THE EFFECT OF ZINC OXIDE AND EUCHEMOL ON MICROORGANISMS
IN THE DENTAL PULP

By

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CURRICULUM VITAE

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INTRODUCTION
The use of zinc oxide and eugenol in the indirect pulp capping technique is apparently quite successful clinically, and the innocuous nature of these agents is well documented.\textsuperscript{1,2,3} There are, however, those who think that zinc oxide and eugenol is irritating to the pulpal tissue.\textsuperscript{4} The sealing and sterilising properties of zinc oxide and eugenol have been demonstrated.\textsuperscript{5,6,7} The antibacterial effect of zinc oxide and eugenol on blood borne microorganisms in the pulp, when the material is used as a capping agent, has been investigated.\textsuperscript{8} The difficulty encountered in contaminating the dental pulp in this fashion has been demonstrated.\textsuperscript{9}

Previous investigations have not demonstrated whether the success of the indirect pulp capping technique is due to the sealing effect of the mixture, to its bactericidal effect or to some other mechanism.

The author is not aware of any reference in the dental literature relating to the degree of bacterial contamination which must exist in the dental pulp before irreparable damage is done to this tissue. In addition, there is no reference, other than theoretical, to the possible effects of eliminating microorganisms in the pulp or to reducing the numbers of such organisms when seeking to validate the rationale of the indirect pulp capping technique.

Whether zinc oxide and eugenol, placed in contact with the pulpal tissue, will, in fact, destroy microorganisms
within the pulp is not known. The possibility exists that this mechanism is responsible, in part, for the success of the indirect pulp capping technique—and was the motivating factor for the present study.
REVIEW OF LITERATURE
The exact role of microorganisms in the development of dental caries has been the subject of scientific study for many years. That such investigations are being conducted at an increasingly greater rate is evident from the volume of reported studies on the subject, none of which can completely answer the problem. It would appear, however, that one aspect of the situation could be partially controlled by the bactericidal or bacteriostatic effect of materials used in the treatment of carious teeth.

That certain materials in use today were known by ancient peoples to have bactericidal properties is documented, although they had no concept of the cause or of the effect produced. The writings of the ancient Chinese and Hindus refer to the use of cloves and pepper for the treatment of toothaches. It is also known that the Egyptians used spices in their oils in embalming.

In the first known mention of cloves in the cavity of a tooth, Balascon of Taranta, in 11490, advocated cleaning a cavity and "placing a bit of pepper or cloves therein."

In 1687 Allen, in the first known book published on dentistry, describes two types of toothache and their remedies.

"You shall know this sort of Tooth-ake by the high beating of your Pulse, the fulness of the Veins, and an often beating in the affected tooth: with a continual, tho not very extreme pain. And then for the cure of it, you must first bleed the
Gums, and sometimes open a Vein in the Arm also, and wash your mouth with Rose-Water and Vinegar, of each equal quantities mixt together; putting a little Cotton dipt in Cyl of Box into the tooth, if it be hollow..."As for its Cure, it may be affected by Sternutation, the friction of the nape of the neck with warm clothes, and the application of aperitive Remedies, to open the pores of the tooth; and if it be hollow, you shall put in't a drop of Cyl of Camphire whereinto has been infused some Henbane-root."

Hunter, in 1773, also described a remedy for toothache:

"The pain, in many cases, being often more than the patient can well bear, warm applications to the part have been recommended, such as hot brandy, to divert the mind; also spices, essential oils, etc. which last are, perhaps the best."

In the sixteenth century Pare wrote of the introduction of oil of cloves into the sufficiently enlarged cavity of a painful tooth.

In 1756, Pfaff, in the first German publication on dentistry, noted that "oil of cloves is a good drug and promotes easy an removal of the decayed portion of a carious tooth."

Bonastre, a nineteenth century French pharmacist, made the first scientific study of oil of cloves. He found that mixing oil of cloves with magnesia oxide produced "a very solid combination which is not crystallizable and is totally insoluble in cold water."
Molnar states that Fauchard mentioned the use of oil of cloves for dental decay when the pulp was not involved.

The first description of the use of zinc oxide and eugenol was made by Flagg in 1875. He experimented with the mixture of oil of cloves and zinc oxides in deep cavities and stated: "My success has thus far been very gratifying, but sufficient time has not yet lapsed for me to be able to give any reliable data in this connection."

Harlan, in 1884, stated that eugenol is a powerful germicide and not dangerous to human life, and advocated its use on exposed or nearly exposed pulps.

Chamberland, in 1887, investigating the antiseptic properties of the essential oils, found that oil of cinnamon, which is composed of 70 to 90 per cent eugenol, was the most effective of the essential oils against the anthrax bacillus.

In 1890, Miller did two studies on the germicidal properties of filling materials. He tested the first group on an ordinary nutrient gelatin plate which had been inoculated with a bacterium from the oral cavity. Samples of the materials were incubated on these plates for 24 to 48 hours. Zones of clear gelatin around the samples indicated inhibition of the organism. In the second test he took extracted teeth and partially removed decay. The test material was placed in the cavity and incubated for three days at 30-40°C. Dentin samples were then placed on sterile nutrient agar-agar and in-
cubated in a moist chamber at or near body temperature. He found that copper amalgam, either fresh mixed or alloys removed from teeth, and even pieces of dentin which had been in contact with copper amalgam, retained bactericidal action for an indefinite period of time. Gold amalgam, when freshly mixed, had a slight action, while old pieces had no effect. Gold foil exerted an inhibitory effect when unannealed; when annealed, the action was absent. Tin-gold was less active than gold alone. Gutta-percha and tin were completely inactive. Oxynaphosphate of zinc had a slight, inconstant action when fresh, and sometimes none at all.

Peck, in 1898, investigated the antiseptic action and irritating qualities of the essential oils. He found that six-tenths of a drop of oil of cloves in 10 c.c. of sterilized mutton broth infected with saliva prevented the growth of microorganisms. He stated: "Sores were produced in guinea pigs and treated with a spray of this oil. This inflammation subsided more rapidly than when treated with any other agent, and the sores healed as readily as they could, simply proving beyond any possibility of doubt that, while effectively destroying microbes, the only action of oil of cloves in contact with irritated, inflamed soft tissue is that of a quieting, soothing agent, serving to reduce the irritation and inflammation, and returning the disturbed tissue to its normal condition."

Sutphen and Luckie, in 1898, advocated the use of
zinc oxide and eugenol as a pulp-capping agent on the basis of its non-irritating, antiseptic properties and its value as an anodyne.

26 Hoffman and Evans, in 1911, investigated the inhibiting action of cinnamon, cloves and mustard on applesauce. They state: "The three spices, cinnamon, mustard and cloves, must be considered important preservatives. Cinnamon and mustard are particularly valuable for they are palatable even when used in proportions that prevent all growth. Cloves in the proportion which prevented growth (1.5 grams to 100 grams of sauce) had too much of a burning taste to be palatable. However, it retards growth in much smaller amounts." They also tested the bactericidal action of varying amounts of cinnamic aldehyde, eugenol and benzoic acid on specific organisms inoculated in tomato broth bouillon containing one per cent sugar and adjusted to 1.5 per cent normal acid. Their results showed that cinnamic aldehyde possesses a more marked antiseptic action than either eugenol or benzoic acid. Cinnamic aldehyde in a concentration of two parts per 10,000 prevented growth; eugenol in a concentration of 10 parts in 10,000 prevented growth.

27 Ames, in 1913, subjected pellets of cement to various media, both sterile and inoculated, to compare their germicidal potency. These pellets were composed of from 25 to 75 per cent copper oxide to zinc oxide. He found that, although
zinc oxide had but little power to inhibit bacterial growth, this slight amount was in excess of any such power possessed by the silicious cements. The actual gemicidal property of set cements was attributed to the formation of a soluble metallic salt.

28 Peetcheke, in 1915, tested the gemicidal efficiency of various dental cements in standard agar inoculated with saliva. He stated: "... the zinc oxide in itself, while not sufficient to produce practical sterilization, shows a gemicidal efficiency of 96.2 per cent."

29 Badhan, in 1916, tested the value of alcoholic extracts of cinnamon, cloves and mustard on Rhizopus, penicillium, aspergillus and Alternaria grown on thaxter's potato hard agar and on B. coli, B. prodigiousus and B. subtillis grown on ordinary nutrient agar. It was found that concentrations of 1:7 or 1:10 of cinnamon or cloves prevented the germination of spores.

30 Wood, in 1920, stated that the phenol coefficient of oil of cinnamon is about 12, oil of cloves about 18 and oil of eucalyptol about one.

31 Myers, in 1927, studied the fungicidal activity of the more commonly used volatile oils, testing the effect of alcoholic solutions of thymol, cinnamon oil and clove oil on various yeasts. It was found that thymol killed yeasts in one minute, cinnamon oil in 25 to 30 minutes and clove oil in 50
minutes. The yeasts did not exhibit any degree of fastness or toleration toward the aqueous of these oils. Thymol was found to be effective against Actinomyces hominis.

De found, in 1929, that the germicidal values of the different phenols and their derivatives decreased in the following order: thymol, 27; Australol, 23; eugenol, 12.7; safrol, 10; elemicin, one.

In 1934, Hill and Rooster conducted tests on commercial cements using S. aureus in broth cultures and on agar plates. It was found that the commercial germicidal cements (Kryptex, Ames black copper, White's silver B., Smith's copper silicate, Fleck's red copper and Gaulk's white copper) are germicidal in broth cultures when mixed in dilutions ranging between one and 0.13 per cent. The relative efficiency of the cements was dependent on their solubility, as well as their bactericidal value. They also found that when phenylmercuric nitrate was added to the powder of a non-germicidal base cement, its germicidal properties were comparable to any commercial cement which depends on the salts of silver or copper for its germicidal efficiency. They also point out that free cement liquid has been shown to have marked bactericidal properties and that consequently all cements are germicidal during the setting period.

In 1935, Sheppard conducted experiments to determine the bactericidal effects of metal fillings and some of their
component metals against microorganisms frequently associated with dental caries. It was found that pure gold and pure silver possessed no antiseptic properties. Pure copper and pure mercury were strongly bactericidal toward all mouth bacteria tested. Most gold inlays had some bactericidal properties while amalgam fillings varied considerably in oligodynamic properties. There was no correlation between the bactericidal power of a filling and the recurrence of dental caries in the tooth structure adjacent to the filling.

35 Kinneer, in 1935, tested the gemicidal action of various materials on agar plates inoculated with one loopful of S. aureus. Pellets of zinc oxyphosphate cement, black copper cement, Kryptex, silver cement, silicate, gold, Acalite, silver amalgam and copper amalgam were used as test materials. It was found that all materials, except gold and Acalite, which they state are inert bacteriologically, had some gemicidal action with silver cement and copper cement best.

36 Topley and Wilson, in 1936, stated that clove oil will kill B. typhosum in 25 minutes.

37 Petrie, in 1936, stated that eugenol has a Ridesal-Walker gemicidal coefficient of 8.6 and that thymol has a coefficient of 20.0.

38 Ireland, in 1939, advocated the application of ammoniacal silver nitrate and eugenol to infected dentin.

39 Easlick stated, in 1939, that most compounds for pulp
capping utilized zinc oxide and eugenol as primary constituents. Baslick says that: "After leathery dentin has been removed from the cavity and exposure is detected, oil of cloves or some similar sedative is sealed under cement for at least 24 hours. Oil of cloves partially sterilizes the cavity and serves to reduce pulp hyperemia."

Grossman found, in 1939, that cements having a zinc oxide-eugenol base were hemostatic sealing agents.

Saltzer, in 1940, studied the removal of bacteria during cavity preparation and concluded that, in shallow cavities, all bacteria could be mechanically removed in more than 50 percent of the cases; that after bacteria has entered the dentin, the chances of removing them during cavity preparation becomes progressively less with increasing cavity size and, that in deep cavities, "the inability to remove all bacteria merely by careful excavation emphasizes the necessity for sterilization of the dentin prior to introduction of the filling material."

Zander, in 1940, conducted a study on bacteria remaining in the dentin after cavity preparation. Ten teeth were prepared under the rubber dam, using Black's preparation. After the decay was removed, the teeth were immediately extracted and fixed. In four of 10 cavities, microorganisms were found in the hard dentin of the cavity floor. Zander states that "sterilization would seem advisable" but "the
effectiveness of our sterilization methods is questionable."

Graham, in 1941, used eugenol as a reducing agent with
Howe's ammoniacal silver nitrate which had been buffered with
ammonium hydroxide to pH 8.5-9.5. He stated that: "Chemically
pure zinc oxide combined with eugenol (U.S.P.) and spatted
ated to a very thick consistency to form a so-called 'cement' has
been used in the dental profession for many years. With this
combination with Howe's silver nitrate solution, we have at
our disposal two materials which are practically indispensable
to modern dental practice."

Selzser, in 1942, recultured the dentin of 93 teeth
which he had treated with various medicaments one year pre-
viously, to determine if any changes had occurred in the bac-
teriologic status. He concluded that bacteria may still re-
main in the dentin after proper cavity sterilization, since
all cases, including controls, were largely positive. He
theorized that sealing of the dentin per se does not sterilize
it and points out that recurrent caries under fillings may be
the result of improper sterilization procedures.

James and Diesenhoff, in 1942, studied histologically
the effect of zinc oxide and eugenol placed in cavity prepara-
tions in the teeth of dogs. They observed no reactions in the
dentin or pulp in 11 of 13 prepared teeth and concluded that
zinc oxide and eugenol would protect the cut tubuli, prevent-
ing reactions in the dentin and pulp.
Van Rysen, in 1943, studied the effects of cavity preparation on sound dogs' teeth. Within 24 to 48 hours he noted hyperemia, edema, and leukocytic infiltration in the area of the pulp opposite the dentinal tubuli extending from the cavity floor. He recommended the use of zinc oxide and eugenol to prevent these changes.

Basis, in 1943, studied the fate of bacteria sealed in dental cavities. This test was conducted in vivo, using occlusal cavities in the molar teeth of white males between the ages of 16 years and 25 years. A sterile technique was used. Small amounts of carious dentin were allowed to remain in the tooth, and cultures were made of the cavity. The cavity was then closed with a dry, sterile cotton pellet, sterile gutta-percha and zinc oxyphosphate cement. The cavities were recultured at intervals of two weeks, some of them being recultured up to a year and a half. Of 10 cases he found viable streptococci in eight cases; lactobacillus in five and staphylococci in two. Of these, lactobacillus died out between two and 10 months, staphylococcus were positive for at least one year, while streptococcus was the most resistant and remained positive for more than one year. Basis concluded that: "It appears as though (a) the carious process in dentin definitely stops or gradually ceases as soon as the lesion is closed from the oral environment even when the organisms remain alive; (b) the bacteria have a tendency to die out; but
(c) in 30 per cent of the cases studied positive cultures of streptococci persisted after being sealed for more than a year. Basing further stated: "Apparently an effective, penetratinig sterilizing agent may be necessary in deep decayed lesions near the pulp not to stop the carious process from progressing because it ceases automatically upon filling the cavity, but to eliminate the possible surviving organisms and eradicate a possible source of bacterial growth that may eventually injure the dental pulp or tissues elsewhere in the body through focal infection."

Dorfman and his co-workers, in 1943, studied the degree of penetration of organisms in carious teeth. It was determined that the extent of infection in carious lesions varied, as shown by culturing dentin samples at different levels in unprepared cavities of freshly extracted teeth. The superficial layers of carious dentin were always infected, intermediate layers were sometimes infected, and the partially decalcified dentin adjacent to sound dentin and the sound dentin were almost always sterile.

Frisbie and Nuckolls, in 1947, showed that the carious process may be active under apparently unbroken and intact enamel surfaces; they stated that this tends to support their hypothesis that: "caries is fundamentally a degradation of the organic matrix of the enamel resulting from the enzymatic action of microorganisms, rather than a simple acid decalcifi-
cation and removal of organic salts."

Kronfeld, in 1949, discussed bacterial penetration and stated: "There are always a few tubules that contain organisms at a level far ahead of the actual decalcification and decay." 51

Bartels, in 1947, evaluated the anti-microbial effect of eugenol and oil of cloves by: (1) Seeding infusion agar with S. aureus and B. coli and placing oil of cloves or eugenol in holes bored in the agar; (2) Infusion agar with pH adjusted to 6.0, 7.0 and 8.0 with eugenol added to give a concentration of 0.5 per cent. Plates were poured and after hardening, one-half of the medium was removed and replaced with plain infusion agar of the same pH. The surface was then streaked across with B. subtilis, S. aureus, B. coli and B. pyocyaneus; (3) Agar in test tubes was cooled to 45°C, seeded with various organisms and poured into plates. Eugenol alone, mixed with zinc oxide or zinc oxide alone was placed on the surface of the medium; and (4) Infusion agar plates were streaked in rows with the various organisms and the materials placed on the plate. He found that: "(1) Microorganisms vary in sensitivity to eugenol and oil of cloves, (2) variations in hydrogen ion concentration between pH 6.0, 7.0 and 8.0 had little effect on the inhibitory qualities of either oil of cloves or eugenol, (3) zinc oxide has no apparent antibacterial effect in the dried state on microorganisms, (4) oil of cloves or eugenol when incorporated into a paste of zinc oxide still possesses
definite inhibitive properties, (5) eugenol in alcoholic solution was more effective than when suspended in water or detergent and (6) oil of cloves and eugenol were comparable in their antibacterial properties."

Hardwick, in 1949, studied the sterilization of carious dentine, utilizing 1,000 tests with 3,000 bacterial cultures. Of 30 different sterilizing agents tested, zinc oxide and eugenol had a bactericidal index of 53 per cent. This use of zinc oxide and eugenol differed from the other materials tested in that most of the agents were applied for two minutes, whereas zinc oxide and eugenol was applied to infected dentin for a period of three hours. Hardwick stated that: "The relatively high germicidal index of zinc oxide and oil of cloves may be explained by: (1) A small quantity of caries having been removed for retention purposes, (2) It was allowed to remain in situ for two and one-half to three hours."

McCue and his co-workers, in 1951, conducted studies on the antibacterial properties of silver amalgam, copper amalgam, gold foil, inlay gold, silicate cement, copper cement, zinc phosphate cement and a quick-setting acrylic resin. They used nutrient agar, nutrient agar with sterile serum and nutrient agar with sterile saliva to which was added microcooccus aureus and E. coli. The materials were found to have varying degrees of bacteriostatic action. In decreasing order of effectiveness these are: (1) silicate cement, (2)
copper amalgam, (3) gold foil, (4) zinc phosphate cement, (5) copper cement, (6) acrylic, (7) silver amalgam and (8) inlay gold. In contradistinction to the work of Sheppard, it was found that gold foil was an excellent bacteriostatic agent. McCue suggested that this difference could be attributed to her use of old gold, whereas they used new gold.  

Seltzer, in 1951, discussing cavity sterilization, stated: "My experiments showed that when dentin at the base of prepared cavities was cultured, bacteria were present in 84 per cent of the cases of medium sized cavities and in 93 per cent of the cases where deep cavities were prepared."

Burnett and Scherp, in 1951, studied the type and distribution of proteolytic and aciduric bacteria in saliva and carious lesions. It was found that: "Proteolytic bacteria, active against resistant proteins such as decalcified dentine, were absent from enamel caries and from the advanced portions of deep dentinal caries. They were relatively abundant in the superficial layers of dentinal caries. They were almost entirely absent from saliva not exposed to carious teeth and were present regularly but only in relatively small numbers in saliva exposed to carious lesions. Proteolytic bacteria, active against less resistant proteins such as casein and gelatin, were found in all types of saliva and throughout the carious lesion." "Lactobacilli and other aciduric bacteria made up a major fraction of the organisms cultivated from
enamel caries and were found regularly, though in a smaller proportion, in deep dentinal caries. In many cases they were absent from the superficial layers of dentinal caries and never made up more than a minor fraction of the organisms at this level. They were present in saliva from mouths containing no detectable caries but were more abundant in saliva exposed to open carious lesions."

In 1952, Turkheim conducted a study on the bactericidal properties of silver amalgam, copper amalgam, zinc oxide and eugenol and zinc oxide and oil of cloves on eight strains of Lactobacillus acidophilus odontolyticus. He stated: "These experiments revealed two facts: the different sensitivity of the 8 strains examined to the amalgams and cements and the bactericidal potency of these materials on the test bacteria in vitro. The same disc of silver amalgam, for example, stayed bactericidal for 14 months (32 subcultures), copper amalgam is still very active when (sic) this report is written (99 passages) and zinc oxide and eugenol and eugenol cement is effective after 13 months (129 subcultures). Other materials, such as silver cements, including the so-called germicidal cements, lost their bactericidal effect after three or four passages or subcultures."

In 1955, Turkheim again investigated the bactericidal effect of zinc oxide and eugenol. In this study, discs of decalcified lower central incisor teeth were inoculated with:
(1) Staphylococcus pyogenes varius aureus, (2) Lactobacillus acidophilus odontolyticus, (3) Escherichia coli and (4) Monilia albicans. These were then placed under a specified (0.5 gm. zinc oxide to 0.1 ml. eugenol) mix of zinc oxide and eugenol. The same procedure was used on carious dentin from extracted teeth. It was found that, in the first test, all microorganisms had been killed at the end of three hours. Growth was evident in all cases on infected dentin in the second test.

In yet another study Turkheim, in 1955, investigated the bactericidal properties of zinc oxide and eugenol reinforced with methyl ammonium chloride, thymol and cellulose acetate. Extracted, non-caries lower incisors and lower pre-molars were decalcified, placed in tubes of sterile glucose broth and incubated at 37°C. for 24 hours to test sterility. The teeth were then sectioned in one mm. slices and each slice was placed in a Kahn tube containing one ml. of sterile glucose broth. These tubes were then inoculated with strains of Staphylococcus pyogenes aureus, Escherichia coli, Monilia albicans, Pseudomonas pyocyanea and Lactobacillus acidophilus odontolyticus and incubated for 72 hours. The dentin slices were then placed in empty, sterile anesthetic capsules and covered with the test cement. They were then incubated at 37°C. for (1) 30 minutes, (2) 50-52 minutes, (3) 60 minutes, (4) 120 minutes and (5) 180 minutes. The
slices were then removed, placed in sterile glucose broth and incubated until growth occurred. If negative they were left in the incubator for up to three weeks. After three hours' contact all cultures were killed. The same test was performed on natural carious dentin from human molars. Nine pieces (A) were covered with normal zinc oxide and eugenol and 25 pieces (B) were covered with reinforced cement. After three or four hours' contact, bactericidal effect showed twice with A and twice with B, bacteriostatic effect once with A and no effect, A and B, once each. The pieces were sterilized by B in $8\frac{1}{2}$ to $20\frac{1}{2}$ hours. Turkheim concluded that the reinforced cement could sterilize natural decayed dentin within ten hours of close contact and determined clinically that the vitality of the healthy pulp was preserved.

In 1956, Shay et al. studied the antibacterial effects of gold foil, cast inlays, silver amalgam, copper amalgam, silicate cement, copper cement, zinc oxyphosphate cement and quick-set acrylic using L. casei, B. fermentans and S. viridans on various media. They found copper amalgam best, followed in order of decreasing effectiveness by copper cement, gold foil, silver amalgam, zinc oxyphosphate cement, silicate cement, inlay gold and quick-set acrylic.

Zawawi, in 1958, studied the connective tissue reaction of rats to implants of various materials. It was found that all materials which contained zinc oxide and eugenol produced
a severe reaction two days after implantation. These reactions tended, however, to become moderate or mild.

Wilkinson, in 1959, studied the antibacterial effects of zinc oxide and eugenol on carious dentin in vivo. He found that after one week 76 per cent of the dentin samples were bacteriologically negative and that after two weeks 100 per cent of the samples cultured were negative.

Mitchell, in 1959, conducted further studies on the connective tissue reaction of albino rats to implants of various dental materials. He found that the reactions to zinc oxide and eugenol were mild for the three time intervals studied.

Mitchell et al., in 1962, also studied pulpal reactions to various dental materials in relation to cavity depth. It was found that: "Zinc oxide and eugenol was associated with the least inflammatory response encountered in comparison with all other materials tested."

It is apparent from the review of the literature that the effect of zinc oxide and eugenol on microorganisms has not been clearly defined—the degree of bactericidal action, as reported by the several investigators, varies greatly.
STATEMENT OF PROBLEM
The purposes of this study were to develop a technique whereby a specific microorganism could be inoculated into the dental pulp of a dog; to determine if this organism would remain viable and could be recovered; and to determine if zinc oxide and eugenol, when placed in contact with the pulpal tissue, would kill this organism.
EXPERIMENTAL PROCEDURE
Before conducting this study it was necessary to perform two preliminary experiments.

The purpose of the first preliminary experiment was to determine the bactericidal effect, if any, of the adhesive* used in the study on the test organism. The procedure of testing was conducted in the following manner.

Serratia marcescens ***, an aerobic, non-spore forming, Gram negative bacillus was selected as the test organism. This bacillus is chromogenic, producing a characteristic red pigment. Five milliliters of sterile tryptocase soy broth*** without glucose, adjusted to pH 7.4, was inoculated with one loop-full of a stock culture of Serratia marcescens and incubated at 75°F. for 72 hours. At this time 0.1 ml. of the culture was pipetted on each of two sterile tryptocase soy agar plates and spread evenly over the surface of the agar with a sterile glass rod.

Five evenly spaced holes were bored in the agar one inch from the periphery of the plate and one hole was bored in the center of the agar plate. These holes extended entirely

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* Eastman 910 Adhesive, Tennessee Eastman Co., Kingsport, Tenn. Procured from John Bleeker, 111 David Street, Cincinnati, Ohio.

** Furnished by the Department of Microbiology, Indiana University.

*** Baltimore Biological Laboratory, Baltimore, Maryland. Furnished by the Department of Microbiology, Indiana University.
through the agar, a distance of approximately five mm., and were produced with a sterile steel dental bur having a diameter of six millimeters. Each hole was completely filled with the adhesive used in the study, Eastman 910 Adhesive, and incubated at 75°F for 72 hours. The plates were examined at this time and the red pigment was observed immediately adjacent to all holes. No zones of inhibition were noted, indicating that, under the conditions of this test, the adhesive exhibited no bactericidal effect on the test organism.

The purpose of the second preliminary test was to determine if the dental pulp of dog's teeth could be successfully inoculated through a hole produced in the coronal portion of the maxillary and mandibular cuspid and second molar teeth and extending into the pulp chamber. It was also necessary to determine if the organism would grow in the dental pulp and remain viable for at least nine days.

The culture was prepared by inoculating five ml. of sterile tryptose soy broth without glucose with one loop-full of Serratia marcescens from a stock culture. It was incubated for 72 hours at 75°F. immediately preceding use.

The animal was anesthetized by intravenous injection of pentobarbital sodium* using a dosage of one ml. per five

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* Nembutal sodium (pentobarbital sodium), 50 mg. (3/4 gr.) per ml., Abbott Laboratories, North Chicago, Illinois.
pounds of body weight.

Throughout the procedure every effort was made to main-
tain a sterile field. The operator wore a sterile gown, ster-
ille face mask and sterile rubber gloves. All materials and
instruments which could be autoclaved were so treated. Bot-
tiles, test tubes, culture tubes, tray tops and other such ap-
purtenances were scrubbed with 70 per cent alcohol.

After the animal was anesthetized, it was placed on the
operating table and covered, with the exception of the head,
with sterile drapes. A cork bite block was placed between
the molar teeth on the side opposite that to be operated to
maintain the mouth in a wide open position. A dental rubber
dam was placed in such a manner that the maxillary and man-
dibular cuspid and second molar teeth were isolated. The dam
was secured by rubber dam clamps on the four teeth to be op-
erated. The edges of the dam were then taped to the animal's
head with adhesive tape. The exposed teeth, rubber dam and
rubber dam clamps were then scrubbed with a sterile four by
four inch gauze pad soaked in 70 per cent alcohol. Sterile
four by four inch gauze pads were then placed in such a manner
that the edges of the rubber dam and adjacent portions of the
animal's head were covered.

At this point the operator assumed a sterile gown, mask
and gloves. Previously, a cotton towel had been prepared by
cutting a hole approximately five inches in diameter in the
center of the towel. This towel was then autoclaved. The
towel was placed in such a manner that the animal's head was
covered with the cut out portion overlaying the mouth. It
was thought that with such a procedure inadvertent contamina-
tion by touching the animal could be avoided.

An air turbine handpiece* and power cord was scrubbed
with 70 per cent alcohol and a sterile #557 friction grip bur
placed therein. This bur was one mm. in diameter. With this
instrument a hole approximately six mm. wide and one mm. deep
was cut in the buccal surfaces of the four isolated teeth.
The bur and handpiece were scrubbed with 70 per cent alcohol
after each hole was cut and dried with a sterile four by four
inch gauze square. At this point each cavity was cultured by
moistening a small cotton pledget with tryptocase soy broth
and touching the pledget to the cavity floor. Care was used
in order that the exterior surface of the tooth or walls of
the cavity were not touched. The pledget was then placed in
five ml. of tryptocase soy broth in a marked test tube.

In the center of the floor of each prepared cavity a
hole approximately three mm. wide was cut and of such a depth
that it appeared to be in close proximity to the pulp. In
the center of this second hole, entrance was carefully made
into the pulp chamber to the extent at which hemorrhage first

* Mid-West Air Drive, Mid-West Dental Manufacturing Company,
Chicago, Illinois.
became apparent. Between each operation the bur and hand-
piece was scrubbed with 70 per cent alcohol and dried with a
sterile four by four inch gauze pad.

The tip of a sterile paper point was then inserted into
the pulp tissue and then placed in a test tube containing
five ml. of tryptocase soy broth.

The purpose of culturing the cavity floor was to deter-
mine the presence or absence of microorganisms at this point.
The purpose of culturing the pulp was to determine if any
contaminating microorganisms were initially present in the
pulp.

The pulp was then inoculated in the following manner. A
33 gauge platinum wire was used as a loop. The loop was
formed on a 10 gauge shaft and compressed to form an ellipsoid
oval to permit easier inoculation. The hemorrhage having been
controlled with sterile cotton pellets, one loop full of the
inoculum was placed in the pulp using standard inoculation
procedures.

A gold disc*, 2.5 mm. in diameter and 0.5 mm. thick was
then placed over the exposure site. One drop of the adhesive
was placed in the cavity and a cotton pellet was used to re-
move most of the fluid, leaving a thin layer of adhesive. A
few grains of zinc oxyphosphate cement were placed in the

* Ney solder contact points, J. M. Ney Co., Hartford, Conn.
cavity to hasten the hardening of the adhesive. The purpose of the adhesive was to prevent displacement of the gold disc. Silver amalgam was then condensed into the cavity and carved to the exterior contour of the tooth.

The cavity and pulp cultures were incubated at 75°F. for 72 hours.

After 72 hours, the animal was again prepared and the alloy carefully removed from the preparation. The cavity floor was then cultured using a cotton pledget in the manner previously described. The pledget was again placed in five ml. of tryptocase soy broth and incubated at 75°F. for 72 hours. The gold disc was then removed, the pulp was cultured with a paper point in the manner previously described, placed in five ml. of tryptocase soy broth and incubated at 75°F. for 72 hours. The cavity floor and pulp were recultured after an additional 72-hour period. Growth and viability of the organism was indicated by the presence of red pigment in the culture tubes and furnished presumptive evidence that the organism would flourish in the dental pulp.

The purpose of the study proper was to determine the bactericidal effect, if any, of zinc oxide and eugenol on a specific microorganism, Serratia marcescens, introduced into the dental pulps of dogs. The maxillary cuspids and second molars were selected as test teeth and the mandibular cuspids and second molars were selected as control teeth.
The animal was prepared in the manner previously described. Initial cavity and pulp cultures were made of the four test teeth and the four control teeth. All teeth were then inoculated and closed with the gold discs, adhesive and silver amalgam.

After 72 hours, the cavities and pulps were recultured. At this point the mandibular cuspids and second molars, the control teeth, were closed with gold discs, adhesive and silver amalgam. The maxillary cuspids and second molars, the test teeth, were closed in the following manner: A creamy mix of zinc oxide and eugenol was placed over and just covering the exposure site and gently compressed with a sterile cotton pellet.

Zinc oxide (96% by weight) containing zinc acetate (4% by weight) as an accelerator was then mixed with eugenol and the cavity completely filled with this mixture. When the mixture hardened it was carved to the external contour of the tooth.

The procedure of culturing test and control teeth, sealing the test teeth with zinc oxide and eugenol and the control teeth with the adhesive, gold disc and silver amalgam, was repeated at 24 hours and at 216 hours.

---

** Zinc oxide, U.S.P., Mallinckrodt, St. Louis, Missouri. Furnished by the pharmacy, Indiana University Medical Center.

*** Zinc oxide, U.S.P., and zinc acetate, U.S.P., Mallinckrodt, St. Louis, Missouri. Furnished by the pharmacy, Indiana University Medical Center.
The maxillary and mandibular cuspid and molar teeth of nine dogs were used in this study, a total of 72 teeth. Thirty-six maxillary cuspids and second molars were used as treatment teeth and 36 mandibular cuspids and second molars were used as control teeth.
RESULTS
Zero hours

Upon initial culture, two (5.5%) of 36 cavity cultures in the treatment group were positive and 34 (94.4%) were negative. In the control group 36 (100%) of the cavity cultures and 36 (100%) of the pulp cultures were negative.

72 hours

At 72 hours, 16 (44.4%) of 36 cavity cultures in the treatment group were positive and 20 (55.5%) were negative. In the treatment group, 25 (69.4%) of 36 pulp cultures were positive and 11 (30.5%) were negative.

In the control group, 19 (52.8%) of 36 cavity cultures were positive and 17 (47.2%) were negative. In the control group, 32 (88.9%) of 36 pulp cultures were positive and four (11.1%) were negative.

144 hours

At 144 hours, three (8.3%) of 36 cavity cultures in the treatment group were positive and 33 (91.6%) were negative. In the treatment group, 11 (30.5%) of 36 pulp cultures were positive and 25 (69.4%) were negative.

In the control group, 13 (33.3%) of 36 cavity cultures were positive and 23 (63.3%) were negative. In the control group, 20 (55.5%) of 36 pulp cultures were positive and 16 (44.4%) were negative.
216 hours

At 216 hours, one (2.8%) of 36 cavity cultures in the treatment group was positive and 35 (97.2%) were negative. In the treatment group 10 (27.7%) of 36 pulp cultures were positive and 26 (72.2%) were negative.

In the control group, eight (22.2%) of 36 cavity cultures were positive and 28 (77.7%) were negative. In the control group, 16 (44.4%) of 36 pulp cultures were positive and 20 (55.5%) were negative.

The preceding data is shown in grouped form in Table I.
### TABLE I

Growth of *Serratia Marcescens*.

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<th>Growth of <em>Serratia Marcescens</em> 72 hours</th>
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| TOTAL     | 2   | 34  | 36  | 36  | 36  | 16  | 20  | 25  | 11  | 19  | 17  | 32  | 4   |
TABLE I (continued)

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<td><strong>TOTAL</strong></td>
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*  = Treatment Group
ULC denotes left maxillary cuspid
ULM " " second molar
URC " right " cuspid
URM " " second molar

(+) = presence of Serratia Marcescens in the culture media.
(-) = absence of Serratia Marcescens in the culture media.
Figure 1. Isolation of treatment and control teeth of a dog with rubber dam. Preparation of cavities with exposure of pulpal tissue is shown.
Figure 2. Closure of pulpal exposure with Ney contact solder point after inoculation of pulp with test organism.
DISCUSSION
The cavity preparations of 34 (94.4%) of the 36 teeth in
the treatment group were bacteriologically negative at the
original culture, as shown in the results. All cavity prep-
parations in the control group and all pulp cultures in both
treatment and control groups were negative at the original
culture. In all probabilities, the presence of the positive
cultures in the cavity preparations of the treatment group
were due to inadvertent contamination by the operator.

At the 72-hour culture, 16 (44.4%) of the treatment group
and 19 (52.8%) of the control group showed positive cavity
cultures. Also, in this period 25 (69.4%) of the treatment
group and 32 (88.8%) of the control group demonstrated posi-
tive pulp cultures. It should be noted at this time that, in
a number of instances, organisms other than Serratia marcescens
were present in either cavity or pulp cultures. A complete
explanation of the effects of these contaminants is not within
the scope of the present work. The terms "positive" and "neg-
ative" in this study indicate the presence or absence of the
test organism, Serratia marcescens. An explanation of neg-
ative pulp cultures in both the treatment and the control
group at the 72-hour interval may be the overgrowth of the
test organism by contaminating bacteria. These results might
also be explained on the basis of varying numbers of organ-
isms in the inoculum. Visual inspection of the culture
broth could only give a qualitative determination of the num-
ber of microorganisms. Positive cavity cultures in this in-
terval, and in succeeding intervals, could have resulted from
inadvertent contamination during inoculation or through the
seepage of blood-borne organisms into areas of incomplete
seal between the exposed pulp and the cavity floor.

At the 1½-hour interval the pulps of the treatment group
had been exposed to the action of zinc oxide and eugenol for
72 hours. The number of positive pulp cultures in the treat-
ment group had dropped from 25 (69.4%) at 72 hours to 11
(30.5%), a reversal from positive to negative of 14 (38.6%),
indicating a bactericidal effect of 38.6%. During this same
period, of the 32 (88.6%) pulp cultures in the control group
which were positive at the 72-hour interval, 12 (33.3%) were
negative at the 1½-hour interval. An analysis of these data
was made using the Chi-square test. The value for Chi-square
was 4.60 with one degree of freedom, consequently .025 < p < .046.
With these results in mind, it is thought, however, that a
trend exists which would tend to indicate a degree of bacteri-
cidal effectiveness for zinc oxide and eugenol. This degree
of bactericidal effectiveness (38.6%) is lower than the bacte-
ricidal index of 53 found by Hardwick. The number of revers-
sals from positive to negative in the pulps of the control
teeth was not foreseen. Such an event might have been the re-
sult of an overgrowth of other contaminating organisms. An-
other possible cause might be the bloodstream clearance of
microorganisms. This phenomenon was investigated by Rogers, who injected known quantities of staphylococci in the marginal ear veins of rabbits. Within 20 minutes following the injection of $5 \times 10^8$ viable staphylococci, the blood concentration had dropped to $5 \times 10^3$. Rogers stated that: "Following the intravenous injection of staphylococci, the majority of microorganisms are swiftly removed from the circulating blood." Rogers' opinion was that the staphylococci are rapidly ingested by rabbit polymorphonuclear leukocytes.

At the 216-hour interval, of 25 (69.4%) positive pulp cultures in the treatment group which were evident at the 72-hour interval, 15 (41.7%) showed a reversal from positive to negative. This represents a drop in positive cultures of one (2.3%) between the 144-hour interval and the 216-hour interval. In this same period, of 32 (88.8%) control pulp cultures which were positive at the 72-hour interval, there was a reversal from positive to negative of 16 (4.4%). The reversal from positive to negative between the 144-hour interval and the 216-hour interval was apparent in four (11.1%) instances. An analysis of these data was made using the Chi-square test. The value for Chi-square was 2.16 with one degree of freedom, consequently $0.03 \ p \ 0.15$. The possible reasons for such effects are the same as those given for the 144-hour interval.

It is recognized that Serratia marcescens is not necessarily representative of those microorganisms usually found
in the mouths of dogs. Furthermore, the reactions of this
organism to zinc oxide and eugenol may vary greatly from the
reactions of those microorganisms which constitute the normal
canine oral flora.

In the opinion of the investigator, this study could be
improved by inoculating the pulp with known concentrations of
microorganisms rather than by use of the somewhat more crude
loop method. It is also probable that the validity of the
study could be improved through refinements of the experimental
technique and more adequate control mechanisms.

In future studies of this type, histological sections of
both treatment and control teeth might give evidence which
would be valuable in the interpretation of results. Also,
sacrifice of animals at various time intervals might reduce
the number of variables in the study. There is, in addition,
a need for more sophisticated methods of statistical analysis.
SUMMARY AND CONCLUSIONS
The maxillary cuspid and second molar teeth of dogs were used as treatment teeth and the mandibular cuspid and second molar teeth in the same animal were used as control teeth. A total of nine dogs was used in which there were 36 treatment and 36 control teeth. Using a sterile technique, openings into the buccal aspects of all teeth were made and the pulp cultured. All pulps were then inoculated with a test organism, Serratia marcescens. After cultures showed evidence of growth of the organism, the pulp exposure in the treatment teeth was covered with a mixture of zinc oxide and eugenol which did not contain an accelerator and the cavity was filled with zinc oxide and eugenol which did contain an accelerator. The pulp exposure in the treatment teeth was covered with a gold disc, after which a layer of Eastman 910 adhesive was placed in the cavity. The cavity was then completely filled with silver amalgam.

At intervals of 72 hours and 144 hours following the application of the zinc oxide and eugenol, the cavities and pulps of all teeth were cultured to determine the presence or absence of the test organism.

Conclusions:

Under the conditions of this study, the results obtained do not indicate that zinc oxide and eugenol has a high degree of bactericidal effect on Serratia marcescens. However, a trend was observed which would tend to indicate that zinc
oxide and eugenol does have a certain degree (38.9%) of bactericidal effectiveness. A need for further investigation using a more refined experimental design is indicated.


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<td>42.</td>
<td>Zender, H.A.</td>
<td>Bacteria in the dentin after cavity preparation</td>
<td>Illinois D. J., 9:207, 1940.</td>
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64. Shrigley, E. W.: Personal communication.

CURRICULUM VITAE
James Pope McKnight

September 19, 1921

Born in Arlington, Tennessee

1940-1942, 1946-1948

B.S., Memphis State University

1946-1951

D.D.S., University of Tennessee College of Dentistry

1951-1952

Postgraduate pedodontics, University of Tennessee College of Dentistry

December 27, 1949

Married Mary Sue Parran

Professional Honors:
Richard Doggett Dean and Marguerite Taylor Dean Honorary Odontological Society

Omicron Kappa Upsilon

Professional Societies

American Dental Association

American Society of Dentistry for Children
ABSTRACT
This study was conducted to demonstrate, in vivo, the bactericidal effect of zinc oxide-eugenol on a specific organism inoculated into the dental pulp of dogs.

An aseptic technique was used throughout the study. The maxillary cuspid and second molar teeth were used as treatment teeth and the mandibular cuspid and second molar teeth were used as control teeth. A six mm. wide and one mm. deep, was cut in the buccal surfaces of the control and treatment teeth. Bacteriologic cultures of the cavity floor were made at this point. The cavity floor was cultured at this point. In the center of the cavity an opening approximately three mm. wide was cut and of a depth that appeared to be close to the pulp. In this second hole, entrance was made into the pulp. The pulp was cultured using a sterile paper point. The pulps were then inoculated with Serratia marcescens. A small gold disc was placed over the exposure site, followed by a layer of Eastman 910 cement and a layer of silver amalgam. At 72 hours, the cavities and pulps were recultured. The pulps of the treatment teeth were covered with a layer of zinc oxide (without an accelerator) and eugenol and the cavity was filled with a zinc oxide (containing an accelerator) and eugenol. The cavities of the control teeth were closed as described above. The cavities and pulps of the treatment and control teeth were recultured at 144 hours and 216 hours and as described at the 72-hour interval. At 144 hours, 11 (30.5%) of 36 pulp cultures in the treatment group were positive and 25 (69.4%) were negative. In the control group, 20 (55.5%) of 36 pulp cultures were positive and 16 (44.4%) were negative. At 216 hours, 10 (27.8%) of 36 pulp cultures in the treatment group were positive and 26 (72.2%) were negative. In the control group, 16 (44.4%) of 36 pulp cultures were positive and 20 (55.5%) were negative. The value for Chi-square at 144 hours was 4.60 with one degree of freedom, consequently .025 p .046. The value for Chi-square at 216 hours was 2.16 with one degree of freedom, consequently .083 p .157. Zinc oxide and eugenol did not exhibit a high degree of bactericidal effectiveness in this study. A need for further investigation is indicated.