tissue to 30 ml 1% PCP) did not completely destroy the bactericidal action, but greatly weakened it. (Fig. 22, 23) This is a common finding with most, if not all, root canal antiseptic or disinfectant agents. (168, 171)

A 2:1 ratio of dentin suspension also evidenced an inhibitory effect. In light of the complete lack of inhibition with a 1:1 ratio and the narrow test range, a true inhibition cannot be assumed. It is also doubtful that such a high ratio of dentin suspension to medication is of any clinical significance. It must also be pointed out that the dentin was obviously greatly altered from its original state because of autoclaving and being ground to a powdery consistency.

Neutralization with saline, saliva and ascites fluid
was minor. Bactericidal activity required only a slightly increased concentration of parachlorophenol and the results could be a manifestation of the test procedure variables. It is, however, readily apparent that 1% PCP does exhibit bactericidal activity against the test microorganism in the presence of a high concentration of these test solutions.

The stability of aqueous parachlorophenol is obvious from the results of the shelflife and thermal extremes experiments. (Fig. 23)

CONCLUSIONS

The in vitro antimicrobial activity thus displayed by this low concentration of parachlorophenol poses serious doubts as to the necessity of using the highly toxic thirty-five percent concentration as used in CPC.

This in no way implies that a low concentration of parachlorophenol would be as effective in vivo. The literature is replete with such "test tube darlings" that failed miserably in the root canal. (27, 29, 63, 70, 79, 80, 86, 95) One cannot help but wonder, however, if the only real advantage in the use of a thirty-five percent concentration in CPC is "ease of trituration". If the ratio of percentages of camphor and parachlorophenol are varied only slightly the solution will not remain stable and will settle out. It is possible, though as yet unproved, that
the camphor aids the effectiveness by acting as a reservoir which releases parachlorophenol slowly over an extended period of time. The camphor is also believed to reduce the surface tension and thereby increase the penetrability of parachlorophenol.

It is a microbiological "rule of thumb" that a five fold increase above the endpoint concentration be used to provide a safety margin for the effects of dilution, neutralization, etc. (173) A 1.5% parachlorophenol concentration provides a nine fold increase beyond the effective in vitro concentration against the most resistant microorganism tested.

The results of this study indicate that: 1) CPC is highly toxic, 2) a reduced parachlorophenol concentration in a vehicle of water is far less toxic than CPC and 3) parachlorophenol 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 as in theory, it could be established by clinical study that a 1.5 or 2.5% parachlorophenol preparation possesses the same clinical "efficiency" in producing negative cultures as does CPC, the use of the latter
could no longer be justified.
CHAPTER VI
SUMMARY

A study of the toxicity of aqueous parachlorophenol as compared to camphorated parachlorophenol and other currently used endodontic medications was conducted. It was determined by conjunctival inflammatory tests and intradermal injection into the abdomens of rabbits, that a 1% aqueous parachlorophenol solution is far less toxic than camphorated parachlorophenol.

The antimicrobial effectiveness of aqueous parachlorophenol was tested in vitro and it was determined that a 1% solution is effective against a variety of microorganisms commonly found in infected root canals. In addition, the inhibitory activity of various environmental conditions on aqueous parachlorophenol was tested.

In the light of the results of these tests, it is concluded that a reduced concentration of parachlorophenol in aqueous and other vehicles should be further investigated in vitro and in vivo for possible future clinical use.
BIBLIOGRAPHY


164. Osol, Arthur; Pratt, Robertson and Altschule, Mark; *op. cit.*, p. 234-235.

165. Osol, Arthur; Pratt, Robertson and Altschule, Mark; *op. cit.*, p. 804-805.


**CONJUNCTIVAL INFLAMMATORY RESPONSE**

**5 HOURS**

<table>
<thead>
<tr>
<th>Degree of Response</th>
<th>Test Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>severe</td>
<td>saline, micocide</td>
</tr>
<tr>
<td>mod-severe</td>
<td>cresatin</td>
</tr>
<tr>
<td>moderate</td>
<td>1% pcp</td>
</tr>
<tr>
<td>mild-mod</td>
<td>2% pcp</td>
</tr>
<tr>
<td>mild</td>
<td>35% cpc</td>
</tr>
<tr>
<td>very mild</td>
<td>eugenol</td>
</tr>
<tr>
<td>none</td>
<td>Mean Response</td>
</tr>
</tbody>
</table>

**Figure 1.** Conjunctival Inflammatory Response

After Five Hours.
Figure 2. Conjunctival Inflammatory Response at Various Time Intervals.
Figure 3. Normal Rabbit Conjunctiva

Figure 4. Conjunctival Response Five Hours After 1% Parachlorophenol.
Figure 5. Conjunctival Response Five Hours After 2% Parachlorophenol.

Figure 6. Conjunctival Response Five Hours After Camphorated Parachlorophenol
Figure 7. Conjunctival Response Five Hours After Cresatin.

Figure 8. Conjunctival Response Five Hours After Microcide.
Figure 9. Conjunctival Response Five Hours After Eugenol.
Connective Tissue Inflammatory Response

24 Hours

<table>
<thead>
<tr>
<th>Degree of Response</th>
<th>Test Solutions</th>
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<tbody>
<tr>
<td>severe 3.5</td>
<td>saline, 1% PCP, 2% PCP</td>
</tr>
<tr>
<td>moderate 2.5</td>
<td>microcide, cresatin</td>
</tr>
<tr>
<td>mild 1.5</td>
<td>NaOCl, eugenol</td>
</tr>
<tr>
<td>very mild 0.5</td>
<td>35% CPC</td>
</tr>
</tbody>
</table>

Figure 10. Connective Tissue Response After Twenty Four Hours.
Figure 11. Connective Tissue Response After Seventy Two Hours.
Figure 12. Saline Control 100x

Figure 13. Saline Control 250x
Figure 16. Microcide 250x

Figure 17. Camphorated Parachlorophenol 250x
Figure 18. Camphorated Parachlorophenol 250x
Figure 19. Effective Concentration of Para-chlorophenol Against Specific Microorganisms.
Figure 20. Effective Concentration of Para-chlorophenol Against Specific Microorganisms.
Figure 21. Effective Concentration of Parachlorophenol Against Positive Root Canal Cultures.
Table 1. Neutralization of Antimicrobial Effectiveness Against Streptococcus faecalis.

<table>
<thead>
<tr>
<th>Test Solutions</th>
<th>PCP Concentration (Gm/Ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dentin</td>
<td>0.0004</td>
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<td>saline</td>
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<td>saliva</td>
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<td>EDTAC</td>
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<td>ascites</td>
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<tr>
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</tr>
<tr>
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<td>0.0028</td>
</tr>
<tr>
<td>1 PCP-1 Test Sol</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

Figure 22. Neutralization of Antimicrobial Effectiveness Against Streptococcus faecalis.
Figure 23. Neutralization of Antimicrobial Effectiveness Against Streptococcus faecalis.
Approval Sheet

The thesis submitted by Dr. John W. Harrison 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 19, 1969
Signature of Advisor

John V. Madonia, D.D.S., Ph.D.