1970

The Effectiveness of Camphorated Paramonochlorophenol, Metacresyl Acetate, and Prednisolone in Combination as an Antimicrobial Agent

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THE EFFECTIVENESS OF CAMPHORATED PARAMONOCHLOROPHENOL, METACRESYL ACETATE, AND PREDNISOLONE IN COMBINATION AS AN ANTIMICROBIAL AGENT

by

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A Thesis in Partial Fulfillment

of the Requirements for the Degree

Master of Science in the Field of Endodontics

I certify that I have read this thesis, and that in my opinion it is fully adequate in scope and quality as a thesis for the degree of Master of Science.

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ACKNOWLEDGEMENTS

I wish to thank all of those who have been so helpful during my graduate education at Loma Linda University. It is unfortunate that I cannot give individual acknowledgement to everyone, but special reference must be made to several individuals who made the completion of this study possible.

A special thanks must be given to the members of my committee--Doctors Ronald E. Buell, Edwin B. Nutting, Judson Klooster, Charles E. Winter and especially to W. Eugene Rathbun whose advice and encouragement proved so beneficial in the preparation of this paper.

I am most grateful to Miss Joyce Ramsey for her laboratory assistance and to Doctor Edward Brady of the University of Southern California School of Pharmacy for his aid in securing the medications used in the project. Mr. Warner Swarner of the Loma Linda University Scientific Computation Facility also gave invaluable assistance with the statistical analysis.

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INTRODUCTION

Postoperative discomfort following endodontic procedures is a too frequent occurrence. Schilder and Amsterdam (1959) suggest that in the absence of periodontal complications, this discomfort is probably the result of one of three vectors or a combination of them: Improper instrumentation, improper medication, and occasionally infection. Attalla and Calvert (1969), Torneck (1961), Schilder and Amsterdam (1959) and Dietz (1957) have reported that commonly used intracanal medicaments are, in varying degrees, inflammatory in themselves. It is therefore reasonable to assume that if inflammation produced by a medication can be significantly reduced, the occurrence of postoperative flare-up can be minimized.

It is imperative that a medicament retain antimicrobial activity while reducing inflammatory potential. Camphorated paramonochlorophenol, metacresyl acetate, and prednisolone in combination (CMP) is advocated to achieve the above requirements. The rationale of its use is that the combining of the corticosteroid with the non-specific germicide combination will result in the reduction of the inflammatory potential of the germicide.

This study was directed toward determining whether CMP retains antimicrobial activity comparable to selected other commonly used non-specific germicides. An in vitro quantitative technique which compared the minimum bacteriostatic concentration versus the minimum bactericidal concentration was utilized.

CHAPTER I

REVIEW OF THE LITERATURE

I. The Role of Microorganisms in Endodontic Disease

On an academic basis, Torneck (1969) divided the relationship of microorganisms to endodontic disease into two phases: a) that of microorganisms, actually establishing disease within the pulp and periapical tissue, often considered a primary effect, and b) that of microorganisms, preventing or impeding post-treatment repair, considered a secondary effect.

Matusow (1967) defines infection as the invasion of the tissue by microorganisms in such a manner as to produce a pathogenic reaction. The obvious significance of the previous sentence is that the mere presence of microbes does not alone constitute infection. Kakehashi, et al. (1965 and 1969) have illustrated in significant studies the role that microorganisms can play in pulpal and periapical disease. Pulp exposures in the molars of 15 conventional rats were left open to oral contamination. Similar pulp exposure in 18 germ free rats were left open in a germ free environment. All molar sections in the conventional rats revealed, on the eighth day, progressive pulp necrosis. Microbial colonies were usually observed. After eight days, all exposed molar specimens revealed complete pulpal necrosis with chronic inflammation and abscess formation in the apical areas. Some specimens revealed microorganisms in the tissues of the periapical region. In marked contrast, all molar specimens of germ free rats revealed no sign of apical abscess formation. Pulpal inflammation was minimal, with complete dentinal bridging noted

in older specimens. From these studies, it was concluded that the pulp is readily injured by microorganisms and that infection of the pulp is a common, if not the major, cause of pulp disease. The studies failed to show that all microorganisms, regardless of their type and number and regardless of the associated environmental conditions, are capable of producing this effect. They showed only that microorganisms as a group play a role.

Three general routes of infection exist: Direct, periodontal, and vascular. The direct route of infection is the primary form of contamination of the pulp, with dental decay being the chief offender. Bacteria and their toxic by-products may initially invade the pulp via the dentinal tubules due to caries. In addition, traumatic crown fractures, erosion, and iatrogenic factors play a significant role in direct microbial involvement of the pulp. Bender, et al. (1959) have demonstrated penetration of dentin by microorganisms may occur during crown or jacket preparation. Chirnside (1961) also has demonstrated the presence of microorganisms in non-vital dentinal tubules.

The second route of infection is periodontal access. It is postulated that microorganisms gain access to pulp tissue via apical and lateral foramina of the root. The important considerations in this hypothesis according to Grossman (1967) are pulpal injury and disease, providing a favorable environment for retrogenic microbial invasion from injured periodontal tissues. Entry into the pulp may also be affected by direct and indirect trauma which includes the mechanical procedures of dental treatment. Robinson, et al. (1950) and Cobe (1954) report that exodontic procedures, prophylaxis and even toothbrushing can cause a transient bacteremia.

Seltzer and Bender (1965) hypothesize that retrogenic pulpitis can occur in the presence of severe periodontitis where intra-alveolar pocket formation exists with access taking place through lateral pulp canals.

The third route of infection is the vascular route where it is postulated that blood-borne bacteria can localize at the site of injured and diseased pulpal and periapical tissue. This phenomenon of bacterial localization at the site of diseased or injured tissue, in association with bacteremia, is termed anachoresis. This phenomenon, according to Zeldow (1965), may explain the relatively common occurrence of an acute exacerbation in an intact non-vital tooth of long duration. Csernyei (1939), Robinson and Boling (1941), Boling and Robinson (1942), Burke and Knighton (1960), and more recently Gier and Mitchell (1968) have conducted significant studies demonstrating this phenomenon of bacterial localization at sites of inflammed tissue.

Matusow (1967) suggests a variety of factors influence the isolation of microorganisms from diseased pulpal-periapical tissues. These include systemic considerations, character of teeth treated, and diagnostic procedures. Systemic considerations could have significance in the isolation of organisms in that systemic infections disease and its characteristic microorganism could be isolated. Brodsky and Klatell (1943) have provided a classic example of the above systemic manifestation. In a series of 362 specimens of periapical lesions from tuberculosis patients, 8 per cent were reported as having pathoses associated with Mycobacterium tuberculosis.

The character or physical condition of teeth treated was significant in terms of the microbial flora isolated. The bacteriologic status

of the root canal in the intact tooth differs from that found in the pulp chamber exposed to its environment. Crawford and Shankle (1961) indicate the flora of the infected root canal and mouth are not identical and that there is a tendency of the flora in the open root canal to simulate more closely the oral microbiota than in the intact infected root canal. In addition, mixed infection is almost universally found in the open canal. Slack (1958) found pure cultures (one organism) in 78.7 per cent of 514 teeth, two organisms in 18.4 per cent, and three organisms in 2.7 per cent of the cases making a total of 21.1 per cent of cases in which there were mixed organisms. In a study of 360 positive cultures from root canals Sciaky and Sultzenu (1961) found two or more organisms in 40 per cent of the cases. Streptococci and staphylococci were most often in combination representing 30 per cent of all mixed cultures, whereas in the infected intact tooth the incidence of pure culture in the root canal is increased. Sulitzeanu (1964) et al. reported about one-fourth of the intact cases yielded cultures of single bacterial strain.

The classification and incidence of microorganisms isolated from the root canals of non-vital teeth represent a major part of the total oral microbial flora. Matusow (1967) in a review of the literature stated these include streptococci, staphylococci, gram-negative and positive cocci, diptheroids, yeast, actinomycetes, lactobacilli, bacteroids, fusiforms, spirochetes, clostridia, coliforms, mycoplasma, and acid-fast microorganisms. Various species may exist as aerobes, obligate anaerobes, and facultative anaerobes. They may occur as contaminants, saprophytes, or pathogenic infectious agents.

Zeldow (1965) has compiled the results of studies by MacDonald,

Hare, and Wood (1957), Mazzarella, Hedman and Brown (1955), Brown and Rudolph (1957), and Winkler and van Amerongen (1959). From 76.4 per cent to 96.4 per cent of the organisms isolated from vital and necrotic pulps of intact teeth are gram-positive. Streptococci (all species, Beta primarily) constituted from 27.8 per cent to 62.9 per cent. Staphylococci constituted 14.4 per cent to 22.5 per cent in these studies. Occasionally, diptheroids, yeasts, lactobacilli, fusiform bacteria, spirochetes, filamentous forms and Bacillus species are isolated. Gram-negative organisms have been consistently found, but much less frequently--3.6 per cent to 23.6 per cent in the above studies. Such organisms as the fusobacterium bacteroides, coli-aerogenes group and pseudomonas have been isolated from infected root canals.

An interesting phenomenon is the detection of spirochetes in the root canal microbiota. Hampp (1957) as an extension of Brown and Rudolph's study observed spirochetes in 21 of 38 unexposed non-vital teeth. Sulitzeanu, et al. (1964) detected spirochetes in 28 per cent of their smears. Mazzarella, et al. (1955) found spirochetes in 13 of 21 samples studied. Zeldow (1965) notes these organisms seemed usually to occur in association with other microorganisms suggesting they may be secondary invaders.

Slack (1958) compiled the studies of Burkett (1938), Grossman (1952), Slack (1953) and Slack (1957). Streptococci constituted 50 per cent to 62 per cent of the specimens while staphylocci 16 per cent to 25 per cent of the total. An interesting finding was that of Grossman (1952) who found 16 per cent yeasts which was reduced to 7 per cent in a later study (1955). Gram-negative organisms ranged from 6 per cent to 9 per cent in the above studies. Substantially similar results have been obtained by Leavitt,

Naidorf and Shugaevsky (1958) for the main groups of organisms.

The anaerobe forms a significant portion of the flora isolated from pulpless teeth. Brown and Rudolph (1957) demonstrated that approximately 25 per cent of the bacterial strains isolated from pulpless teeth are obligate anaerobes. MacDonald, Hare, and Wood (1964) showed 32 per cent while Leavitt, et al. (1958) reported anaerobes in approximately 33 per cent of the infected canals. Sulitzeanu, et al. (1964) reported 25 per cent of specimens studied were obligatory anaerobic bacteria.

It is obvious that there are certain discrepancies in the above studies but it is also obvious that there is agreement on certain basic points such as the predominance of the gram-positive organism and that the anaerobe is an important part of the flora isolated from the root canal.

These discrepancies depend in part upon several experimental variables. Zeldow (1965) includes among these variables the selection of the medium for primary isolation, the physical integrity of the tooth, the technique used for obtaining a representative sample, and whether the specimen was obtained immediately upon opening into the pulp chamber, after instrumentation, or at a subsequent visit. It is apparent that the frequency of microbial isolation can vary with culturing and microscopy procedures and thus is limited only by the scope and sophistication of the laboratory procedures utilized.

The relation of the microbial flora of root canals to pulp infection depends partially on the state of diseased root canal tissue. Pulpal inflammation and progressive disease arise from bacterial, traumatic, or chemical factors. Matusow states the diseased pulp tissue can provide a

suitable environment for microbial existence and growth. A balance in the numbers of microorganisms and their virulence, mediated by the resistance of the host, help determine the severity of infectious disease according to Ingle (1963), thus obviously the numerical reduction of infectious microbes during root canal debridement may allow the elimination of residual microbes by host defense mechanisms. Accepting the above concept thus leads into the second phase of the role of microorganisms in endodontic disease as defined by Torneck--that of microorganisms preventing or impeding post-treatment repairs considered a secondary effect.

Fish (1939) performed a monumental experiment in which he provided a definitive rationale for endodontic treatment. He established foci of infection in the jaws of guinea pigs by drilling holes in the mandibles and inserting cotton pledgets saturated with a broth culture of Staphylococcus aureus, or alpha-streptococcus, or beta-hemolytic streptococcus. The animals were sacrificed after varying periods of time, from 4 to 46 days, and what he found in histologic examination of the tissues is of great importance in defining the role of microorganisms in bone infection and repair. Four distinct yet interrelated areas around the focus of infection which he established in the bone were visualized, each one having a characteristic type of cell. In the focus itself were microorganisms, and at the periphery of the focus were polymorphonuclear leukocytes. This area is called the zone of infection. Beyond the zone of infection leukocytes could be seen, bone cells were destroyed in this area, and in many places, the lacunae were empty. This was the zone of contamination. Around it was still another zone characterized by osteo-

clasts, which were digesting bone, and histiocytes, which were dissolving the collagen framework. This area was termed the zone of irritation. Finally, in the most peripheral area the characteristic cells were the osteoblasts and fibroblasts; this area was called the zone of stimulation.

The significance of Fish's study from an endodontic standpoint is profound in that it explains the disease process occurring in bone and repair following endodontic treatment. Grossman (1967) interprets the above experiment to mean that the zone of infection is in the root canal and that so long as this zone exists, zones of contamination and irritation with breakdown of bone will develop, and will manifest itself in the form of periapical pathology. When the microorganisms in the root canal are eliminated, repair of the periapical tissues will occur. The periapical tissue therefore is essentially sterile because as the microorganisms grow out of the root canal and into the periapical tissue, they generally are destroyed by the polymorphonuclear leukocytes. Thus, when an area of rarefaction is present, it does not mean that the bone is infected. In a series of 109 cases in which areas of rarefaction were present requiring root resection, Grossman (1959) isolated microorganisms from the periapical bone in only 15 per cent of the cases. In an earlier study, Hedman (1951) attempted to determine the relationship between pulpal and periapical infection in intact teeth manifesting pulpal-periapical pathogenesis. He used a cannula culture technique and revealed several significant observations. The first was that viable bacteria were isolated in both pulpal and periapical areas in 68.5 per cent of the cases; 23 per cent were negative. The second was that viable bacteria were isolated in the pulp canal but not from the periapical areas in 8.5 per cent of the

cases. The third observation was that all cases demonstrating streptococci in the pulp canals also revealed streptococci in the periapical areas. The last observation was that the elimination of the viable microbes in the pulp canal during root canal treatment resulted in negative cultures from the periapical areas.

The obvious conclusions according to Matusow (1967) that can be drawn from the above observations are the following: 1) the pathological sequence of events in the pulp leading to periapical pathosis may be caused by physical and chemical factors without microbial involvement; and 2) that periapical pathosis cannot be unequivocably related to infection.

It is obvious then that bacteriologic control must be a significant factor in the ultimate healing of that tooth undergoing endodontic therapy.

The primary tool used in bacteriologic control is the culture. As used in endodontics, it provides a means of determining the microbiologic status of diseased root canals. As generally interpreted, a negative culture indicates effective removal of the microorganisms producing endodontic infection. Bender, et al. (1964) questions the validity of culturing as a routine procedure and has raised significant questions as to its value as a routine procedure.

With Onderdonk (1901) came the first recommendation of a routine culture test prior to filling the root canal. As the technique found favor, the second, third and fourth decades witnessed its significant role in successfully refuting the indictment of pulpless teeth as foci of infection.

Logan (1937) commented that:

> ...the terms 'presence' of microorganisms, and 'infection' are not to be regarded as synonyms. The finding of bacteria in a tissue does not necessarily indicate that the organ or a tissue is infected. Bacteria are often present in normal tissues without having pathological significance.

Hatton (1931) and Dixon and Rickert (1938) had demonstrated histologically that pulpless teeth properly treated and filled were not a source of infection.

Prior to 1940, teeth showing periapical changes roentgenographically were considered to be infected by such investigators as Haden (1925) and Cramer and Reith (1932). Ennis (1931) even stated that some lesions diagnosed roentgenographically could be linked to certain species of streptococci. The above studies reported were based on cultures of extracted teeth which were generally invalidated by Fish's study of 1936.

Sommer and Crowley (1940) showed that the roentgenographic diagnosis of pulp-involved teeth did not agree with bacteriologic cultures obtained from such teeth in situ. Negative cultures were obtained from all types of roentgenographic diagnosed lesions. It was also noted that positive cultures were obtained from pulp-involved teeth which appeared roentgenographically normal. The types of organisms isolated from different periapical lesions had no relation to the type of lesion. It was obvious from the above study that within the limits of the culture technique that one could not diagnose infection from an x-ray only.

Morse and Yates (1941) reported 53 per cent negative cultures of cases showing periapical involvement by roentgenographs. Morse and Yates (1942) found that a negative culture is often obtained on first opening into a root canal. Ostrander and Crowley (1948) further reported that

38.6 per cent of cases studied showing periapical involvement were sterile with limits of the technique, while 42.6 per cent showing no periapical involvement were likewise negative within the limits of the technique. No correlation between clinical symptoms and bacterial cultures could be made. Bender (1954) has found that 38 per cent of cases showing periapical involvement gave a negative culture. Bender conjectured from this that areas of rarefaction in the apical region can be produced by chemical and mechanical irritations as well as bacterial means.

It is apparent that a significant number of cases may be sterile within the limits of the culture technique when first seen by the dentist. An empirical method that has been used to determine if a tooth can be filled is the detection of an odor by the operator. Grossman (1936) generally invalidated the concept when he reported no correlation existed between the presence of odor, character of exudate, and the presence of microorganisms in the canal.

Many studies have been performed to determine if teeth that have demonstrated a negative culture prior to filling, reveal a high degree of clinical and roentgenographic success. Appleton (1932) analyzed data published by Rhein, Krasnow and Gries (1926) using roentgenographic interpretation as the criteria. There was a 9 per cent increase in failure in those cases filled with a positive bacteriological culture. Buchbinder (1941) on the basis of radiographic evidence, found a 10 per cent increase in failure. Abramson (1961) reported an increase in failure of 12.4 per cent. Zeldow and Ingle (1961-1963) on the basis of radiographic and clinical criteria reported a significant increase in failure rate of 11.2 per cent. Oliet (1962) reported an increase in failure rate of

15 per cent.

The above selected studies show a correlation between clinical and roentgenographic success and a negative culture. Seltzer, et al. (1963-1964) indicated that after a two year observation period there appeared to be no difference in the overall repair of periapical tissue following endodontic treatment of the root canal, despite the presence or absence of microorganisms as shown by preobliteration culture testing.

It was reported, however, that under certain treatment conditions a delay in the healing of periapical areas of rarefaction occurred in the first six months. The investigators subsequently concluded that where differences in incidence of healing did occur, they were primarily attributable to the type of root canal filling used, the apical position of the root canal filling, the preoperative status of the periodontium, and variances in the operating ability of the clinician and not to the presence or absence of residual microorganisms. The investigators were cautious to point out, however, that their conclusions were based solely on the admitted limited accuracy of the dental radiograph and that a reduction in size and not the complete elimination of an area of rarefying osteitis was one of the criteria used for determining success.

It is apparent that the correlation of negative cultures to endodontic success is very much in question and such factors as microbial concentration and virulence, host resistance, and various endodontic techniques preclude a significant correlation of negative or positive culture with clinical success.

Myers, et al. (1969) reported a study in which culture reversal was noted immediately prior to obliteration which would certainly cast

some doubt upon the validity of the cultures of many of the above studies. Root canals scheduled to be filled following one negative culture yielded 25.9 per cent positive cultures when cultured again at the time of root filling. Root canals filled following two successive negative cultures produced a reversal rate of 13.2 per cent. Bender and Seltzer (1964) reported similarly when they revealed that among the root canals of teeth which in the previous visit had yielded a negative culture, 16.6 per cent yielded positive cultures immediately prior to the filling of the canal.

In order for studies such as the above to be valid, the accuracy of the culture technique as a means of determining the presence of microorganisms must be established. There certainly is evidence that there is a significant margin of error in the accuracy of culture tests in reflecting the microbiological status of the root canal. Ingle and Zeldow (1953) stated that there is a 4.6 per cent minimum probability error of this technique to depict a true negative culture when incubated for 48 hours. The authors concluded that this 4.6 per cent was due to the error introduced by virtue of sampling. If the paper point did not contact the organisms in the canal, or if too few bacteria are present in the inoculum, as may be the case immediately after instrumentation and irrigation, a false negative culture may be obtained. Slack (1958) gives several weaknesses of the bacteriological test. They are the following: 1) the inoculum may not be a true sample of the canal, 2) the length of time taken to obtain the results, and 3) the possibility that the medium used may not support the growth of the more fastidious bacteria. It may be that the most valid use of the culture within its technical limits in endodontics is that of antibiotic sensitivity testing as suggested by

Zeldow and Ingle (1962) and Goldman and Pearson (1962).

A histological study relating the effect that residual microorganisms have on influencing periapical repair has been reported by Seltzer, et al. (1964). It showed no differences in the rate or type of periapical repair following the completion of root canal therapy in dogs' teeth, despite the presence or absence of residual microorganisms. However, caution must be exercised in directly relating the results of animal experiments to man, since experimental animals are not always susceptible to microorganisms pathogenic to man and animals do not always respond in the way man responds immunologically.

Thus the entire subject of the residual effect of microorganisms is at an impasse on an academic basis and Torneck (1969) has summarized the subject well by stating:

1. There are several etiological factors which can delay or prevent healing in the periapical tissues following treatment of the root canal. One of these factors is the microorganism. However, the effect of these microorganisms seems to be secondary and cumulative. They must co-exist with some other factor that would prevent repair, but once so established, the microorganisms would augment the difficulty.
2. Not all microorganisms or groups of microorganisms or their by-products will have this influence. Even among these that do have this influence, a critical number must be present. The type and number may vary as to the pre-existing or concurring environmental conditions.

II. Role of Medicament

At the beginning of the twentieth century, the role of the drug in root canal therapy was based upon its strength and penetrability and the greater the strength of the drug, the more desirable it was. Due to the lack of standardization of instruments and minimal or missing roentgenographic examination, the debridement of the root canal was performed on a most primitive basis, and because the usual disinfectants of the time--carbolic acid, formalin, creosote, arsenic and sulfuric acid and others--seemed to provide palliative relief on a temporary basis, they were used extensively with little or no regard for possible injury to periapical areas.

Buckley introduced formocresol in 1906 and recommended it for chemical control of the gaseous products of pulp decomposition and as a strong disinfectant for the treatment of all pulpless teeth. Its use was opposed by some investigators (Grove, 1913) because of the effect of formalin on living tissue cells in the periapical area. Chlorophenol, recommended by Walkhoff in 1891 in Germany was introduced in the United States by Prinz in 1899. This was the first strong germicide to enter the field of endodontics which could be used with relatively little tissue damage in the periapical area. It still is highly recommended currently as a non-specific germicide (Schilder, 1965, Ostrander, 1966, and Luebke, 1967).

Electromedication was popularized in America by Rhein (1897). Dr. Rhein had avoided the use of extreme caustics because of his concern

about the damaging of the periapical tissues and the influence of this pioneer in encouraging the use of electromedication was considerable. Sommer (1953) states that electromedication was an important step in endodontic treatment because it aided the movement away from strong caustic medication and also helped to refute the "foci of infection" theory which resulted in many unnecessary tooth extractions in the early part of the twentieth century. However, Ostrander (1958) states that as new effective antibacterial agents and germicides were developed, the time consuming disadvantage of the electromedication technique became the chief reason for its disfavor in use.

It is apparent during the era of the 1920's and 1930's and 1940's that the basic premise for the use of the intracanal drug had changed from that of a chemical premedication where Buckley was concerned primarily with neutralizing the end products of pulp breakdown, with bacteria still considered secondary, to the role of the drug seen today as an effective antibacterial drug used in concert with debridement and irrigation.

The drugs mentioned previously were of the non-specific type which are general protoplasmic poisons which act by destroying cell proteins. Most of these drugs were oily liquids of relatively low surface tension. They are effective against a wide spectrum of microorganisms depending on the individual efficacy of the medicament. However, in the 1940's and early 1950's as the role of the intracanal drug changed to that of an anti-microbial agent rather than a cauterizing agent, there was a trend to the use of specific chemotherapeutic agents in an attempt to employ therapeutic agents that would be specific against the flora commonly found in the root canal.

Adams (1943) used a hot solution of sulfanilamide, the active ingredient of prontosil, and this was one of the first instances of a chemotherapeutic agent used as an intracanal medicament. Casey, Gurney and Rapp (1947) developed a derivative of sulfanilamide, sulfamylon, which they named Benzylog. More recent suggested uses of sulfa derivatives are by Best, et al. (1949) who suggested Micro-cide A (formerly known as Endocide) and regarded it as equal to or superior to previously existing endodontic bactericidal agents. Frank, et al. (1968) reported a favorable double-blind study in the clinical effectiveness of sulfathiazole. The sulfa derivatives in light of present clinical usage, apparently require a thorough re-evaluation as to their role as intracanal medicaments.

As the antibiotics developed, it was only natural for the dentist to use these new drugs in the root canals. Among many others, Ostrander, et al. (1947) and Grossman (1948) used penicillin as an intracanal drug, but it was soon discovered that the individual antibiotics were highly selective in action and their spectrum of activity very limited (Dinin, 1955). Thus as new antibiotics were developed, they were quickly combined into antibiotic pastes and were used in endodontics. Bender and Seltzer (1950) advocated the use of streptomycin and chloramphenicol in propylene glycol. Stewart (1953) introduced a combination of perazil (an antihistamine), penicillin, chloromycetin, and propylene glycol in neobase or xylocaine ointment. The most popular paste was Grossman's PBSC (1951), which consisted of a combination of penicillin, bacitracin, streptomycin, and caprylate sodium in a silicone base. The rationale for the use of these drugs was that since most antibiotics have a limited spectrum of action, it became obvious that the intracanal use of a single antibiotic

might lead to abundant overgrowth of nonsusceptible organisms. Laboratory and clinical experimentation led to the development of several polyantibiotic mixtures effective against organisms encountered in the root canal.

However, for several reasons, there was some disenchantment with the use of the polyantibiotic pastes. The first was concerning the use of antibiotics on a topical basis. Disadvantages against the use of topical antibiotic therapy were: 1) the possibility of sensitizing patients to penicillin or other antibiotics sealed into the canal, 2) the possibility of producing reactions in patients who are already sensitized to penicillin or other antibiotics. Bender and Seltzer (1954) reported two known reactions in more than 2,500 cases. Pirila (1960) reported a reaction to bacitracin-neomycin and Fox and Moodnik (1964) reported a systemic reaction to PBSC. 3) Direct drug toxicity is significant for particularly two drugs: The first is streptomycin which can affect the eighth cranial nerve, and the second is chloramphenicol which has been indicated as the drug responsible for several cases of aplastic anemia (Jawetz, 1955). 4) The causing of superimposed infections in the form of either antibiotic resistant organisms or in the form of fungal overgrowth (Zeldow and Ingle, 1962, Goldman and Pearson, 1962, 1969, Goldberg, 1970), and 5) it was also found that PBSC will produce irritant effects comparable to, or even slightly greater than some of, the common non-specific drugs such as camphorated paramonochlorophenol (CMC) and metacresyl acetate (Cresatin) (Schilder and Amsterdam, 1959, and Torneck, 1961).

The second reason was the high rate of the false-negative culture. Bender and Seltzer (1954) showed that when a polyantibiotic mixture was used, cultures read at the end of 48 hours were 31 per cent inaccurate,

and that even at the end of a week, there were still 13 per cent false negative cultures.

The advantages of using inactivators is limited because penicillinase in solution is not stable at room temperature for more than one week. Also, the inactivation for bacitracin is limited. Streptomycin has complete inactivation only after several hours in the culture media (Grossman, 1970). Buchbinder and Bartels (1951) also demonstrated a broad-spectrum antibiotic can be active in the root canals for as long as two weeks following its application, and that it could be carried over into culture media.

The third reason was particularly interesting in that several investigators found no significant advantage in the use of polyantibiotics as compared to the non-specific type of drug. Ostrander (1958) stated that his results with the antibiotic combinations have not been outstanding as compared with results with non-specific drugs. Zeldow (1959) also reported no statistically significant difference in the rapidity with which negative cultures were obtained when a polyantibiotic mix was used as compared to the use of camphorated parachlorophenol.

Fox and Isenberg (1967) suggest that antibiotics should be used in endodontics, as in other fields of medicine and dentistry, only after identification and susceptibility studies have been performed, so that the proper antibiotic may be selected for maximum effectiveness. They suggest also that in the interim required for these studies, treatment with the non-specific chemical agents may be employed. If there is laboratory or clinical evidence of the inadequacy of these chemical agents, treatment with specific antibiotics, in adequate doses, may then

be instituted. Lane (1955) went so far as to state that the dangers of development of antibiotic resistant strains may destroy any possible advantages gained by the use of these agents for root canal sterilization.

During this same era of the 1950's, as Glick (1968) has noted, Auerbach (1953) helped retard the rush to combine various drugs by re-emphasizing the importance of meticulous cleansing of the canal rather than depending solely upon drugs. Auerbach found 78 per cent negative cultures after debridement and irrigation with a chlorinated soda solution only. The importance of mechanical cleaning and debridement has also been stressed by others. Stewart (1955) found 78 per cent of teeth that originally gave positive cultures upon initial opening gave negative cultures after chemomechanical debridement.

Goldman and Pearson (1962) showed 82 per cent negative cultures after thorough chemomechanical cleansing of the canal. Grossman (1953) in a reply to Auerbach's condemnation of PBSC, restated that biomechanical instrumentation is the most important phase of root canal therapy and that adequate mechanical preparation of the root canal rather than reliance upon antiseptics cannot be stressed too strongly. Stewart, Cobe, and Rappaport (1961) showed a 97.7 per cent rate of negative cultures after initial chemomechanical cleansing and 65.7 per cent negative cultures with the initial culture at the second visit. Stewart, Kapsimalas, and Rappaport (1969) obtained a 97.2 per cent negative culture at the end of the chemomechanical cleansing using an EDTA-urea peroxide combination, and a 94.4 per cent negative culture rate with the initial culture at the second visit. Marshall, Massler, and Dute (1960) reported an interesting study in which hydrogen peroxide and sodium hypochlorite solutions, used

alternately, produced a significant increase in dentine permeability to isotopes. Hydrogen peroxide alone and sodium hypochlorite alone also increased dentine permeability, but to a much lesser degree than when the two medicaments were combined. The above study is of obvious significance when the dentist is using an intracanal medicament which depends upon diffusion through the root canal to perform its function. These studies do not belittle the importance of medication, but rather emphasize the importance of biomechanical cleansing of the canals. Winkler and van Amerongen (1959) state the above premise well when they state that what is taken out of the canal is at least as important as what is put in it.

In determining the role of the intracanal medicament today, Luebke (1967) prefers the term "pulp cavity disinfection" rather than "root canal sterilization." He bases this conclusion upon the apparent fact that, regardless of method of treatment, the truly sterile pulp cavity is a rarity. As Appleton (1953) has stated, negative cultures cannot be considered as absolute values in the sense that all bacteria have been destroyed. It merely suggests that the number of microorganisms has been reduced; thus, the body may more easily dispose of any surviving bacteria. Thus when instrumentation and irrigation have physically removed the microorganisms in the root canals and the substrate upon which they live, circumstances are right for the placement of intracanal drugs for effective disinfection. Luebke assents that canal medication is a "holding action" between appointments rather than a sterilizing process eliminating all bacteria. The rationale for the above statement is the assumption that drugs sealed into a clean pulp cavity will diffuse from the pulp chamber

into the canals, maintaining asepsis while the bacteria in the periapical soft tissues are being eliminated by phagocytic and immune mechanisms.

It is apparent that in the selection of an intracanal drug, there must be other properties considered in addition to antimicrobial activity. Schilder (1965) and Luebke (1967) urge the selection of the canal dressing be placed on the pharmacological rather than on the antimicrobial properties of the drugs. Schilder states that post-operative "flare-up" is more often caused by over-instrumentation and over-medicating, and only occasionally by infection. Thus the role of the drug is such that while maintaining antimicrobial efficiency, it should be selected on the basis of tissue tolerance so as to avoid irritation of periapical tissues. It should also encompass anodyne or sedative properties so as to provide palliative therapy to residual pulp and/or inflammed periapical tissues.

III. Development and Rationale of Use of CMP

Camphorated paramonochlorophenol, metacresyl acetate, and prednisolone in combination is known as CMP and was first described by Fry, Watkins, and Phatak (1960) and later employed by Mosteller (1962 and 1963). The drug formula is an altered eutectic combination composed of a non-specific germicide mixture to which a 1 per cent by weight amount of prednisolone-USP is added. The formula consists of the following components:

1. Paramonochlorophenol--25 per cent by weight
2. Metacresyl acetate--25 per cent by weight, hereafter known as Cresatin
3. Gum camphor-USP--50 per cent by weight
4. Prednisolone-USP--1 per cent by weight.

The medication has the odor and viscosity of camphorated paramonochlorophenol. Brady (1970 pers. comm.) states the shelf life is at least one year.

The above non-specific germicide combination without the prednisolone is commercially known as XP-7. Dietz (1957) reported that XP-7 is compatible with 33 per cent hydrogen dioxide-ethereal and sodium hypochlorite solutions. It has excellent penetrability which is ascribed to the depolymerization action of the Cresatin which also contributes anodyne properties to the formula. Dietz also reports that XP-7 possesses an extremely low surface tension and negligible volatility. Cummings and Hockin (1970 pers. comm.) indicated the inflammatory potential of XP-7 is less than that of camphorated paramonochlorophenol (CMC).

The corticosteroid component was first added to the germicide formula by Fry, et al., for the relief of dentinal and pulpal pain hypersensitivity and for conserving the vitality of exposed dentin and pulp tissue. A rationale for the specific use of prednisolone as the corticosteroid component is that Stewart (1958) stated that prednisolone is believed to have much less mineral corticoid activity and a much greater anti-inflammatory effect than hydrocortisone. Laskin and Kolodny (1965) found prednisolone to be much more effective than hydrocortisone in inhibiting exudate formation in rats. Schroeder (1965) stated prednisolone represented a considerable advance over cortisone and hydrocortisone. It had been possible to secure a substantial enhancement of the desired action and at the same time a reduction of the less welcome side effects.

Mosteller recommended CMP be used prophylactically on exposed dentin to prevent thermal sensitivity. Stanley, Swerdlow and Driscoll (1965) used

CMP as a pulp-capping agent and suggested that the germicide vehicle and the prednisolone acted synergistically, the prednisolone capable of modifying the initial inflammatory response within the pulp but the vehicle being necessary to maintain it so. Despite the application of steroid medication, the phenomenon of tissue repair was not hindered at these dosage levels and number of applications.

Wolfsohn (1954) used hydrocortisone acetate in the root canal after treatment by the electrosterilization procedure. He reported that in the presence of overt infection in the periapical tissues and in the pulp canal, the hormone did not seem to be effective, and his impression was that it actually was responsible for the exacerbation of the disease process in a significant number of cases. He also suggested continued research to evaluate the effectiveness of hydrocortisone in combination with other available drugs and the antibiotic suspensions.

The rationale for the inclusion of an antimicrobial agent in therapy when a corticosteroid is administered in the presence of infection has been demonstrated repeatedly. Stewart and Chilton (1958) demonstrated that failure to take this precaution in endodontic and periodontic surgery resulted in the development of severe cellulitis requiring external incision and drainage.

Keefer (1954) recommended that a corticosteroid should be administered with an effective antimicrobial agent. Hendershot (1963) stated anti-infectives are necessary because the resistance of tissues to infection can be markedly lowered by the corticosteroids. Schroeder (1963) emphasized that, due to the interference of the defense mechanism of the body, corticoid therapy must be supplemented by an antimicrobial agent.

The rationale for the use of the corticosteroid to combat inflammation is that vascular permeability is reduced, thereby limiting fluid accumulation. The corticosteroids have been used because of their ability to suppress inflammation, regardless of its etiology. Corticosteroids preserve vascular integrity, thereby restoring capillary permeability to normal and suppressing the cellular exudate. There is increased vascular tone, decreased loss of fluid and cells from the blood vessels and diminished adherence of leucocytes to endothelium. Hendershot (1963) stated that the corticosteroids can inhibit the growth of mesenchymal tissues when administered on a sustained basis. Thus these drugs impair vascularization and interfere with the formation of fibroblasts, granulation tissue, and ground substance. Menkin (1953) stated that cortisone can suppress the inflammatory response in a localized infected area and can allow microorganisms to multiply freely because of the antiphlogistic effects of corticosteroid therapy. This is the result of the reduced capacity for localization of organisms and the reduced influx of phagocytes. This presumably is accomplished by inhibiting the action of leukotaxine in increasing capillary permeability and in inducing leucocytic migration. Bacterial fixation is of interest, and Menkin (1940) considered the fixation primarily due to mechanical obstruction caused by a fibrin network and thrombosis of efferent lymphatics.

Thomas (1965) explains this mechanism on the basis of lysosomes. He states that lysosomes are bags of fluid, enclosed in a semi-permeable membrane, which are kept in a stable state by a corticosteroid. Granules of polymorphonuclear leucocytes have a similar enzymatic effect and probably even have the same composition. Lysosomes contain acid hydrolases

which become active on disruption of the membrane and are concerned with autolysis and digestion of cell metabolites as well as damage to tissue as in inflammation. There are at least a dozen hydrolases implicated in the process, varying in number and kind from tissue to tissue. The principle enzymes are acid phosphatases, beta-glucuronidases, and cathepsin.

Bunim (1960), however, states that in a properly nourished individual receiving conventional therapeutic dosages of corticosteroids, no significant interference with normal wound healing following surgery or trauma is usually encountered.

Oversuppression or modification of the inflammatory response is very significant in that the ability of the inflammatory cells to phagocytize the periapical area of the tooth is limited and thus may result in infection. It is therefore pertinent to note that the average dosage of prednisolone within the germicide vehicle per endodontic treatment is approximately 1/10 mg which is a decidedly minute amount when compared to systemic dosage even on a dosage/weight basis. This amount of corticosteroid was determined on the basis of Stewart's volumetric studies (1948) which determined that the average volume of medication ranged from 0.0088 ml in the mandibular central incisor to that of the maxillary first molar which was 0.0412 ml.

The CMP formula has a weight of approximately 1.02 gm/ml and with the average volume of medication being 0.0088 ml to 0.0412 ml, the approximate amount of prednisolone as mentioned is 1/10 mg to 4/10 mg maximum.

There are several contraindications to the use of corticosteroids even in very small quantities. Hendershot (1963) states an absolute contraindication to the use of corticosteroids is the presence of infection

which cannot be controlled with chemotherapeutic agents. Tuberculosis, even if arrested, and some viral diseases are among these conditions. The systemic administration of corticosteroids should be avoided in persons with psychoses, severe psychoneuroses, or peptic ulcer. The systemic use of these drugs is also contraindicated in persons with diabetes, renal or cardiac disease, thrombophlebitis, convulsive disorders or osteoporosis. Dental procedures have been indicted as a factor in the occurrence and/or relapse of rheumatic heart disease and/or subacute bacterial endocarditis. The use of corticosteroids is contraindicated in this instance because there is no absolute assurance that the corticosteroid does not significantly reduce immunological activity, even in such minute dosages.

The subject of bacteremia as a result of pulpal and endodontic manipulation must be discussed in reference to the root canal because of possible systemic effects. Bender and Pressman (1945), Robinson, et al. (1950), Rhoades, Schram, and Adair (1950), Cobe (1954), and Eisenbund (1962), have shown that the manipulation of oral tissue produces transitory bacteremias of short duration which may result in serious embolic processes such as subacute bacterial endocarditis, in a significant number of patients. These investigations included the effects of trauma by rocking of teeth, prophylaxis, chewing, toothbrushing, exodontia, and surgical procedures.

Klotz, Gerstein, and Bahn (1965) reported a significant finding when they found a systemic bacteremia in 21 per cent of the patients whose pulp exposures were experimentally treated with Streptococcus faecalis and prednisolone. Previous studies had indicated that bacteremia of pulpal origin was only possible when the periapical areas were trauma-

tized, Beechen, Laston, and Garbarino (1956) reported no significant bacteremia occurred during or following vital pulpotomy. Kennedy, Hamilton, and Syverton (1957) reported an interesting study in which hemolytic streptococci were introduced into root canals of monkeys. After removal of the pulp, the root canal was reamed and filed; instruments were carried beyond the end of the tooth to traumatize the periapical area. The root canal was then inoculated. It was shown that transient bacteremias could be produced by such inoculation and that the inoculated organisms were recoverable from root canals at terminal examination. However, and most significantly, the experimental treatment did not result in cardiac changes typical of rheumatic heart disease. Bender, Seltzer, and Yermish (1960) reported a study in which the incidence of bacteremia following vigorous filing for ten minutes under non-sterile conditions was 12 per cent in 50 cases. If the manipulation was kept within the confines of the root canal, there was no demonstrable bacteremia in 26 cases. Of 24 cases in which manipulation was purposely done beyond the apex of the canal, six (25 per cent) displayed positive blood cultures. Blood samples taken ten minutes after endodontic manipulation revealed no positive blood cultures in the 50 cases thus indicating a short transitory bacteremia. Cobe (1954) stated that ten minutes appears to be the maximum duration of transitory bacteremia. Bender, et al., interpreted these findings to mean that for patients with a history of valvular heart disease, endodontics should be the treatment of choice whenever possible.

Since Klotz, Gerstein, and Bahn did not inflict periapical trauma in the pulpally exposed teeth, they interpret the etiologic factor responsible for the bacteremia to be the prednisolone. This work suggests the

use of an antimicrobial agent in addition to the prednisolone when treating open pulps might be indicated. Additional findings by de Deus and Han (1967) have shown that tritiated cortisone after application in the exposed dental pulps of hamster incisions was detected in the liver by radioautographic techniques within two minutes of application.

These previous studies are most significant and cannot be minimized. They show that great caution must be used in the selection of patients for whom a corticosteroid is to be used as there are obvious medical contraindications in that the antiphlogistic action of the corticosteroid may facilitate the liberation of microorganisms into the bloodstream. This provides a rationale for the combining of the corticosteroid with an antimicrobial agent. Klotz (1966) stated it well when he noted that drugs, including the corticosteroids are no panacea but they can be useful tools. From this it is apparent that their judicious and rational use is justified in the hands of well trained, intelligent dentists.

CHAPTER III

METHODS AND MATERIAL

The following non-specific germicides were studied: Camphorated paramonochlorophenol (CMC), metacresyl acetate (Cresatin), camphorated paramonochlorophenol and metacresyl acetate in combination (XP-7) and camphorated paramonochlorophenol, metacresyl acetate and prednisolone in combination (CMP). These substances were prepared in the following manner:

1. CMC was prepared on a percentage basis by weight of 35 per cent paramonochlorophenol crystals to 65 per cent USP gum camphor crystals. The crystals were mixed together to form a eutectic solution.
2. Cresatin was used in the pure state as metacresyl acetate (which is a colorless, oily liquid with a characteristic phenolic odor).
3. XP-7 was prepared on a percentage basis by weight of 25 per cent paramonochlorophenol crystals, 25 per cent Cresatin, and 50 per cent USP gum camphor crystals. It was prepared in the same manner as CMC to which was added the metacresyl acetate.
4. CMP is composed of the XP-7 formula to which a 1 per cent by weight amount of prednisolone--USP--a powder--is added. This was done according to the scheme of Mosteller (1962) and modified by Brady (1970 pers. comm.). The prednisolone powder may require 24 to 48 hours to dissolve.

The following organisms obtained from the American Type Culture Collection (ATCC) were used for testing:

1. Streptococcus faecalis--ATCC No. 8043
2. Streptococcus salivarius--ATCC No. 13419
3. Staphylococcus aureus--ATCC No. 12600
4. Escherichia coli--ATCC No. 11775
5. Clostridium perfringens--ATCC No. 3624
6. Candida albicans--ATCC No. 10231
7. Nocardia asteroides--ATCC No. 3308

The broth dilution test tube method was used to determine both the minimum inhibitory concentration (bacteriostatic) hereafter known as MIC, and the minimum bactericidal concentration. Spectrophotometric readings were used to measure turbidity for the minimum inhibitory concentration (bacteriostatic) with the exception of Nocardia asteroides which was recorded on a 0-4 basis visually because of the clumping of the organism. Subcultures were used to determine the minimum bactericidal concentration. The dilution scheme was set up as follows:

1. 9.9 ml of Todd Hewitt broth to tube No. 1 and 5 ml broth to the remaining tubes.
2. 0.1 ml of the test medicament was added to tube No. 1 and shaken well.
3. Doubling dilutions were made in the remaining tubes from 1/100 dilution in the first tube to 1/5120 dilution in the tenth tube.
4. The tenth tube was used as a sterility control for the medicament, broth, and technique.

5. Three trials for each organism and medicament combination were made.

The inoculum was set up as follows:

1. A 16-18 hour Todd-Hewitt broth culture of each test organism was diluted to the density of a MacFarland Nephelometer tube No. 1 based upon visual accuracy, and 0.1 ml of the inoculum was added to tubes one through nine.
2. A loopful of the inoculum was spread on a Sheep Blood Agar Petri dish with Difco Blood Agar Base as a control to ascertain if there was possible contamination in the inoculum.

Readings:

A spectrophotometer (Bausch and Lomb Spectronic 20) was used with the reading scale set to measure transmittance between 20 and 80--80 indicating bacteriostasis at a wave-length of 540 nm. A reading for each tube was taken after 24 hours incubation at 37 degrees Centigrade with the exception of Nocardia asteroides which was incubated for 48 hours. Clostridium perfringens--an obligate anaerobe--was incubated anaerobically in a Precision Thelco Anaerobic Incubator which was evacuated to 25 inches of Mercury twice and was washed with a gas mixture composed of 10 per cent CO_2, 10 per cent H_2 and 80 per cent N_2. The methylene blue indicator was used to determine strict anaerobic conditions prevailed.

Subcultures for viability:

After the spectrophotometric readings, one loopful of broth from each tube which had no growth and all tubes which did not have maximum growth was streaked on blood agar plates. These were

incubated for 24 hours at 37 degrees with the same exceptions and variations as noted for the spectrophotometric readings. The observations were recorded as follows:

No growth: NG

Few colonies: +

Moderate: ++

Many: +++

Confluent: ++++

To confirm the validity of the subculture readings, the following technique was performed after the previous subcultures from the third trial had incubated.

1. The last two test tube dilutions which had previously shown no growth, after the 24 hour subculture, were centrifuged.
2. The supernatant broth was removed leaving about 0.5 ml.
3. Four ml of Todd-Hewitt Broth were added; the tube was shaken and centrifuged again.
4. The supernatant broth was removed leaving about 0.5 ml.
5. Four ml of fresh Todd-Hewitt Broth were added and incubated aerobically and anaerobically for the specific microorganisms at 37 degrees Centigrade for 48 hours.
6. Subcultures were taken of all tubes on blood agar plates. These were incubated appropriately as noted above for 48 hours and the observations were measured and recorded.

The MIC (bacteriostatic) and the minimum bactericidal concentration for each drug-microorganism combination was determined for each trial by dividing the weight per milliliter of the drug by the greatest dilution

of the antimicrobial agent which exhibited no visible growth by means of the spectrophotometer or subculture methods. The answer was expressed in micrograms/milliliter.

The readings and observations were measured and recorded. A statistical analysis was performed utilizing the two-way analysis of variance. Significant differences were assumed when the probability (P) was less than 0.01 (one per cent).

CHAPTER IV

RESULTS

The spectrophotometric and subculture readings as recorded in Figures 1-7 are average figures based on three readings for each organism and compound combination studied.

Consistent in spectrophotometric readings in Figures 1-7 is that camphorated paramonochlorophenol (CMC), camphorated paramonochlorophenol and metacresyl acetate in combination (XP-7) and camphorated paramonochlorophenol, metacresyl acetate, and prednisolone in combination (CMP) do not achieve complete transmittance until the 1/800 dilution factor or tube No. 4. These medicaments do not dissolve until some point between 1/400 and 1/800 dilution.

Metacresyl acetate (Cresatin), without exception, achieves complete transmittance at the 1/400 dilution factor or tube No. 3. Cresatin dissolves at some point between 1/200 and 1/400 dilution.

Candida albicans (refer to Figure 1)

Spectrophotometric Readings:

(a) CMC, XP-7, and CMP demonstrated complete transmittance through the 1/1600 dilution factor.

(b) Cresatin demonstrated complete transmittance through the 1/800 dilution factor and very high transmittance through the 1/1600 dilution factor.

Subculture Readings:

(a) The inoculated tubes containing CMC and CMP demonstrated no

growth through the 1/1600 dilution factor.

(b) The inoculated tubes containing XP-7 and Cresatin demonstrated no growth through the 1/800 dilution factor. The tube containing XP-7 demonstrated a few colonies in the 1/1600 dilution factor while the tube containing Cresatin demonstrated many colonies in the 1/1600 dilution factor.

(c) It is significant to note that while the spectrophotometer exhibited lower readings in tubes Nos. 1 through 2 or 3 depending upon the solubility of the medicament, the subcultures without exception show no growth at these dilutions.

Escherichia coli (refer to Figure 2)

Spectrophotometric Readings:

(a) CMC, XP-7, and CMP demonstrated complete transmittance through the 1/1600 dilution factor. CMP on one trial demonstrated bacteriostatic activity through 1/3200 dilution.

(b) Cresatin demonstrated complete transmittance through the 1/800 dilution on two trials and 1/400 on the third trial.

Subculture Readings:

(a) The inoculated tubes containing CMC and XP-7 demonstrated no growth through the 1/1600 dilution factor. The inoculated tubes containing CMP demonstrated no growth through the 1/800 dilution and a few colonies at the 1/1600 dilution. The inoculated tubes containing Cresatin demonstrated no growth at the 1/400 level, moderate growth at the 1/800 level and confluent growth at the 1/1600 dilution factor.

Nocardia asteroides (refer to Figure 3)

Turbidity:

(a) Readings were made on a visual basis utilizing the 0-4 scale as utilized in the subculture. The organism appeared in large clumps and was not amenable to the use of the spectrophotometer.

(b) The inoculated tubes containing CMC, XP-7, and CMP demonstrated no visible growth through the 1/1600 dilution factor while those containing Cresatin demonstrated no visible growth through the 1/800 dilution level.

Subculture Readings:

(a) The inoculated tubes containing CMC, XP-7, and CMP demonstrated no visible growth through the 1/1600 dilution factor while those containing Cresatin demonstrated no visible growth through the 1/800 dilution level.

Staphylococcus aureus (refer to Figure 4)

Spectrophotometric Readings:

(a) CMC and XP-7 demonstrated complete transmittance through the 1/1600 dilution factor. CMP demonstrated complete transmittance on one trial at the 1/1600 level and two trials at the 1/800 dilution level.

(b) Cresatin demonstrated complete transmittance at the 1/400 level on all trials but on only one trial at the 1/800 level.

Subculture Readings:

(a) The inoculated tubes containing CMC, XP-7, and CMP demonstrated no visible growth at the 1/800 dilution factor.

(b) Cresatin demonstrated bactericidal activity in only one trial through the 1/400 dilution level. All other trials demonstrated growth at all dilutions indicating bacteriostatic activity only.

Streptococcus salivarius (refer to Figure 5)

Spectrophotometric Readings:

(a) CMC, XP-7, and CMP demonstrated complete transmittance through the 1/1600 dilution level.

(b) Cresatin demonstrated complete transmittance on all three levels through the 1/400 level of dilution while at the 1/800 level two trials showed complete transmittance while one trial was recorded at 36.

Subculture Readings:

(a) The inoculated tubes containing CMC, XP-7, and CMP demonstrated no visible growth through the 1/1600 dilution level.

(b) The inoculated tubes containing Cresatin demonstrated no visible growth through the 1/400 dilution level, a few colonies at the 1/800 level, and confluent growth at the 1/1600 level.

Streptococcus faecalis (refer to Figure 6)

Spectrophotometric Readings:

(a) CMC, XP-7, and CMP demonstrated complete transmittance through the 1/800 dilution level.

(b) Cresatin demonstrated complete transmittance through the 1/400 dilution level.

Subculture Readings:

(a) The inoculated tubes containing CMC and XP-7 demonstrated no visible growth through the 1/800 dilution level.

(b) The inoculated tubes containing CMP demonstrated no visible growth through the 1/400 dilution and at the 1/800 level demonstrated a few colonies in only one trial while the other two trials demonstrated complete inhibition of growth.

(c) The inoculated tubes containing Cresatin demonstrated no visible growth in two of three trials at the 1/100 level with a few colonies demonstrable in the third trial at 1/100 dilution level. Subsequent dilutions without exception showed varying degrees of growth.

Clostridium perfringens (refer to Figure 7)

Spectrophotometric Readings:

(a) CMC, XP-7 and CMP demonstrated complete transmittance through the 1/1600 dilution factor.

(b) Cresatin demonstrated complete transmittance through the 1/400 dilution factor.

Subculture Readings:

(a) The inoculated tubes containing XP-7 and CMP demonstrated no visible growth through the 1/1600 dilution level.

(b) The inoculated tubes containing CMC demonstrated no visible growth through the 1/800 dilution level and a few colonies in one trial at the 1/1600 dilution level. The other two trials demonstrated no visible growth at the 1/1600 level.

(c) The inoculated tubes containing Cresatin demonstrated no visible growth through the 1/400 dilution level.

The controls on the validity of the subcultures were performed on the third trial of each microorganism-drug combination and the subcultures

from the washings correlated extremely well with the original subcultures from the diluted medicaments with the exception of two instances of contamination with other microorganisms.

Table 1 is a comparison of the mean minimum inhibitory concentrations (bacteriostatic) based upon spectrophotometric readings for six microorganisms and visual observations for Nocardia asteroides. The mean is expressed in micrograms/milliliter of four intracanal medicaments against seven microorganisms. It is a compilation of the previously expressed results in a chart form. The units are means of the three trials per organism per medicament expressed as the minimum inhibitory concentration (bacteriostatic) and the adjacent number is the natural log equivalent of the minimum inhibitory concentration values. This table was a model for the statistical analysis employed.

Table 2 is a comparison of the mean minimum bactericidal concentrations based upon subculture readings for the seven microorganisms. The mean is expressed in micrograms of medicament per milliliter of broth for the four intracanal medicaments against the seven microorganisms. It is a compilation of the previous results expressed in a chart form. The units are means of the three trials per organism per medicament expressed as the minimum bactericidal concentration and the adjacent number is the natural log equivalent of the minimum bactericidal concentration value. This table was a model for the statistical analysis employed.

Statistical Analysis

A two-way analysis of variance was performed to a level of significance less than .01--the two factors being microorganisms and intracanal medicaments.

The hypothesis that there was no significant difference between the four drugs (expressed as their means) was rejected on the basis of this analysis--whether on original observations (minimum inhibitory concentration--bacteriostatic--or minimum bactericidal concentrations) or using log-transformed observations. This analysis was performed both for the spectrophotometric and subcultures values and the results were identical.

This analysis also permitted the testing of the hypothesis that there are no significant differences in the drug resistance of the seven microbial species. This hypothesis was rejected in all the above analyses. Streptococcus faecalis was the most resistant organism with Staphyloccus aureus slightly more resistant than the remaining organisms.

When the above analysis was repeated eliminating Cresatin, there was no significant difference observed among the three remaining drug-means. The only significant differences that were observed were in the resistance among the seven organisms. This can be interpreted to mean that CMC, XP-7, and CMP demonstrate no significant differences in their effectiveness against the seven microorganisms while Cresatin is significantly weaker against the same spectrum of organisms.

Significant interaction between organism and drug combinations were observed in all of the above analyses.

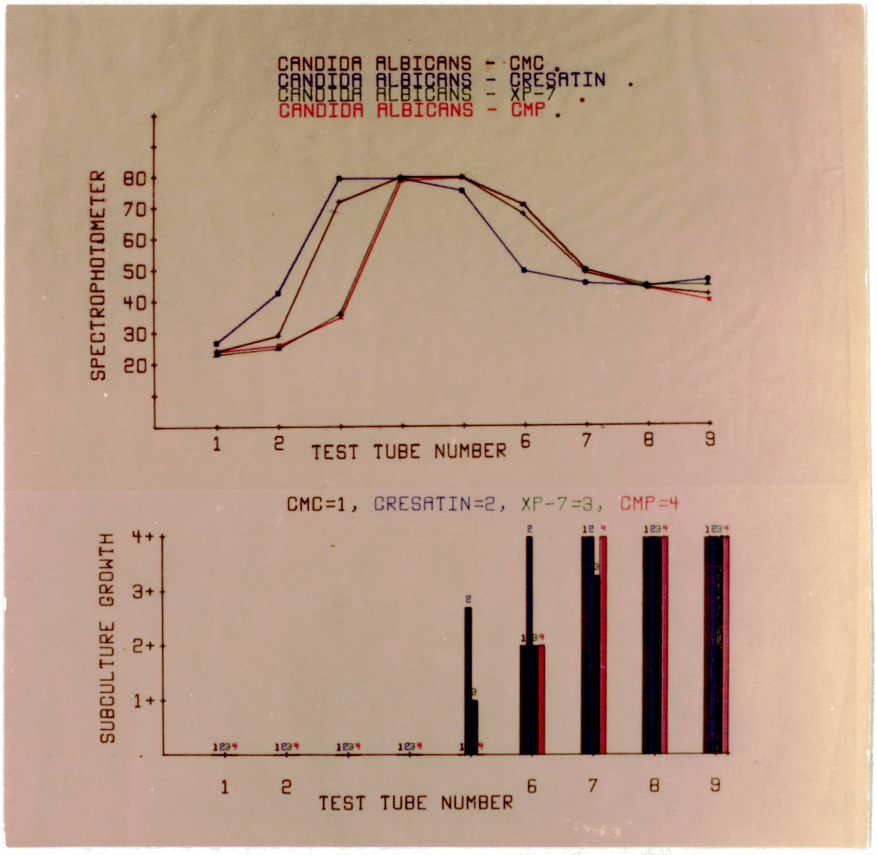

FIGURE 1

A Comparison of Spectrophotometric (Bacteriostatic) and Subculture (Bactericidal) Effectiveness of CMC, Cresatin, XP-7, and CMP against Candida albicans

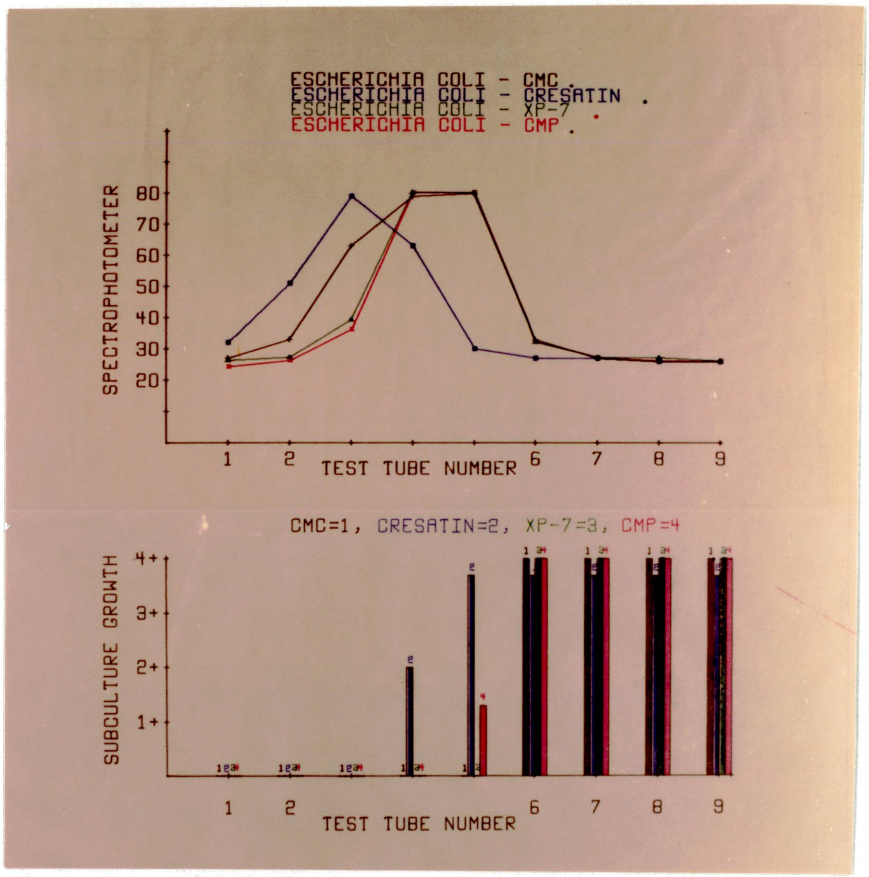

FIGURE 2

A Comparison of Spectrophotometric (Bacteriostatic) and Subculture (Bactericidal) Effectiveness of CMC, Cresatin, XP-7, and CMP against Escherichia coli

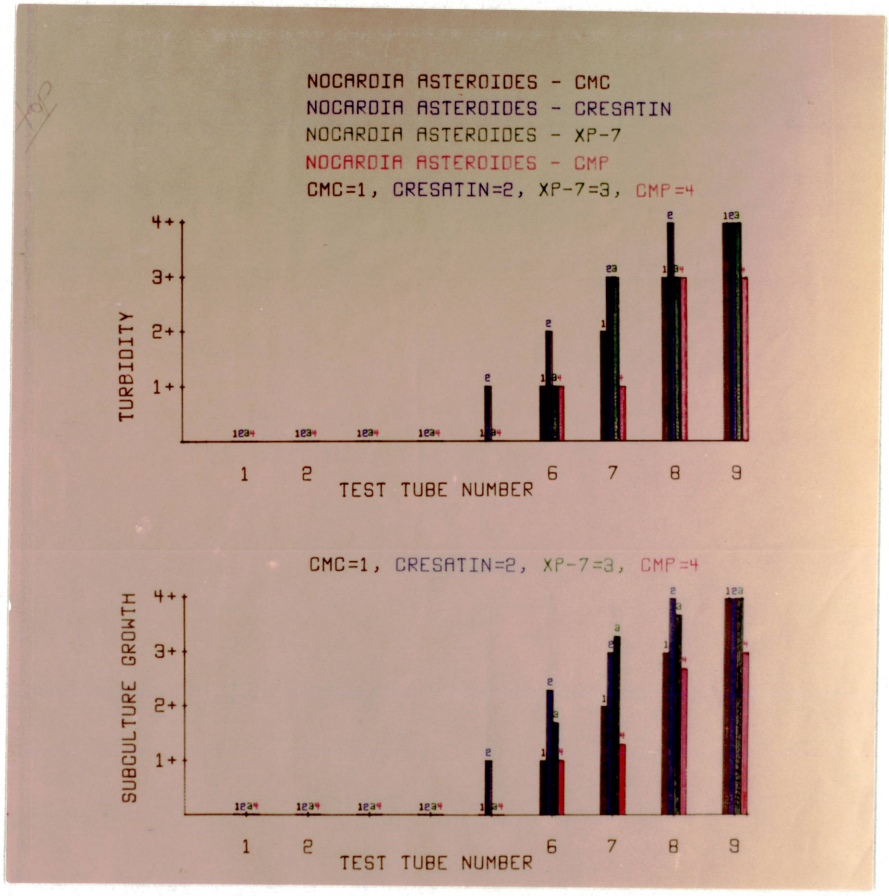

FIGURE 3

A Comparison of Spectrophotometric (Bacteriostatic) and Subculture (Bactericidal) Effectiveness of CMC, Cresatin, XP-7, and CMP against Nocardia asteroides

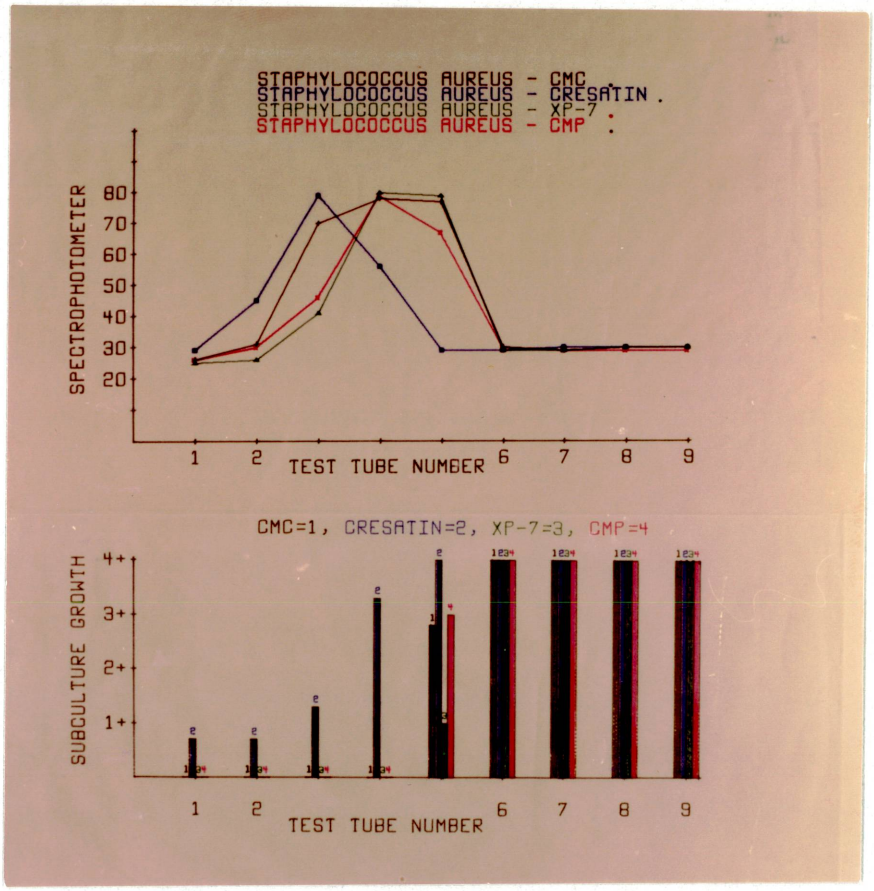

FIGURE 4

A Comparison of Spectrophotometric (Bacteriostatic) and Subculture (Bactericidal) Effectiveness of CMC, Cresatin, XP-7, and CMP against Staphylococcus aureus

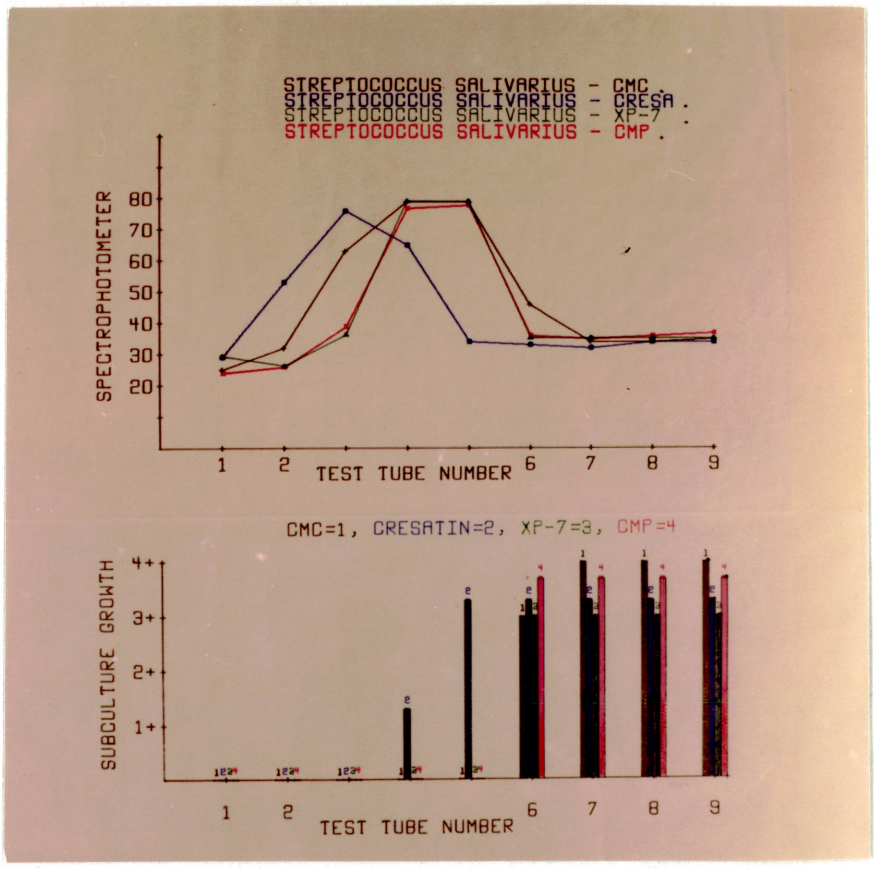

FIGURE 5

A Comparison of Spectrophotometric (Bacteriostatic) and Subculture (Bactericidal) Effectiveness of CMC, Cresatin, XP-7, and CMP against Streptococcus salivarius

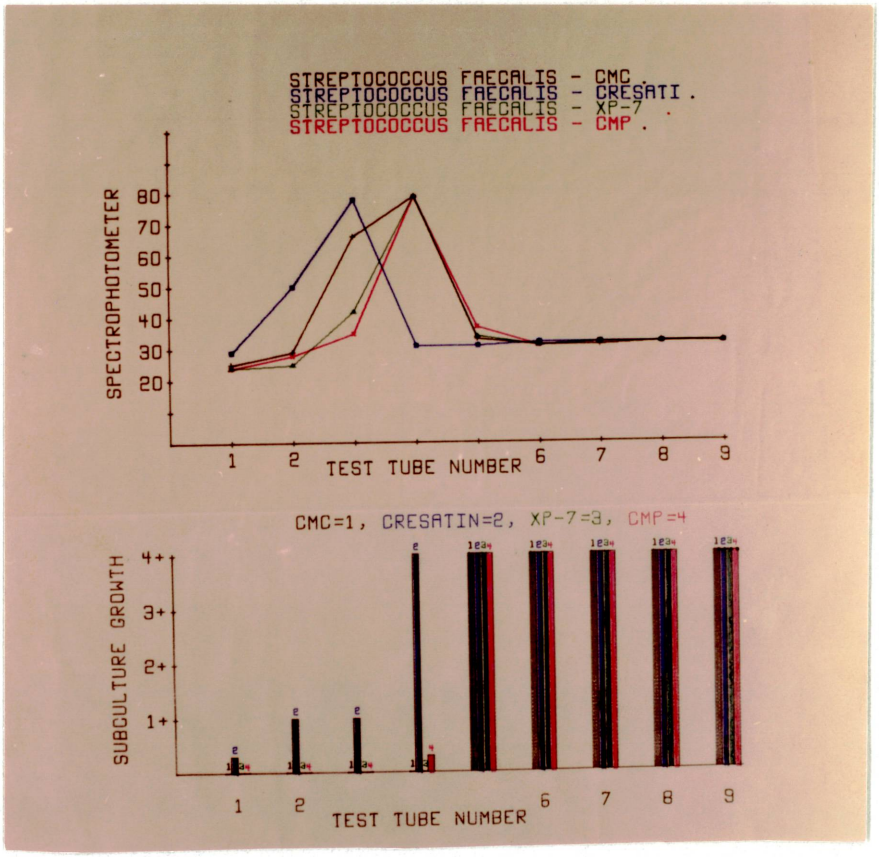

FIGURE 6

A Comparison of Spectrophotometric (Bacteriostatic) and Subculture (Bactericidal) Effectiveness of CMC, Cresatin, XP-7, and CMP against Streptococcus faecalis

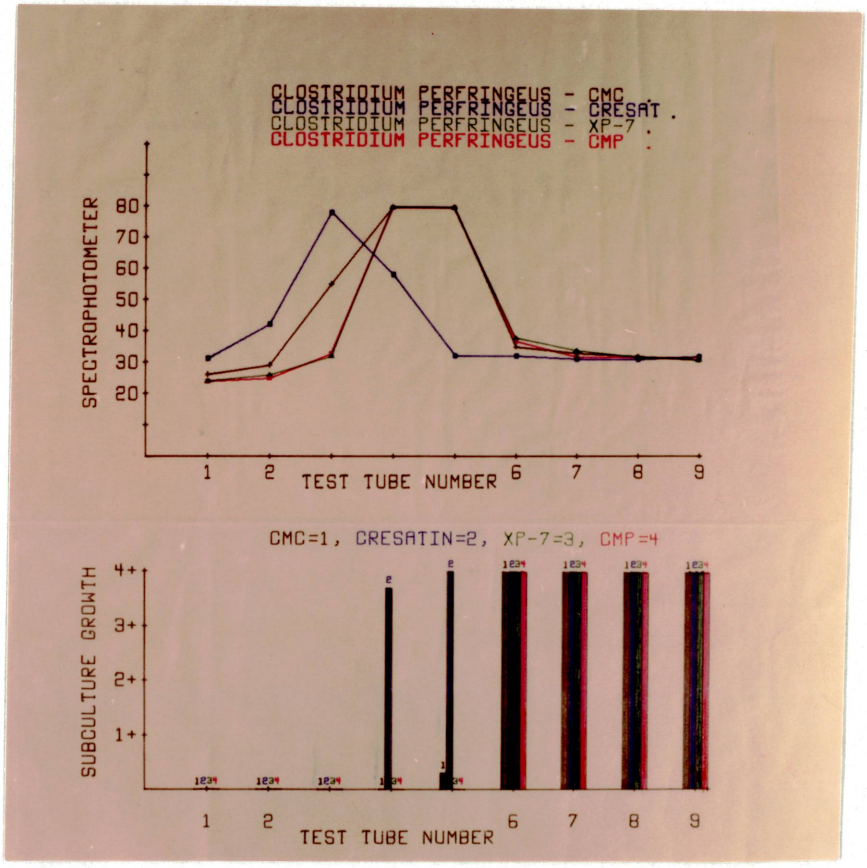

FIGURE 7

A Comparison of Spectrophotometric (Bacteriostatic) and Subculture (Bactericidal) Effectiveness of CMC, Cresatin, XP-7, and CMP against Clostridium perfringens

TABLE 1

A COMPARISON OF THE MEAN MINIMUM INHIBITORY CONCENTRATION (BACTERIOSTATIC) EXPRESSED IN MICROGRAMS/MILLILITER OF FOUR INTRACANAL MEDICAMENTS AGAINST SEVEN MICROORGANISMS

	CMC		Cresatin		XP-7		CMP		Sum of the Means	
	Obsv.	Log_e	Obsv.	Log_e	Obsv.	Log_e	Obsv.	Log_e	Obsv.	Log_e
C. albicans	641	6.46	856	6.69	633	6.45	635	6.45	2755	26.05
E. coli	641	6.46	1708	7.39	633	6.45	529	6.22	3511	26.52
N. asteroides	641	6.46	1281	7.15	633	6.45	529	6.22	3084	26.28
S. aureus	641	6.46	2135	7.62	633	6.45	1059	6.91	4468	27.44
S. salivarius	641	6.46	1708	7.39	633	6.45	847	6.68	3829	26.98
S. faecalis	1281	7.15	2562	6.85	1266	7.14	1271	7.15	6380	28.29
C. perfringens	641	6.46	2562	7.85	633	6.45	635	6.45	4471	27.21
Sum of the Means	5127	45.91	17403	50.94	5054	45.84	5505	46.08		

TABLE 2

A COMPARISON OF THE MEAN MINIMUM BACTERICIDAL CONCENTRATION EXPRESSED IN MICROGRAMS/MILLILITER OF FOUR INTRACANAL MEDICAMENTS AGAINST SEVEN MICROORGANISMS

	CMC		Cresatin		XP-7		CMP		Sum of the Means	
	Obsv.	Log_e	Obsv.	Log_e	Obsv.	Log_e	Obsv.	Log_e	Obsv.	Log_e
C. albicans	641	6.46	1281	7.15	1266	7.14	635	6.45	3823	27.20
E. coli	641	6.46	2562	7.85	633	6.45	1059	6.91	4895	27.67
N. asteroides	641	6.46	1281	7.15	633	6.45	635	6.45	3190	26.51
S. aureus	1281	7.15	7686	8.77	844	6.68	1271	7.15	11082	29.75
S. salivarius	641	6.46	1708	7.39	633	6.45	635	6.45	3617	26.75
S. faecalis	1281	7.15	10249	9.23	1266	7.14	1694	7.38	11490	30.90
C. perfringens	854	6.69	2562	7.85	633	6.45	635	6.45	4684	27.44
Sum of the Means	5980	46.83	27329	55.39	5908	46.76	6564	47.24		

CHAPTER V

DISCUSSION

The broth dilution test tube method is a quantitative technique for determining the minimum inhibitory concentration (bacteriostatic) in micrograms of an antimicrobial agent that will inhibit the growth of an organism in vitro. The principle of the tube dilution method is the inhibition of the growth of the test organism by an antimicrobial agent incorporated in a broth medium. Tubes may be arranged and the agent serially diluted in double-dilution sequence.

The broth dilution method has one great significant advantage over the agar plate diffusion method in that it permits the determination at both the minimum inhibitory concentration (bacteriostatic) and the minimum bactericidal concentration. The latter can be determined by subculturing to appropriate culture media from those tubes in the series which show visible inhibition of growth.

Stock cultures of individual American Type Culture Collection (ATCC) microorganism were selected rather than mixed flora cultured and isolated from the oral cavity. The reasons were the following: The first was that the study is replicable with stock microorganisms. The second reason was that a mixed flora may or may not be representative of the typical flora encountered in the root canal.

Microorganisms were selected to represent the basic groups of microorganisms found in the root canal as indicated by studies cited in

the review of literature concerning the role of microorganisms in endodontic disease. Clostridium perfringens, though not a normal member of the root canal flora, was used in this study because it was a readily available obligate anaerobe with which to conduct such a study. The more delicate anaerobes such as anaerobic streptococci and bacteroide are unreliable for such tests since they are too easily destroyed by the mechanics of serial dilution in which they are unavoidably subjected to aeration.

The volume of medications used in this study are considered valid as they are based upon Stewart's volumetric studies (1948) of teeth. The smallest volume was that of the mandibular central incisor which averaged 0.0088 ml. The volume was composed of the space occupied by the entire root canal and that part of the pulp chamber not being used in sealing the medication. The volumes ranged to the maximum which was of the maxillary first molar and was 0.0412 ml. The bactericidal ranges of the studies with the standardized inoculum ranged, with the exception of Cresatin, from a dilution factor of 1/400 to 1/1600. The respective quantities of intracanal medicaments were 0.0025 ml; .00125 ml and .00067 ml. It is apparent that this study used volumes comparable to those used clinically. Based upon these same volumetric comparisons, it has been mentioned previously in the review of literature that the amount of prednisolone used per treatment is approximately 1/10 mg-4/10 mg.

The minimum inhibitory concentration (bacteriostatic) and the minimum bactericidal concentration was determined using the values of the weight per milliliter of the four intracanal medicaments obtained by weighing one milliliter of each drug upon a Christian-Becker analytical

balance, The same drug sample was used throughout the entire study and the respective weights were the following:

1. CMC - 1.025 gm/ml.
2. Cresatin - 1.025 gm/ml.
3. XP-7 - 1.013 gm/ml.
4. CMP - 1.017 gm/ml.

The bacteriostatic and bactericidal concentrations are based upon determining the greatest dilution of the antimicrobial agent which exhibits no visible growth. At this dilution level, the values for the minimum inhibitory concentration (bacteriostatic) and minimum bactericidal concentrations were determined by dividing the weight by the volume and expressing the concentrations in micrograms/milliliter. The exception to the above formula were the subculture readings in which Cresatin was not bactericidal and the maximum value of 10,249 micrograms/milliliter of broth was assigned to Cresatin for statistical purposes.

There are certain inherent weaknesses in using the minimum inhibitory concentration (bacteriostatic) and minimum bactericidal concentration as a quantitative value. The first reason is that there is an inherent error of one tube either way in a serial dilution; the end point is not an exact figure. The averaging of trials is a second reason that can create an error when using doubling dilutions, but the author minimized this error by using natural log equivalents of the observed data. The third possible reason for error is in the arbitrary assigning of values for subculture growth on a 0-4 scale based upon visual comparisons. In this study the readings were so obviously different that it is felt that little error was introduced by this means. Regardless of the above pos-

sibilities, the tube dilution method utilizing the weight/volume basis is a far more accurate means of determining antimicrobial effectiveness than the agar diffusion technique.

The conclusions reached by this study correlate well with previous studies. Dietz (1957) found CMC and XP-7 virtually equivalent against the test bacteria in his study (agar diffusion). He found Cresatin to be weaker in most cases--no statistical analysis was performed. Stewart and Gautieri (1962) found in an agar diffusion study that Cresatin produced considerably smaller zones of inhibition than CMC. No statistical analysis was performed. Grossman (1970) states that the "sterilizing effect of Cresatin is probably not so marked as some of the other phenolic compounds." Schilder (1965) considered Cresatin to be a mild antimicrobial agent.

It is of interest to note that these drugs are true non-specific drugs in that all demonstrated bacteriostatic or bactericidal activity in varying degrees to all the various microorganisms tested. The drugs were effective within the same range to the yeast as to the bacteria. They were as effective against the anaerobe as against the other bacteria. The more resistant organisms as demonstrated were consistant with previous literature. Crawford and Shankle (1961) found streptococci to be the most persistent form during endodontic treatment. Zeldow and Ingle (1962) found the enterococci displayed a high incidence of resistance along with several species of staphylococci. Goldman and Pearson (1969) found enterococcus to be the most persistent organism in endodontic therapy.

This study is generally consistent with previous literature. Its

findings have been determined by more quantitative methods, control, and replicability than previously reported studies in endodontic literature.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Injudicious use of intracanal medicaments can be one of the factors responsible for post-treatment discomfort. Camphorated paramonochlorophenol, metacresyl acetate, and prednisolone in combination is advocated to reduce the inflammatory potential of the germicide and thus reduce the occurrence of post-treatment discomfort. It is imperative that a medicament retain antimicrobial activity while reducing inflammatory potential.

This study was directed toward determining on an in vitro quantitative bactericidal versus bacteriostatic basis by use of the broth dilution test tube method whether camphorated paramonochlorophenol, metacresyl acetate, and prednisolone in combination retains antimicrobial activity comparable to selected other commonly used non-specific germicides. Four drugs--camphorated paramonochlorophenol (CMC), metacresyl acetate (Cresatin), camphorated paramonochlorophenol and metacresyl acetate in combination (XP-7) and camphorated paramonochlorophenol, metacresyl acetate and prednisolone in combination (CMP)--were tested against seven organisms: Candida albicans, Escherichia coli, Nocardia asteroides, Staphylococcus aureus, Streptococcus salivarius, Streptococcus faecalis, and Clostridium perfringens.

Based upon a statistical analysis, the following conclusions were made:

1. There was no significant difference in the effectiveness of CMP compared to CMC and XP-7.

2. The addition of prednisolone to the non-specific germicide made no significant difference in the effectiveness of the drug as an antimicrobial agent.
3. Cresatin was significantly less effective than CMC, XP-7, and CMP at the levels and dilutions used against the spectrum of microorganisms in this study.
4. Streptococcus faecalis was the most resistant organism with Staphylococcus aureus slightly more resistant than the remaining organisms.

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Graduate School

THE EFFECTIVENESS OF CAMPHORATED PARAMONOCHLOROPHENOL, METACRESYL ACETATE, AND PREDNISOLONE IN COMBINATION AS AN ANTIMICROBIAL AGENT

by

Maurice Cutler

An Abstract in Partial Fulfillment

of the Requirements for the Degree

Master of Science in the Field of Endodontics

THE EFFECTIVENESS OF CAMPHORATED PARAMONOCHLOROPHENOL, METACRESYL ACETATE, AND PREDNISOLONE IN COMBINATION AS AN ANTIMICROBIAL AGENT

Post-treatment discomfort following endodontic procedures can be caused by inflammation due to injudicious use of intracanal medicaments. Camphorated paramonochlorophenol, metacresyl acetate, and prednisolone in combination is advocated to reduce the inflammatory potential of the germicide and thus reduce the occurrence of post-operative pain. It is imperative that a medicament retain antimicrobial activity while reducing inflammatory potential.

This investigation was undertaken toward determining on an in vitro quantitative bactericidal versus bacteriostatic basis by use of the broth dilution test tube method whether camphorated paramonochlorophenol, metacresyl acetate, and prednisolone in combination retains antimicrobial activity comparable to selected other commonly used non-specific germicides. Four drugs--camphorated paramonochlorophenol (CMC), metacresyl acetate (Cresatin), camphorated paramonochlorophenol and metacresyl acetate in combination (XP-7) and camphorated paramonochlorophenol, metacresyl acetate and prednisolone in combination (CMP)--were tested against seven organisms: Candida albicans, Escherichia coli, Nocardia asteroides, Staphylococcus aureus, Streptococcus salivarius, Streptococcus faecalis, and Clostridium perfringens.

Based upon a statistical analysis, the following conclusions were made:

1. There was no significant difference in the effectiveness of CMP compared to CMC and XP-7.
2. The addition of prednisolone to the non-specific germicide made no significant difference in the effectiveness of the drug as an antimicrobial agent.
3. Cresatin was significantly less effective than CMC, XP-7, and CMP at the levels and dilutions used against the spectrum of microorganisms in this study.
4. Streptococcus faecalis was the most resistant organism with Staphylococcus aureus slightly more resistant than the remaining organisms.