Treatment Of Infected Dental Pulps Of Monkeys With Vancomycin And Calcium Hydroxide

By

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INTRODUCTION

It is generally accepted that pulp-capping procedures should be limited to young, vital teeth which have been exposed for brief periods. The principle involved is that the exposed dental pulp tissue will not form a dentin bridge over the pulp exposure in the presence of large numbers of bacteria and bacterial infections. A healthy pulp can repair minute injuries even in the presence of small numbers of bacteria. Calcium hydroxide has long been used for this minute pulpal exposure, but is not effective on an infected pulp.

Physiologic repair of the exposed dental pulp is the desired result of pharmacologic treatment. The major objectives in achieving repair are the control and inhibition of infection, and the stimulation of odontoblasts to form reparative dentin over the exposure site.

Many techniques and medications have been employed to meet these objectives. With the rapid growth of the pharmaceutical industry in recent years, numerous drugs have been available for pulp-capping procedures. Antibiotics, corticosteroids, sulfonamides, and fungicides are among the drugs that have been used, alone or in various combinations. Usually these materials are placed directly over the exposed

pulp. Unfortunately, most of these procedures have been assessed clinically, and do not correlate well with histologic findings. Most histologic studies have been inconclusive and require further investigation.

This study is designed to evaluate the histologic findings using an antibiotic alone and in combination with calcium hydroxide. It is hoped that this will enhance our knowledge of direct pulp therapy.

REVIEW OF THE LITERATURE

Antibiotics were used in the treatment of infected dental pulps soon after their first clinical application in medicine. In 1942, penicillin therapy, as an entity, first appeared in the Quarterly Cumulative Index Medicus.[1] At approximately the same time, the Index to Dental Literature[2] listed several dental publications which made reference to the drug. Kolmer[3] recognized the potential role of antibiotic compounds in pulp therapy and gave intra-muscular injections of penicillin while treating acute early pulpitis. This systemic use of penicillin was the first study describing an antibiotic effect on pulpal infections.

Bonner[4] reported the first clinical use of an antibiotic in pulp-capping[a] procedures. He moistened crystalline penicillin with glycerin and applied this paste over the exposed pulps. He reported that out of 162 cariously exposed teeth, only eight pulpal deaths resulted. Of the 162 teeth, 113 were treated with penicillin alone, and the rate of success was 100 per cent. Six teeth treated successfully with penicillin were reopened and vital tissue was found. The only criterion for selecting teeth to be treated was a painful response when the pulp was touched by an explorer.

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[a]Pulp capping - the application of a medicament directly to the dental pulp.

In 1950, Kutscher[5] reported the use of a penicillin capping compound on vital cariously exposed pulps. Clinical evaluation of 54 teeth six weeks post-operatively showed a 98 per cent success rate. The author concluded that the dental pulp can continue to function as a normal tissue after exposure, if the source of infection is eliminated, or if the resultant infection is controlled.

Gilberg,[6] in 1951, did not find age to be a significant factor in pulp-capping with penicillin. He placed a drop of sterile water over the carious exposure and then dissolved 25,000 units of crystalline penicillin in the water. Another 25,000 units of crystalline penicillin was mixed with zinc oxide-eugenol and sterile water and placed over the exposure site. He reported five failures among 205 teeth treated in this manner, for a 97 per cent success rate. He selected teeth with negative vitality and periapical infections, and from patients ranging in age from two to 50 years.

Burkman, Schmidt and Crowley[7] used penicillin mixed with camphorated monoparachlorophenol in indirect pulp-capping procedures. They reported 75 per cent success in sterilizing carious dentin with no attempt to remove the deeper layers of decay.

The carious dentin treated in this manner was later removed from the tooth and cultured on eight different media. In 93 per cent of these cariously exposed teeth, clinical success was reported.

In 1950, Webb[8] used intramuscular injections of 300,000 units of procaine penicillin for the treatment of painful pulpitis and hyperemia. He stated that one injection usually was sufficient, but two were sometimes needed. After two years of observation, the author claimed clinical success since the teeth remained vital and apparently normal. No indication was given as to the number of teeth treated or the number of failures.

Roberts[9] reported on the adjunctive use of intramuscular penicillin injections in the treatment of exposed vital pulps. Calcium hydroxide was the pulp-capping agent, and 300,000 units of penicillin were injected intramuscularly on each of two consecutive days. After 60 days, 94 per cent were evaluated clinically as being successful. After three years, 23 of the original 44 treated pulps were re-evaluated. Of this number, 21 were still vital.

Kutscher[10] reported in 1953 on the use of terramycin alone, or terramycin mixed with zinc oxide-eugenol as dressings on 20 pulp exposures. No clini-

cally deleterious results were recorded over the three to fifteen month investigation. In all cases, the medicament was considered an irritant because pain resulted when the dressing was applied.

Seelig et al[11] were the first to histologically evaluate an antibiotic compound used for pulp-capping. Penicillin G in a paste was forced into the preparations of 11 of the 20 test teeth of a Rhesus monkey. Of the remaining teeth, four received zinc oxide-eugenol, one had zinc phosphate cement, two were left open, and two were used as controls. A non-sterile technique was used in which coronal pulps were macerated with a bur. Sacrifice and extraction were accomplished seven days post-operatively, and seven of the 11 phosphate cement coverings, over the antibiotic paste, were missing and another was loose. When zinc oxide-eugenol contacted the pulp, inflammation and subsequent abscess formation resulted. A similar reaction was seen when zinc phosphate was placed in direct contact with the pulp. A normal pulp was observed under all the applications of the penicillin capping compounds. The teeth were removed after only seven days and yet bridge formation was evident in the penicillin treated teeth. They described this bridge as being from dentin chips held

by a homogeneous mixture. Their sample size was quite small, and the loss of the seven protective coverings over the experimental drug leaves their results open to question.

Amler[12] claims success in 15 of 16 teeth which had deep carious lesions and vague intermittent pain pre-operatively, and after treatment with systemic antibiotics. Therapy was initiated within two days of the onset of pain, and consisted of administration of buffered long-acting penicillin and terramycin. Amler's rationale was to control bacteria via high blood levels of the antibiotics, and the drugs were administered every six hours up to a maximum of 96 hours.

In 1956, Morales[13] reported the use of sulfonamide, streptomycin, and penicillin as a pulp-capping mixture for anterior teeth. Chloramphenicol mixed with chlortetracycline was recommended as the capping agent for posterior teeth. He placed the antibiotic over the exposure site, then placed vitamin C, calcium hydroxide, zinc oxide and eugenol, and finally zinc phosphate cement over the antibiotic. Four failures were reported in the 150 treated teeth. Two of these failures were attributed to improper diagnosis, and the other two to the breakdown and loss of the cement fillings.

Englander, Massler, and Cortes[14] performed pulpotomies using various antibiotic and calcium salt combinations. This clinical evaluation consisted of a sample of 228 teeth, of which the majority had been associated with a history of pain and had been ordered for extraction. The pulp stumps were covered with one of the following: a polyantibiotic paste, tetracycline in various calcium salts, tetracycline in a non-calcium diluent, and penicillin powder. The average post-operative observation was 43 days. The tetracycline in calcium salts (unspecified) and the polyantibiotic pastes showed 100 per cent clinical success. The tetracycline in non-calcium dilutants showed 82 per cent success, and the penicillin powder showed 64 per cent success. The chloramphenicol was not successful in any of the treated teeth. The authors stated that 94 per cent of the teeth treated with calcium salts were successful, and 80 per cent of the teeth treated with antibiotics were successful. Thus, the calcium salts were more effective as a pulp-capping agent than the antibiotics.

James et al[15] did a histologic evaluation of 175 teeth with vital pulp exposures due to caries. Of these teeth, 131 were treated with calcium salts,

various antibiotics, or a combination of calcium salts and antibiotics. The teeth were sedated for from one to seven days prior to the pulpotomy procedure, by the application of zinc oxide-eugenol. The data of this report indicated a direct relationship between the severity of inflammation and the incidence of internal resorption, and an inverse relationship between the degree of inflammation and the incidence of bridging. Of nine teeth treated with penicillin, 11 per cent showed calcific bridge formation, 56 per cent demonstrated internal resorption, and 89 per cent showed moderate to severe inflammation. Of all medicaments tested, 11 to 69 per cent showed some form of bridging. The authors noted that when calcium salts were added to the antibiotic, the reaction more closely resembled what was seen with the antibiotic alone, rather than with the calcium salts alone.

By 1957, corticosteroids were commercially available and Sidky[16] described their use in pulpotomy therapy. He described the paste consisting of hydrocortisone, omnacillin, and calcium hydroxide as being very effective when used in asymptomatic teeth of young healthy patients.

Kiryati[17] used polyantibiotics in combination with hydrocortisone in pulp-capping surgically ex-

posed pulps of rat molars. Different combinations of the drugs were used and the results evaluated histologically. The most effective combination of drugs which were evaluated from eight to 12 weeks was hydrocortisone, oxytetracycline and chloramphenicol. With this combination, there was complete healing in 52 per cent of the teeth, incomplete healing in 38 per cent, and necrosis in 12 per cent. The antibiotic mixture without the hydrocortisone, but still in combination with calcium hydroxide or zinc oxide-eugenol, showed 40 per cent complete healing, 30 per cent incomplete healing, and 30 per cent necrosis. Kiryati's description of incomplete healing described calcific bridging in the primary stages of formation, with irregular fibrous calcification and irregular dentin formation. The author stated that pulpal healing was dependent upon the degree of infection.

Seltzer and Bender[18] in 1958 pulp capped surgically exposed dogs' teeth with an aqueous solution of 250,000 units of potassium penicillin. Following this application, the exposure site was covered with asbestos fibers, and amalgam was used to restore the tooth. Necrosis and the development of an apical granuloma occurred in all of the 52 treated teeth. These findings were observed as early as seven days and as late as 90 days post-operatively.

Schroeder and Triadan[19] claimed clinical success after pulp-capping with a mixture of triamcinolone, chloramphenicol, four per cent xylocaine solution, and an ointment base. Of the 214 teeth treated in this manner, all were free from pain in two to three hours after treatment, and remained asymptomatic and vital during the three week observation period.

In 1962, Kiryati[20] described the effects of streptokinase in combination with oxytetracycline and cortisone. He studied the effect of proteolytic enzymes on inflamed pulps in rat molars. He mentioned that streptokinase or streptodornase did not increase or decrease the rate of the pulpal reparative process. However, he said that the greatest success was obtained when streptokinase, oxytetracycline, and cortisone were used in combination. This success included the formation of an osteoid-like substance just beneath the area of destruction.

In 1962, Vigg[21] reported that he was still having success after 20 months of observing 66 teeth treated with an experimental drug combination. The drug consisted of one per cent hydrocortisone and three per cent oxytetracycline in an ointment base. His criteria for evaluation were negative radiographic pathological findings and a lack of pain.

Fiore-Donno and Baume[22] stated that the favorable clinical results obtained by the direct capping procedure with compounds containing corticosteroids plus antibiotics were not corroborated by histological evidence. After studying 123 human pulps capped with these compounds, the authors recommended caution in their use. They found an absence of a solid barrier at the exposure site even after long periods of time. This opening at the exposure site was described as a route for re-infections, as most filling materials have a decreased sealing ability with age. Most of the 123 teeth tested were judged clinically successful, but histologically they could not be considered successful. It was concluded that glucocorticosteroids could be the cause of the fibrotic metaplasia and inhibitions of collagenic activity seen with the experimental pulps.

In histologic sections of 31 teeth, Tabin[23] compared the healing of dental pulps capped with calcium hydroxide with the healing of pulps capped with terramycin. He reported that placing terramycin on amputated pulp stumps produced an area of chronic inflammation and retarded dentin bridge formation. This effect was described histologically on teeth extracted seven to 60 days post-operatively. Dentin bridge formation was

found under all medications, but in the 16 teeth treated with calcium hydroxide, and distant from the site in those treated with terramycin. Tabin concluded that aqueous calcium salts were not needed for bridge formation.

Gardner et al[24] reported a histological evaluation of Neosporin[a] (Polymixin B, Neomycin, and Bacitracin) used as a pulp-capping compound on induced pulp exposures of dogs. Of the 27 tooth samples, one-half were treated with Neosporin in combination with calcium hydroxide, and the other one-half with Neosporin in combination with paramonochlorophenol. Twenty-six of these 27 teeth had a histologic picture of degenerative changes, including acute pulpitis in 24 of the teeth, and one with an abscess.

Burke and Knighton[25] surgically exposed dental pulps in rat molars and placed an antibacterial agent in combination with zinc oxide and eugenol over the pulpal stumps. Staphylococcus aureus was then injected intravenously to produce bacteremias in each animal. Pulpal contents were then cultured at one and seven days post-operatively. When the pulp-capping compound contained penicillin, 47.5 per cent of the specimens at one day and 6.9 per cent of the specimens

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[a]Neosporin, Burroughs, Wellcome and Company Inc., Tuckahoe, New York.

at seven days had bacterial growth. Bacitracin,[a] when used in the pulp capping agent, resulted in bacterial growth in 50.9 per cent of the one day media, but at seven days no growth was recorded. Bacterial colony formation at one day ranged from 84.4 per cent to 93.2 per cent for other antibacterial compounds tested. At seven days, these compounds had a range of colony growth of from 71.4 per cent to 96.6 per cent.

Ninety per cent success was reported by Gason-Zade[26] while using antibiotics to treat pulpitis. Chlortetracycline was placed as an ointment on the cavity floor after removing all the carious dentin. If pain persisted after this treatment, local anesthetic was administered in conjunction with 100,000 units of penicillin. The dressing over the pulp was changed on the second day, and chlortetracycline in combination with 20 per cent oil of camphor was placed.

Using a blind control procedure, Lawson and Mitchell[27] examined 54 teeth, about half of which were treated with antibiotics and a glucocorticoid mixture. The 27 control teeth treated with starch and polyethylene glycol had 55 per cent successful

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[a]Bacitracin, Eli Lilly and Company, Indianapolis, Indiana.

responses when re-examined in three months. Of the 27 teeth treated with the corticoid-antibiotic, 100 per cent met with clinical success, indicating that the experimental drug combination (erythromycin estolate,[a] streptomycin sulfate, and flurandrenolone) might have a definite therapeutic value in the treatment of painful pulpitis. Seven of these teeth were studied histologically, and although the corticoid-antibiotic treated teeth did not always show normal appearing pulps, the controls invariably revealed persistent pulpitis. The authors concluded that painful pulpitis seems to be a reversible process when treated, that painless pulpitis can occur without clinical symptoms, and that histologic interpretations do not necessarily correlate with clinical findings.

Mager,[28] in 1964, treated pulpitis in 20 teeth with a synthetic steroid in combination with an antibiotic.[b] Only one of the 20 teeth treated was considered a failure over the six month observation period. Immediate relief of pain was described after the initial treatment, and in no cases were side effects observed.

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[a] Erythromycin estolate, Eli Lilly Company, Indianapolis, Indiana.

[b] Ledermix Dental Compound, Lederle Laboratories (A Division of American Cyanamide Company) Pearl River, New York.

Olsen[29] described loss of vitality in only three of 379 teeth treated with a corticoid and demethylchlortetracycline (Ledermix).[a] The teeth were treated for hyperemia, pulpitis, and apical periodontitis, and were observed from six to 12 months.

In a clinical study, Ehrman[30] reported that 16 of 22 teeth treated with a corticosteroid were non-vital when tested six months after pulp-capping. These teeth were treated for acute suppurative pulpitis and remained free from pain during the six month post-operative period. Bridging did not occur and the author concluded that a symptomless vital tooth with an unhealed exposure is still preferable to a non-vital endodontically treated tooth.

Haldi and John[31] administered sulfonamide subcutaneously, and injected penicillin intravenously, to observe pulp fluid levels of these drugs. They found that when used in this manner, these drugs appeared in the dental pulp fluid in approximately the same concentration as in the blood.

In a double-blind clinical pulp-capping study, Schneider and Lawson[32] attempted to evaluate results with a mixture of corticoid and antibiotic, as against

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[a]Ledermix Dental Compound, Lederle Laboratories (A Division of American Cyanamide Company) Pearl River, New York.

results with calcium hydroxide alone. The corticoid-antibiotic mixture resulted in 76 per cent clinical success rate, while the calcium hydroxide met with 90 per cent success.

In 1966, Janiszewska[33] reported treating 41 vital teeth which had deep carious lesions and a history of pain with a combination of antibiotics and hyaluronidase. The paste consisting of Declomycin,[a] erythromycin, Kanamycin, and hyaluronidase was placed over exposed pulps and left for up to 12 months. Toothaches recurred in four of the 41 teeth, but all retained their vitality. The author recognized that this 90 per cent success rate was fraught with certain dangers, and he warned his readers to withhold general use of this technique until a histological and long term study could be evaluated.

The 100 per cent success rate described by Lawson and Mitchell[27] prompted a follow-up of this study by Mullaney, Lawson and Mitchell.[34] Originally, this 100 per cent rate was reported clinically after three months of observations. Many of the same teeth were re-examined as long as two years, four months, and ten days post-operatively. Over this longer period, the

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[a] Declomycin, Lederle Laboratories (A Division of American Cyanamide Company) Pearl River, New York.

corticoid-antibiotic mixture resulted in 71.4 per cent success. This was a marked reduction of 28.6 per cent from the original three month observation. These authors also reported a laboratory study of 28 monkey teeth. One-half of these served as controls, while the others received the cortico-antibiotic mixture. All teeth were extracted at 90 or 180 days, and histologic sections prepared. All but two of the teeth revealed normal pulps, and these two control teeth were abscessed. The experimental teeth seemed to allow for a more extensive dentinoid bridge formation, and less inflammation was apparent in them.

The evidence presented by Fiore-Donno and Baume[35] led them to conclude that pulp-capping with corticosteroids is contraindicated. The authors reviewed 30 pulps which had a history of spontaneous pain prior to treatment with corticosteroids. Histologically, it was apparent that dentinogenesis was arrested at the exposure site. Bridging never occurred and residual chronic inflammation increased as the post-operative period increased.

In a clinical study by Weine,[36] an 89.3 per cent success rate was declared when using a combination of Nystatin and oxytetracycline as a pulp-capping agent. Oxytetracycline used alone over the exposed pulp resulted

in a 96 per cent success rate. Evaluation was dependent upon an electric pulp tester, radiographs, and clinical symptoms. A histologic study was initiated for further evaluation of the pulp's reaction to these drugs. The author concluded that oxytetracycline is more effective as a pulp-capping agent than oxytetracycline in combination with Nystatin.

In 1966, Baker[37] disclosed his findings on the treatment of infected monkey teeth with topical antibiotics. His modified double-blind method of investigation was used after 52 monkey teeth were surgically exposed, left open for 24 hours to become infected, and then pulp-capped. One-half of these teeth were treated with a combination of erythromycin estolate 10 per cent and streptomycin sulfate 10 per cent. The other 26 teeth were treated with starch as a control. The teeth were extracted at 30 or 90 day intervals after treatment and histologically evaluated. Inflammation of varying degrees was seen in all teeth treated. However, the teeth treated with the antibiotic capping compound exhibited much less inflammation than did the controls. Abscess formation and necrosis were often found in the teeth capped with starch. There were no complete bridges at the exposure site, but attempted calcific repair was observed.

In 1968, Compton[38] again evaluated 18 of the teeth that were initially studied by Lawson and Mitchell,[27] in 1964, and re-evaluated by Mullaney, Lawson and Mitchell,[34] in 1966. Thirteen of the teeth were treated with erythromycin estolate, streptomycin sulfate, and flurandrenolone, and the remainder were controls treated with starch. Six of the teeth successfully treated with the corticoid-antibiotic mixture were evaluated as still clinically successful after five years. All of the control teeth were still clinically successful. The author concluded that painful pulpitis in permanent teeth of adults is reversible. The corticoid-antibiotic mixture did not seem to be superior to the starch control over the long term of this study. Also, the lack of correlation between clinical and histologic findings was emphasized.

In 1968, Eggers[39] undertook a histological investigation of direct pulp therapy using an antibiotic and an anti-inflammatory enzyme. The study required the contamination of 56 pulps of monkeys' teeth, by surgically exposing the pulpal tissue and leaving them open to the oral environment for 24 hours. A satisfactory response was observed in 92.2 per cent of the teeth treated with vancomycin,[a] starch, and

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[a]Vancocin, Eli Lilly and Company, Indianapolis, Indiana.

hyaluronidase;[a] in 71.5 per cent of the teeth treated with vancomycin, starch, and water; and in 42.9 per cent of the teeth treated with both starch and water and starch and hyaluronidase. None of the teeth treated with vancomycin, starch, and water and vancomycin, starch, and hyaluronidase became necrotic, while 35.7 per cent of the teeth treated with starch and water or starch and hyaluronidase became necrotic.

A number of other studies[40-52] have been reported but will not be included in this review. While these authors demonstrated interest in the treatment of dental pulps with antibiotics, their studies lacked controls, were of a short duration, or included many variables. Also, most of these studies supplied little data, involved only a few teeth, or did not include histologic evidence. For these reasons their results could not be accepted with any confidence.

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[a] Alidase, G.D. Searle and Company, Chicago, Illinois.

STATEMENT OF THE PROBLEM

The review of the literature has demonstrated the wide range of results obtained by those who have pulp-capped with antibiotics. It becomes apparent that additional investigation into this area is warranted, for the results have tended to be inconclusive and conflicting.

Bacteria within the oral cavity are considered to play a prominent role in the initiation of pulpal pathology when the vital dental pulp is exposed. It has been suggested that the presence of bacteria is the most significant factor in prohibiting healing. It has also been noted that pulp-cappings of infected pulps with calcium hydroxide is usually unsuccessful.

If an anti-microbial agent were strong enough to be effective against the pathogenic organisms within an infected pulp, and if calcium hydroxide could stimulate reparative dentin over the exposure site in the presence of this anti-microbial agent, the vital pulp would overcome the infection, repair the damage, and survive.

This histologic evaluation should expand the existing knowledge of the pulp's response to antibiotics in combination with other drugs, when they are applied over exposed, vital, contaminated pulp tissue.

PRELIMINARY STUDIES

Antibiotic Inactivation Test

Before the principal study of capping infected pulps with vancomycin and calcium hydroxide was attempted, it was necessary to determine what concentrations of the two drugs would be compatible and yet would maintain their individual characteristics. Kutscher and Yigdall[53] stated that the antibacterial activity of penicillin is nearly destroyed when incorporated with calcium hydroxide. They reported also that Aureomycin, streptomycin, and Terramycin retain some of their antibacterial effect when mixed with calcium hydroxide. No reference was found in the literature to any earlier study concerning the effect of calcium hydroxide on vancomycin.[a] Vancomycin's effect on gram positive microorganisms is well documented in the literature.[54,55]

Methods and Materials:

This preliminary study was done in the Eli Lilly Microbiological Laboratory at Marion County General Hospital, Indianapolis, Indiana. Since vancomycin is effective against oral microorganisms at 2.5 mg/ml,[54] a mixture of 5% vancomycin[a] and 94% calcium

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[a] Vancomycin (Vancocin), Eli Lilly and Company, Indianapolis, Indiana.

hydroxide[a] along with 1% methyl cellulose[b] was the lowest concentration tested. Other concentrations tested were 10, 15, 20, 25, and 30% vancomycin with a proportional decrease in calcium hydroxide or starch, and with the methyl cellulose maintained at 1%. The methyl cellulose was used because it gave body to the mixtures and because it appears in some commercial preparations of calcium hydroxide. Vancomycin, starch, and calcium hydroxide with methyl cellulose were all tested alone on each of the agar plates.

These medicaments were placed on both trypticase soy agar and trypticase human blood agar in two forms: (1) a freshly mixed thick creamy paste, applied with a bacteriological smearing technique, and (2) a dry pellet 1mm by 2mm that was made up the day before the study. Two methods were used to see whether the mixtures would lose any of their antibacterial effect upon drying.

Tissue Compatibility Test

It was imperative that the materials used in this pulp-capping study be compatible with the living

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[a]Calcium hydroxide, Mallinkrodt Chemical Works, St. Louis, Missouri.

[b]Methyl cellulose, Dow Chemical, Division of Dow-Corning Corporation, Greensboro, North Carolina.

tissue, in order to preclude any adverse responses. In using pulp-capping agents, the clinician or investigator has not always attached sufficient importance to the biologic assay of the material. Thus, a study of the tissue response to vancomycin and calcium hydroxide, and the two materials in combination, was attempted before their use was advocated in pulp-capping procedures in animals.

The impetus given by Mitchell,[56] and Boyd and Mitchell,[57] and the fact that this method of assay permits an exact interpretation with the least number of variables, brought about the selection of the tissue implant technique for this preliminary study.

Methods and Materials:

Eighteen adult Wistar rats, which appeared clinically healthy, were used in this preliminary study. The animals were anesthetized by peritoneal injections of 3.5 mg pentobarbital per 100 gm. body weight.

Four areas on the dorsal surface of the animals were shaved with electric shears, scrubbed with 70 per cent alcohol, and dried. One centimeter horizontal incisions were then made with a Bard-Parker #5 blade in a Bard-Parker #3 handle. A plastic preforming matrix was used to make cylindrical pellets 2mm long and 2mm in diameter of the desired concen-

trations of the materials. The subdermal supramuscular tissues were spread apart by the use of a hemostat and the preformed pellets placed 1 cm. away from the incision with a cotton forceps. The incision was then closed and sutured with a curved non-cutting needle and 000 silk suture material (Figure 1).

After sacrifice of the animals at 2, 16 and 32 days post-operatively by overdose of pentobarbital, about one square inch of skin and connective tissue around and including the embedded pellet was excised and placed in 10 per cent formalin. After fixation these specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histologic evaluation was then done to determine the amount and type of inflammation.

The gross appearance and the microscopic findings were recorded after histologic sections for each were read "blind." Mitchell[56] used these criteria to illustrate acute (2-4 days), chronic (14-16 days), and long-term (28-32 days) inflammatory responses. The assay included the types and relative numbers of leukocytes; vascularity of the area, description of membranes, if any, in contact with the materials, and the relative thickness of the fibrous capsule around the specimens.

Materials and concentrations:

Animal No.	Material	Sacrifice Day	No. of Test Sites
1	Vancomycin	2	4
2	Vancomycin	16	4
3	Vancomycin	32	4
4	99% Ca(OH)2 & 1% methyl cellulose	2	4
5	99% Ca(OH)2 & 1% methyl cellulose	16	4
6	99% Ca(OH)2 & 1% methyl cellulose	32	4
7	Starch	2	4
8	Starch	16	4
9	Starch	32	4
10	5% Vanc. & 95% starch	2	4
11	5% Vanc. & 95% starch	16	4
12	5% Vanc. & 95% starch	32	4
13	5% Vanc. & 94% Ca(OH)2, 1% methyl cellulose	2	4
14	5% Vanc. & 94% Ca(OH)2, 1% methyl cellulose	16	4
15	5% Vanc. & 94% Ca(OH)2, 1% methyl cellulose	32	4
16	94% Ca(OH)2, 5% starch, 1% methyl cellulose	2	4
17	94% Ca(OH)2, 5% starch, 1% methyl cellulose	16	4
18	94% Ca(OH)2, 5% starch, 1% methyl cellulose	32	4

Perfection of Surgical Technique and Bacterial Contamination of 48 Hour Pulp Exposures

A stump-tailed Macaque monkey (Macaca Speciosa) was prepared for surgery in the following manner:

1. The animal was removed from its cage with the aid of a net and weighed.

2. An intraperitoneal injection of nembutal sodium was given using a 20 gauge short needle. The recommended dosage of 1.00 ml. (60 mg.) for every three pounds of body weight was used.
3. When profound anesthesia was achieved, radiographs of the four maxillary bicuspids and maxillary central incisors were taken and developed.
4. The animal was then placed in a supine position on the operating table with its head tilted back. No difficulty was experienced in maintaining an unobstructed airway.
5. A careful clinical and radiographic examination was made and any pathologic or unusual conditions and the morphology of the dental pulps were noted.
6. Class V cavities were prepared in three maxillary bicuspids and one maxillary central incisor using a #35 friction grip bur rotating at high speed without water. No attempt was made to operate under aseptic conditions, for contamination of the exposure was desired.

7. A well was then made in the cavity floor using a #4 bur at slow speed. Compressed air was used to remove dentin chips and debris as the cavities were being prepared.
8. The cavities were irrigated with water and dried with air.
9. A #1 Jacquette scaler or a #23 shephard's crook explorer was used to expose the dental pulps by applying firm pressure with the point of the instrument to the thin dentin floor.
10. Care was taken to avoid deep penetration of the explorer or Jacquette scaler into the coronal pulp. Saliva from the vestibular area and tongue was carried to the exposures to aid in contamination. The exposures were left open to the oral environment.
11. The animal was then returned to its cage and observed for postsurgical complications.

Forty-eight hours later the animal was again prepared for the following surgical procedures:

1. The four maxillary bicuspids were extracted using a straight elevator to free the attached gingiva from the teeth and a pedodontic forcep.
2. The extracted teeth were immediately placed in a 10 per cent formalin solution for initial fixation.
3. The animal was returned to its cage and was observed for postsurgical complications.
4. The extracted teeth were trimmed from the mesial aspect with a #700 friction grip bur rotating at high speed. The pulps were approximated by this trimming with care taken to avoid penetration of the pulp chamber. The teeth were returned to the formalin for further fixation.

Specimens were decalcified and seven microns thick serial paraffin sections were made through the exposure sites. Staining was done with hematoxylin and eosin on alternate sections and the Brown and Brenn[58] stain was used on selected sections. Other selected sections were stained by the John Hopkins modification of the gram staining technique.[59]

PRINCIPAL STUDY

Formulation of Medicaments

1.	Powder:	vancomycin hydrochloride (Vancocin^{R})[a]	10%
		calcium hydroxide[b]	89%
		methyl cellulose[c]	1%
	Liquid:	U.S.P. water	
2.	Powder:	calcium hydroxide	99%
		methyl cellulose	1%
	Liquid:	U.S.P. water	
3.	Powder:	vancomycin hydrochloride	10%
		starch	
	Liquid:	U.S.P. water	q.s.
4.	Powder:	starch	
	Liquid:	U.S.P. water	q.s.

Sufficient powder was mixed with one or two drops of the liquid to produce a thick creamy mix.

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[a] Vancocin^{R}, Eli Lilly and Company, Indianapolis, Indiana.

[b] Calcium hydroxide, Mallinkrodt Chemical Works, St. Louis, Missouri.

[c] Methyl cellulose, Dow Chemical, Division of Dow-Corning Corporation, Greensboro, North Carolina.

This portion of the study was originally designed for a modified double-blind control testing procedure. However, during the preliminary studies, the author became familiar with the test agents' physical properties, and it was impossible not to know which drug was being applied to the pulp exposures. Therefore, to eliminate the bias each drug was tested in a random quadrant of the animal. Double-blind control procedures were initiated during histologic evaluation.

Surgical Procedure

One Macaca Speciosa monkey and two Macaca Nemestrina monkeys were used for this experiment. These monkeys were to have a full compliment of 28 permanent teeth. However, when the monkeys were received from the supplier, several permanent teeth had not erupted. The handling of the animals before, during, and after surgery was as outlined by Spedding.[58]

Each animal was operated a total of three times: once to expose the pulps and let them become infected, 48 hours later to place the medicaments, and 30 or 90 days later for sacrifice. The two Macaca Nemestrina monkeys served as 30 day animals, and the one Macaca Speciosa was sacrificed 90 days post-operatively.

The following procedures closely follow the operating procedure used by Spedding,[60] and were not changed from the preliminary study.

Each animal was removed from its cage with the aid of a folding net. This net and heavy gloves provided safety for the operator. The animals were weighed before each procedure to calculate the proper dosage of anesthetic solution. Recommended dosage of nembutal sodium[a] is 1.0 cc. for every three pounds of body weight.

The anesthetic was injected intravenously with a 20 gauge needle and left in the folding net to avoid injury during onset of anesthesia. Profound anesthesia was obtained in about 20 minutes. Since the average operating time was 2½ to 3 hours, additional 0.5 to 1.0 ml. of anesthetic solution was often needed.

After a suitable level of anesthesia was acquired, the net was removed and the animal placed on the operating table. Full mouth radiographs were taken before the first operation and on the day of sacrifice. The tongue was tied to the lip with 4-0 black silk to insure an unobstructed airway.

The rubber dam was not used in any of the operative procedures, for the purpose of this procedure was to provide an infected pulp. A classic Class V cavity

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[a]Veterinary Nembutal Sodium, Abbott Laboratories, North Chicago, Illinois.

preparation was prepared on the buccal or labial surface of the animals' permanent teeth. A high speed rotary instrument with a #35 inverted cone bur was used to prepare the cavity closely approximating the pulp. No water spray was used with the high speed rotary instrument. Short blasts of air were used to clear the area of debris. A #4 round bur was then used to remove additional dentin to a point just short of entering the pulp.

The exposure was made with a shepherd's crook explorer (#23)[a] to minimize the number of dentin chips pushed into the pulp. Bleeding was sought as an indication of exposure, but was not apparent in all cases.

Forty-eight hours later, the same preparatory procedures were carried out. Any debris in the preparations was removed with a small spoon excavator and short air blasts. The cavity preparation was then wiped clean with a cotton pledget moistened with saline solution. The preparations were then dried and ready for the pulp capping agent.

The capping agents were mixed with U.S.P. water to a thick creamy paste. These materials were placed over the exposure sites, and tapped to place with a

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[a]Tarno Explorer #23 (Shepherd's Crook), S.S. White and Company, Philadelphia, Pennsylvania.

FP3 plastic instrument (Tarno).[a] Excess material was removed from the edge of the preparations, Copalite[b] applied over the margins and the cavity filled with amalgam. The pulp capping agents were one of the following compounds: (1) 10 per cent vancomycin, 89 per cent calcium hydroxide, and one per cent methyl cellulose mixed with U.S.P. water; (2) 10 per cent vancomycin and 90 per cent starch mixed with U.S.P. water; (3) 99 per cent calcium hydroxide and one per cent methyl cellulose in combination with U.S.P. water; (4) starch, q.s., in combination with water. All drug measurements were calculated by weight.

The animals were then identified by tattoo and returned to their cages. The animals were kept on a normal diet of Monkey Chow[c] until sacrifice.

Brief oral observations were made at intervals of two or three days for detection of gross oral lesions, or restorations that had been lost.

On the days of sacrifice, complete oral examinations were made. Soft tissues were checked for

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[a]Tarno FP3, S.S. White and Company, Philadelphia, Pennsylvania.

[b]Copalite, Cooley and Cooley, Ltd., Houston, Texas.

[c]Monkey Chow, Ralston Purina, St. Louis, Missouri.

pathoses, restorations for intactness, and each tooth for mobility. Each animal was weighed and this figure compared to its preoperative weight. Radiographs were again taken to disclose apical bone changes. The teeth were removed by first removing the labial or buccal plate of bone with an automatic surgical mallet (Reiter)[a] and large round burs rotating at high speeds. A number 101 pedodontic extraction forcep[b] and surgical elevators were used and every effort to avoid tooth fracture was made. One proximal surface was then flattened by use of the high speed rotary instrument with a #700 fissured bur to facilitate fixation and sectioning. The teeth were then placed in a 10 per cent formalin solution.

Histologic Evaluation

Alternate slides of the seven micron serial sections through the exposures were stained with hematoxylin and eosin, and were evaluated using a modified double-blind control. Pulp necrosis, abscess formation, and inflammation were recorded as unsatisfactory responses. The quantity of reparative dentin was recorded. This interpretation was based on an empirical

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[a]Reiter, Union Broach Automatic Surgical Mallet, Union Broach Company, Long Island City, New York.

[b]Tarno 101 Pedodontic Forceps, S.S. White and Company, Philadelphia, Pennsylvania.

judgement by the author. All stained serial sections were evaluated twice. Interpretations of each evaluation were compared, and any variations in the findings of the same specimens were evaluated a third time. This evaluation was made on the following basis:

Reparative dentin

1. Large amount--complete coalescence and bridging.
2. Large amount--incomplete coalescence and bridging.
3. Moderate amount--smaller amount of dentin is present and coalescence is less pronounced.
4. Small amount--less formed and little coalescence.
5. Negligible amount--minimal or none.

Inflammatory status of the pulp

1. Mild--inflammation limited to the immediate vicinity of the exposure site, with few cells involved and those of a chronic type. Remainder of pulp is normal.
2. Moderate--inflammation extending beyond the immediate exposure site but still limited to the coronal pulp. Cells are increased in number over mild, but are chronic in nature. Remainder of pulp is normal.
3. Severe--inflammation wide spread and penetrating deep into the pulp. There is heavy concentration

of mixed inflammatory cells. Abscess formation may be seen.

4. Partial necrosis--widespread necrosis but vital tissue still remains.
5. Complete necrosis--the pulp is completely necrotic.

One hundred and forty-eight previously unstained sections of 74 teeth were selected for Brown and Brenn[58] staining, as well as the John Hopkins Modifications of gram staining.[59] These sections were stained in this manner to demonstrate by histologic means the presence or absence of bacteria in the exposed dental pulps, and to compare these two techniques.

The 90 day animal was given an intraperitoneal injection of Procion brilliant red H-8BS[a] dye as an *in vivo* hard tissue marking agent. This fluorescent red dye was injected into the monkey 30 days post-operatively with a dosage of 100 mg. per Kg of body weight. This procedure was used to determine the quantity of reparative dentin deposited between 30 and 90 days post-operatively, and to see if a complete bridge existed at 30 days.

Histologic examination of unstained decalcified paraffin sections using fluorescent light was

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[a]Harlan, Indianapolis, Indiana.

performed and compared to the sections of the tooth stained with hematoxylin and eosin, and examined under ordinary light microscopy. Further information regarding vital dye staining may be found in Tomich.[61]

RESULTS

Preliminary Studies

Antibiotic Inactivation Test

Table I lists the 20 strains of microorganisms tested for susceptibility to 15 different concentrations and variations of the test drugs. Zones of inhibition were recorded at 48 hours, but were apparent as early as 24 hours, and did not change when observed after 72 hours. Three trypticase soy agar plates and three trypticase soy human blood agar plates were tested for each bacterial strain.

Vancomycin is especially active against gram-positive bacteria, thus zones of inhibition were apparent around all concentrations of the drug.

Starch was used as a control and by itself was ineffective against all of the microorganisms tested.

Vancomycin alone was effective against all the gram-positive organisms in the study, but was effective against only a few of the gram-negative organisms.

Five per cent vancomycin mixed with 95 per cent starch also proved to be effective against gram-positive organisms used.

Calcium hydroxide was effective against both gram-positive and gram-negative microorganisms. This was undoubtedly due to its high pH.

Five per cent vancomycin combined with 94 per cent calcium hydroxide and one per cent methyl cellulose was effective against all gram-positive organisms. The higher concentrations of vancomycin mixed with proportionally declining concentrations of calcium hydroxide gave the same results. This combination of medicaments was also effective against the gram-negative bacteria, but less effective at the lower concentrations of calcium hydroxide. The zones of inhibition were larger in most of these combinations than with either of the drugs independently. This complementing finding is unexplained.

It became apparent from this study that five per cent vancomycin along with 94 per cent calcium hydroxide in combination with one per cent methyl cellulose, would be effective against most of the gram-positive microorganisms involved with infected dental pulps. Ten per cent vancomycin in combination with 89 per cent calcium hydroxide and one per cent methyl cellulose was chosen for the principal pulp-capping study. This concentration was chosen to provide a five per cent leaway and to correlate the study with the efforts of Eggers,[39] who used 10 per cent vancomycin in his pulp-capping study.

Tissue Compatibility Test

Two day implant specimens of the five pulp-capping agents were relatively easy to locate at the time of sacrifice of the rats. Only two of the 20 implants could not be found clinically and all were located histologically. In the 16 day animals, three of the 20 implants were not apparent upon gross observation of the implant areas. Serial histologic sections revealed all of the implants.

Three of the 20 implants in the 32 day animals were not found clinically, but small crystals and a mild inflammatory response could be seen histologically around all.

Twenty-one of the 60 implants caused a moderate inflammatory response (Figure 3). This was seen mainly in the vancomycin implants, but was also seen when vancomycin and calcium hydroxide were implanted together. These moderate responses were characterized by a thin fibrin capsule and small numbers of inflammatory cells.

Forty of the implants caused a mild inflammatory response (Figure 4). A thin fibrin capsule and a general lack of inflammatory cells were noted (Table II).

Perfection of Surgical Technique and Bacterial Contamination of 48 Hour Pulp Exposures

Histologic sections of the three maxillary bicuspids and one maxillary central incisor stained by the Brown and Brenn[58] technique and the Johns Hopkins[59] modifications of gram staining the method demonstrated the presence of bacteria in the cavity preparations and in the coronal pulp in the immediate area of the exposure (Figures 5 and 6). Bacteria could not be demonstrated in the deeper layers of the pulps.

Microorganisms could not be distinguished from the cellular elements of these pulps when the sections were stained with hematoxylin and eosin. These sections did demonstrate dentin chips in the exposure areas and the overall appearance of the pulps was normal.

Principal Study

Clinical and Radiographic Findings

Clinical oral examinations were made of each animal prior to surgery and every 14 days until sacrifice. No signs of pathoses were observed during these examinations. All monkeys appeared healthy

and gained weight between the day of pulp exposure and the day of sacrifice. Only three of the 74 restorations were defective. Two were totally lost from one of the 30 day animals and one was loose at the time of sacrifice of the 90 day animal. These were eliminated from the study and were not evaluated.

Radiographic examinations were made prior to surgery the day the pulps were exposed, and the day the teeth were extracted. Apical pathosis appeared in the 90 day animal in three of the teeth treated with the starch control, and one of the teeth treated with vancomycin, starch and water.

Histologic Evaluation of the Teeth Treated with the Starch and Water Control

A satisfactory response at 30 days was found in only one of the 14 teeth treated with the starch and water control (Table III). A large amount of reparative dentin and dentin chips were observed in this tooth. Six teeth demonstrated an unsatisfactory response with inflammatory cells around the dentin chips pushed into the pulp by the exposure instrumentation. No bridging was apparent, and only small to moderate amounts of reparative

dentin were observed. Six necrotic teeth revealed large numbers of inflammatory cells and generally a lack of pulp tissue (Figure 7).

At 90 days postoperatively all the teeth were necrotic and three of the four had apical granulation tissue with massive numbers of leukocytes and macrophages (Figure 8).

Histologic Evaluation of the Teeth Treated with Vancomycin, Starch and Water

Among the teeth treated for 30 days with vancomycin, starch and water, five proved satisfactory, six unsatisfactory, and three were necrotic (Table IV). Generally, those with a satisfactory response displayed large amounts of reparative dentin, but no complete bridges were formed. There were few inflammatory cells (Figures 9 and 10). The unsatisfactory responses displayed little reparative dentin formation and many inflammatory cells (Figures 11 and 12). The necrotic pulps were characterized by large numbers of leukocytes and macrophages, a lack of pulp tissue, and apical granulation tissue. Pyogenic membranes composed of fibrin and vascular tissues were seen in two of the necrotic teeth and pus was also apparent.

Only one of the teeth treated for 90 days was satisfactory and the rest were necrotic. A partial bridge composed of dentin chips and reparative dentin was seen in the satisfactory response.

Histologic Evaluation of the Teeth Treated with Calcium Hydroxide, Methyl Cellulose and Water

All but one of the 14 teeth treated for 30 days with calcium hydroxide, methyl cellulose and water had a satisfactory response (Table V). Eight of these teeth showed large amounts of reparative dentin formation and complete bridges.[a] Five teeth gave a satisfactory response, but only partial bridging was apparent. Bridging appeared to be dependent upon the number of chips in the exposure area and was vertical and irregular in nature (Figure 13). These satisfactory responses demonstrated few inflammatory cells and relatively normal pulps. However, just beneath the exposure area on those with partial bridging, there appeared an amorphous hematoxyphylic particulate material (Figures 14 and 15). This proved not to be bacteria. The one unsatisfactory response was characterized by large numbers of leukocytes and macrophages.

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[a] A complete dentin bridge is one which is seen microscopically to be complete across the pulp exposure in each serial section of the tooth.

All teeth treated for 90 days responded in a satisfactory manner, but only two of the four had complete dentin bridges.

Histologic Evaluation of the Teeth Treated with Vancomycin, Calcium Hydroxide Methyl Cellulose, and Water

At 30 days all of the teeth treated with vancomycin, calcium hydroxide, methyl cellulose, and water gave a satisfactory pulpal response (Table VI). Large amounts of reparative dentin were seen in the 12 satisfactory teeth and were described as complete bridging. These bridges were generally retracted away from the exposure site, were horizontal in nature, and were complete in each of the serial sections of the tooth (Figures 16 and 17). Odontoblasts lined the bridge and a predentin layer of the bridge was continuous with that of the predentin of the rest of the tooth (Figure 18). A few dentin chips were incorporated into the bridge in some of the teeth. A lack of inflammatory cells in these 12 teeth was recorded and the remaining pulp appeared normal.

All of the five 90 day specimens showed a satisfactory response and a complete dentin bridge. This bridge was as described for the 30 day specimens, but much thicker (Figure 19).

Histologic Evaluation of the Sections Stained with Brown and Brenn and the Modified Gram Stain

There appeared to be no difference in results between the two staining techniques. The modified gram stain is easier to perform and was preferred.

Bacteria were not apparent in any of the pulps with satisfactory responses, or in the pulps containing calcium hydroxide or vancomycin which were unsatisfactory (Figures 20 and 21). However, one necrotic tooth containing vancomycin did reveal gram-positive organisms. Gram-negative organisms were rare or difficult to identify, and therefore may have been overlooked. All teeth that were capped with starch and water and which became necrotic had large colonies of bacteria (Figures 22 and 23). In seven of these teeth, the colonies extended down the pulp canal toward the apical area to a pyogenic membrane which separated the apical vital tissue from the necrotic pulp.

Histologic Evaluation of Procion Brilliant Red H-8BS Dye

All of the 90 day teeth revealed an excellent fluorescent Procion marking. All of the teeth treated with vancomycin, calcium hydroxide, methyl cellulose, and water revealed complete bridges. Due to the completeness of the fluorescent Procion marking, it was apparent these bridges were completely formed at the time of the 30 day injection (Figures 24 and 25). Only one of the four teeth treated with calcium hydroxide, methyl cellulose and water appeared to have a complete bridge at 30 days. None of the others were similar (Figures 26 and 27).

Between 30 and 90 days, large amounts of reparative dentin were laid down in response to the vancomycin, calcium hydroxide, methyl cellulose and water capping. Calcium hydroxide, methyl cellulose and water gave a similar pattern, but the dentin was not as regular. In an untreated tooth that erupted into the oral cavity during the test period, the amount of coronal dentin deposition was much less (Figure 28). Dentin chips in the treated teeth were outlined well by the fluorescent dye and made apparent their incorporation into some bridges.

TABLES AND ILLUSTRATIONS

Preface to Table I

Key to Headings

VH - vancomycin hydrochloride

CH - calcium hydroxide

MC - methyl cellulose

Pre-formed pellets (all mixed with U.S.P. water 24 hours before test)

A - starch q.s.

B - crystalline VH

C - 99% CH and 1% MC

D - 5% VH and 95% starch

E - 10% VH and 90% starch

F - 15% VH and 85% starch

G - 20% VH and 80% starch

H - 25% VH and 75% starch

I - 30% VH and 70% starch

Smears (all mixed with U.S.P. water)

1. 5% VH, 94% CH, 1% MC
2. 10% VH, 89% CH, 1% MC
3. 15% VH, 84% CH, 1% MC
4. 20% VH, 79% CH, 1% MC
5. 25% VH, 74% CH, 1% MC
6. 30% VH, 69% CH, 1% MC

Symbols

TSA	-	Trypticase soy agar
TSAHB	-	Trypticase soy-human blood agar
-	-	Gram negative
+	-	Gram positive
R	-	Resistant
S	-	Sensitive

TABLE I

Bacterial Sensitivity to Test Agents

Organism	Gram Stain	Pellets of Test Materials A	B	C	D	E	F	G	H	I
α hemolytic strep	+	R	S	S	S	S	S	S	S	S
α hemolytic strep	+	R	S	S	S	S	S	S	S	S
β hemolytic strep	+	R	S	R	S	S	S	S	S	S
β hemolytic strep	+	R	S	S	S	S	S	S	S	S
β hemolytic strep	+	R	S	S	S	S	S	S	S	S
Pneumococcus	+	R	S	S	S	S	S	S	S	S
Pneumococcus	+	R	S	S	S	S	S	S	S	S
Staph (coagulase positive)	+	R	S	S	S	S	S	S	S	S
Staph (coagulase positive)	+	R	S	S	S	S	S	S	S	S
Staph (coagulase positive)	+	R	S	S	S	S	S	S	S	S
Staph (coagulase positive)	+	R	S	S	S	S	S	S	S	S
Staph (coagulase positive)	+	R	S	S	S	S	S	S	S	S
Staph (coagulase negative)	+	R	S	S	S	S	S	S	S	S
Proteus mirabilus (mucoid)	-	R	R	S	R	R	S	S	S	S
Proteus mirabilus (spreader)	-	R	R	S	R	R	R	R	R	R
Aerobacter aerogenes	-	R	R	S	R	S	S	S	S	S
Herellea vaginicola	-	R	R	S	R	R	R	R	R	R
Pseudomonas aeruginosa	-	R	R	S	R	R	R	R	R	S
Pseudomonas pseudomallei	-	R	R	S	R	R	R	R	R	R
α strep (very little growth)	+	R	S	S	S	S	S	S	S	S

(continued)

TABLE I (continued)

Bacterial Sensitivity Using Smears of the Test Materials

Organism	Gram Stain	Smear of Test Materials 1	2	3	4	5	6
α hemolytic strep	+	S	S	S	S	S	S
α hemolytic strep	+	S	S	S	S	S	S
β hemolytic strep	+	S	S	S	S	S	S
β hemolytic strep	+	S	S	S	S	S	S
β hemolytic strep	+	S	S	S	S	S	S
Pneumococcus	+	S	S	S	S	S	S
Pneumococcus	+	S	S	S	S	S	S
Staph (coagulase positive)	+	S	S	S	S	S	S
Staph (coagulase positive)	+	S	S	S	S	S	S
Staph (coagulase positive)	+	S	S	S	S	S	S
Staph (coagulase positive)	+	S	S	S	S	S	S
Staph (coagulase positive)	+	S	S	S	S	S	S
Staph (coagulase negative)	+	S	S	S	S	S	S
Proteus mirabilus (mucoid)	-	S	S	S	S	S	R
Proteus mirabilus (spreader)	-	S	S	S	S	S	R
Aerobacter aerogenes	-	S	S	S	S	S	R
Herellea vaginicola	-	S	S	S	S	S	R
Pseudomonas aeruginosa	-	S	S	S	S	S	R
Pseudomonas pseudomallei	-	S	S	S	S	S	R
α strep	+	S	S	S	S	S	S

Preface to Tables II-IX

Specimen - identification of serial sections of a given tooth.

Tooth Number - universal numbering system.

Implant Material - symbol represents the drug placed subcutaneously in the connective tissue of rats.

Method of Exposure - method used to make the initial penetration into the pulp.

Capping Agent - the medicament placed over the exposure.

Reaction of the Pulp - the histologic evaluation of the response of the dental pulp to experimental procedures.

Symbols

Exp - with the point of an explorer the initial pulp exposure was made.

#4 - with a #4 bur rotating at slow speed the initial exposure was made.

1J - with a #1 Jacquette scaler the initial exposure was made.

SW - starch q.s. mixed (with U.S.P.) water.

VW - Vancomycin hydrochloride (with U.S.P.) water.

Symbols (continued)

VSW - Vancomycin hydrochloride 10%, starch and water.

CMW - calcium hydroxide 99%, methyl cellulose 1% and water.

VCMW - Vancomycin hydrochloride 10%, calcium hydroxide 89%, methyl cellulose 1% and water.

+++ - large amounts of reparative dentin on canal walls and around dentin chips with an attempt at complete bridging.

++ - moderate amount of reparative dentin on canal walls and around dentin chips.

+ - small amount of reparative dentin on canal walls and around dentin chips.

\- - no reparative dentin formation present.

Sat - satisfactory response to the experimental procedure.

USat - unsatisfactory response.

Nec - all or major part of pulp has become necrotic.

TABLE II

Histologic Response to 60 Implants in Subcutaneous Connective Tissue of Rats

Specimen	Time in Days	Implant Material	Histologic Response
8562	2	VW	Mod
8563		VW	Mild
8564		VW	Mod
8565		VW	Mod
8566		CMW	Mild
8567		CMW	Mod
8568		CMW	Mild
8569		CMW	Mild
8570		SW	Mod
8571		SW	Mod
8572		SW	Mod
8573		SW	Mod
8574		VSW	Mod
8575		VSW	Mod
8576		VSW	Mod
8577		VSW	Mod
8578		VCMW	Mod
8579		VCMW	Mod
8580		VCMW	Mild
8581		VCMW	Mild
8593	16	VW	Mod
8594		VW	Mod
8595		VW	Mod
8596		VW	Mod
8597		VW	Mild
8598		CMW	Mild
8599		CMW	Mild
8600		CMW	Mild
8601		SW	Mild
8602		SW	Mild
8603		SW	Mild

(continued)

TABLE II (continued)

Specimen	Time in Days	Implant Material	Histologic Response
8604		SW	Mild
8605		VSW	Mild
8606		VSW	Mild
8607		VSW	Mild
8608		VSW	Mild
8609		VCMW	Mild
8610		VCMW	Mod
8611		VCMW	Mod
8612	16	VCMW	Mild
8667		VW	Mild
8668		VW	Mild
8669		VW	Mild
8670		VW	Mod
8671		CMW	Mild
8672		CMW	Mild
8673		CMW	Mild
8674		CMW	Mild
8675		SW	Mild
8676	32	SW	Mild
8677		SW	Mild
8678		SW	Mild
8679		VSW	Mild
8680		VSW	Mild
8681		VSW	Mild
8682		VSW	Mild
8683		VCMW	Mild
8684		VCMW	Mild
8685		VCMW	Mild
8686		VCMW	Mild

TABLE III

Histologic Findings in the 18 Control Teeth

Treated with Starch and Water

Specimen Number	Tooth Number	Method of Exposure	Reparative Dentin Formation	Bridge Formation (None -, Partial +, Complete ++)	Reaction of Pulp
30 Days Postoperative					
8796	7	Exp	-	-	Nec
8800	5	Exp	-	-	Nec
8805	24	1J	-	-	USat
8810	22	Exp	-	-	Nec
8811	21	Exp	-	-	Nec
8819	8	1J	+++	+	Sat
8824	6	Exp	+	-	Nec
8829	2	Exp	+	-	USat
8836	23	#4	+++	-	Sat
8840	20	Exp	-	-	USat
8841	19	#4F	+	-	USat
8845	18	Exp	++	-	USat
8849	3	Exp	-	-	Nec
8850	4	Exp	-	-	USat
90 Days Postoperative					
8851	8	Exp	-	-	Nec
8858	23	Exp	-	-	Nec
8871	30	#4F	+	-	Nec
8874	15	Exp	-	-	Nec

TABLE IV

Histologic Findings in 18 Teeth Treated with Vancomycin, Starch and Water

Specimen Number	Tooth Number	Method of Exposure	Reparative Dentin Formation	Bridge Formation (None -, Partial +, Complete ++)	Reaction of Pulp
0 Days Postoperative					
8802	12	Exp	++	-	Sat
8806	25	Exp	+++	+	Sat
8807	26	#4F	+	-	USat
8809	27	Exp	++	-	USat
8815	29	Exp	++	-	Nec
8816	30	#4F	+	-	USat
8820	9	#4	+++	+	USat
8822	10	Exp	++	+	Sat
8823	11	Exp	+	-	Nec
8825	13	1J	+++	+	USat
8826	14	Exp	+++	+	Sat
8830	15	Exp	+	-	Sat
8838	28	Exp	-	-	Nec
8847	31	1J	+	-	USat
0 Days Postoperative					
8854	24	1J	+	-	Nec
8855	7	Exp	+++	+	Sat
8870	14	Exp	-	-	Nec
8876	31	#4	-	-	Nec

TABLE V

Histologic Findings in 18 Teeth Treated with Calcium Hydroxide, Methyl Cellulose and Water

Specimen Number	Tooth Number	Method of Exposure	Reparative Dentin Formation	Bridge Formation (None -, Partial +, Complete ++)	Reaction of Pulp
0 Days Postoperative					
8795	25	Exp	+++	+	Sat
8797	26	1J	+++	++	Sat
8801	28	Exp	+++	++	Sat
8803	29	Exp	+++	++	Sat
8808	10	Exp	+++	+	Sat
8813	12	Exp	+++	++	Sat
8814	13	Exp	+++	-	USat
8817	25	Exp	+++	++	Sat
8828	30	Exp	+++	++	Sat
8831	31	1J	+++	+	Sat
8833	9	Exp	+++	++	Sat
8843	14	Exp	+++	+	Sat
8844	15	Exp	+++	+	Sat
8848	11	#4	+++	+	Sat
0 Days Postoperative					
8853	25	Exp	+++	++	Sat
8856	10	Exp	+++	++	Sat
8869	3	#4	+++	+	Sat
8875	18	1J	+++	+	Sat

TABLE VI

Histologic Findings in 20 Teeth Treated with Vancomycin in Combination with Calcium Hydroxide Methyl Cellulose and Water

Specimen Number	Tooth Number	Method of Exposure	Reparative Dentin Formation	Bridge Formation (None -, Partial +, Complete ++)	Reaction of Pulp
30 Days Postoperative					
8798	22	Exp	+++	++	Sat
8799	21	Exp	+++	++	Sat
8804	20	1J	+++	++	Sat
8812	5	Exp	+++	++	Sat
8818	24	Exp	+++	++	Sat
8821	23	Exp	+++	++	Sat
8827	19	#4F		(Restoration Lost)	
8832	18	#4F	+++	++	Sat
8834	8	Exp	+++	++	Sat
8835	7	Exp	+++	++	Sat
8837	6	1J		(Restoration Lost)	
8839	4	Exp	-	-	Nec
8842	3	Exp	+++	++	Sat
8846	2	#4F	+++	++	Sat
90 Days Postoperative					
8852	9	#4	+++	++	Sat
8857	26	Exp	+++	++	Sat
8865	29	Exp	+++	++	Sat
8866	20	Exp	+++	++	Sat
8872	19	#4F		(Restoration Lost)	
8873	2	Exp	+++	++	Sat

TABLE VII

Pulp Response to Each Medicament

At 30 and 90 Days

Capping Agent	Treatment Days	Response: Satisfactory	Unsatisfactory	Necrotic
VCMW	30	12	0	0
VCMW	90	5	0	
VSW	30	5	6	3
VSW	90	1	0	3
CMW	30	13	1	0
CMW	90	4	0	0
SW	30	2	6	6
SW	90	0	0	4

TABLE VIII

Reparative Dentin Response to Each Medicament at 30 and 90 Days

Capping Agent	Treatment Days	Reparative Response: None	Small	Moderate	Partial Bridging	Complete Bridging
VCMW	30	0	0	0	0	12
VCMW	90	0	0	0	0	5
VSW	30	1	5	4	5	0
VSW	90	2	1	0	1	0
CMW	30	1	0	0	5	8
CMW	90	0	0	0	3	1
SW	30	8	3	1	1	0
SW	90	3	1	0	0	0

TABLE IX

Percentage Comparison of All Teeth
Treated with Each Medicament

Capping Agent	Responses of the Pulp in Per Cent: Satisfactory	Unsatisfactory	Necrotic
VCMW	100.0	0	0
VSW	33.3	33.3	33.3
CMW	94.4	5.6	0
SW	11.2	33.3	55.5

Figure 1. Implants in place immediately after surgery.

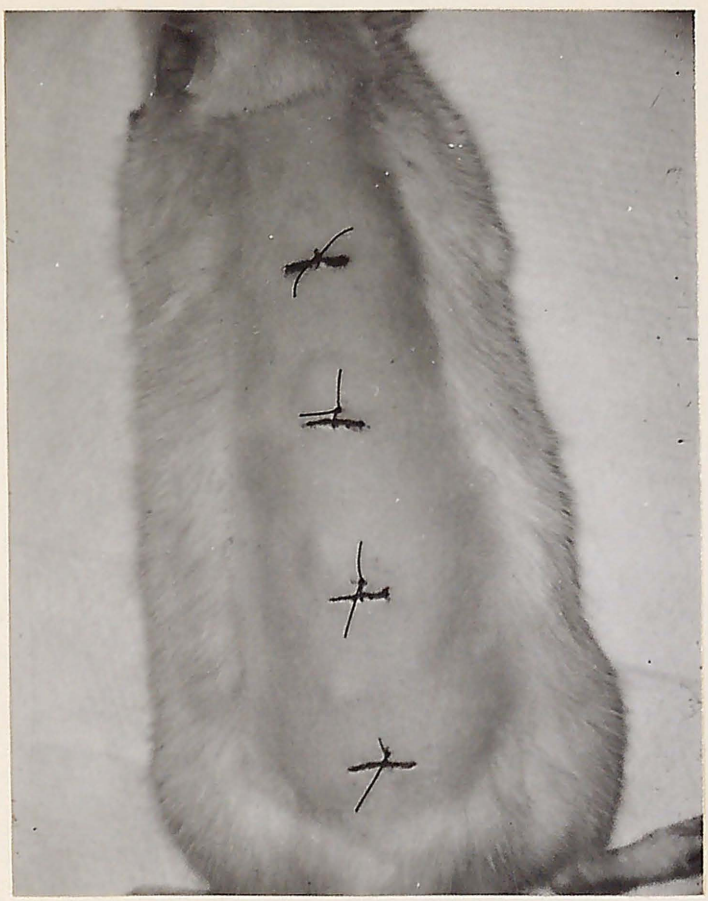


Figure 2. Cavity preparations and pulp exposures in an experimental monkey.

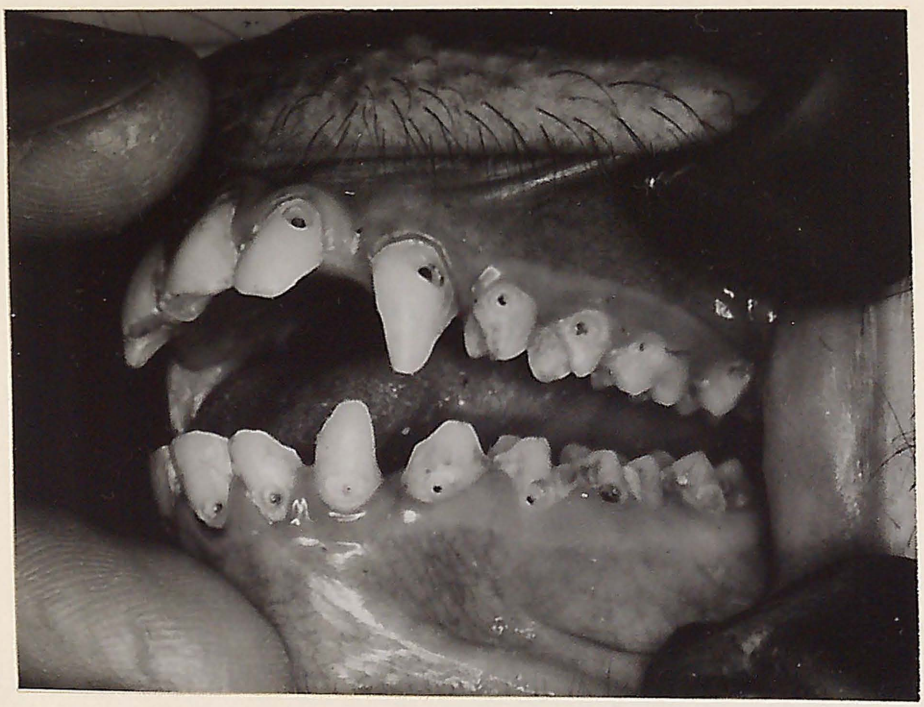
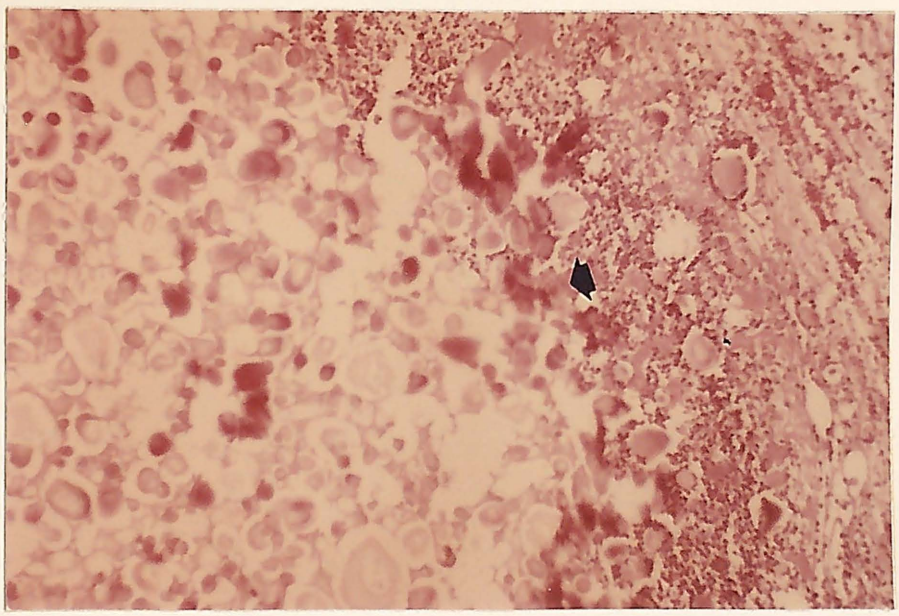


Figure 3. Photomicrograph of a section through a subcutaneous implant of vancomycin, calcium hydroxide, methyl cellulose and water at 32 days demonstrates a moderate response. Arrow indicates implant material. (Hematoxylin and eosin stained, original magnification x 100).

Figure 4. A section through a 32 day implant specimen of calcium hydroxide, methyl cellulose and water indicating a mild response. Arrow indicates implant material. (Hematoxylin and eosin stained, original magnification x 100).



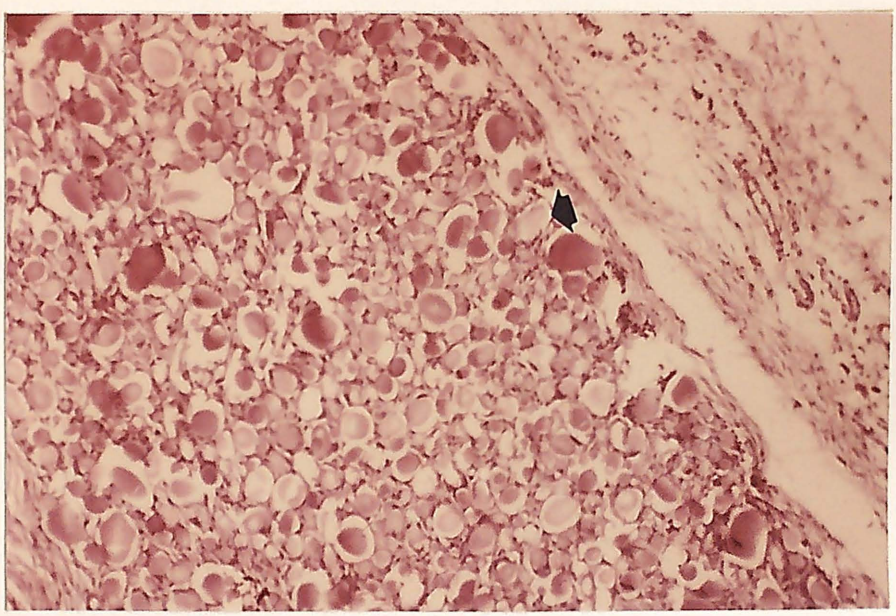
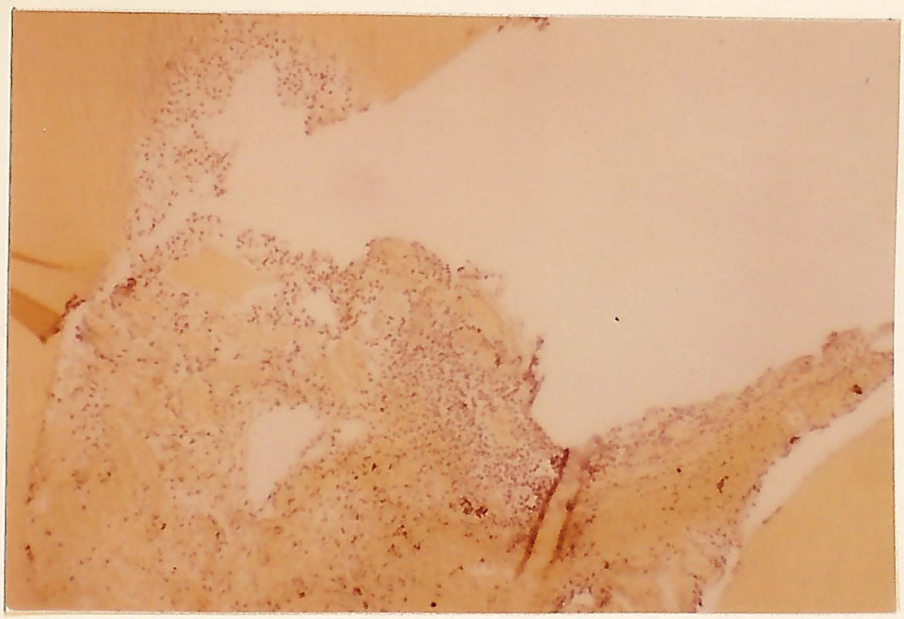


Figure 5. A section through a maxillary central incisor demonstrating the pulp response to the oral environment at 48 hours. Note inflammatory cells and dark staining bacteria. (Brown and Brenn stained, original magnification x 100).

Figure 6. A section through the pulp of the same tooth as Figure 5. Note the red staining inflammatory cells and dark staining bacteria. (Brown and Brenn stained, original magnification, x 1000 oil).



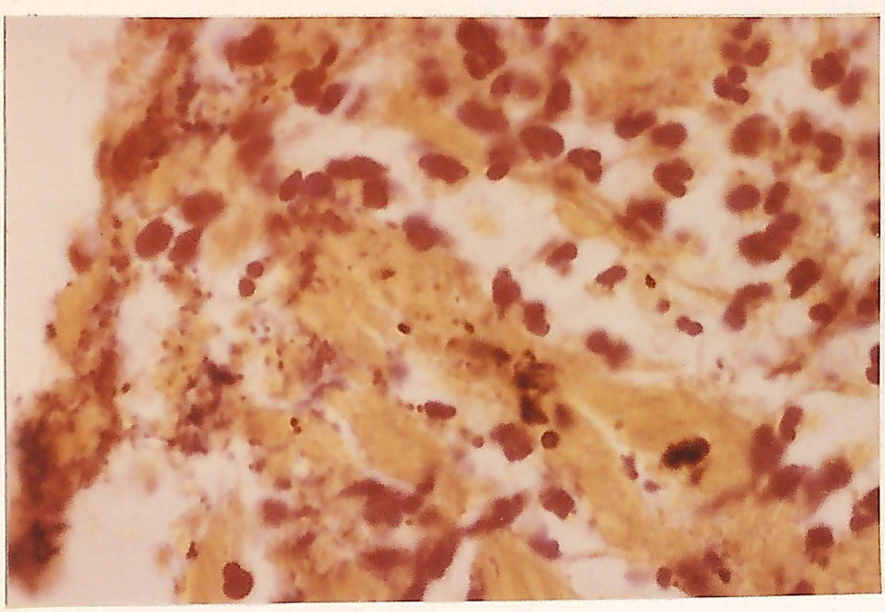
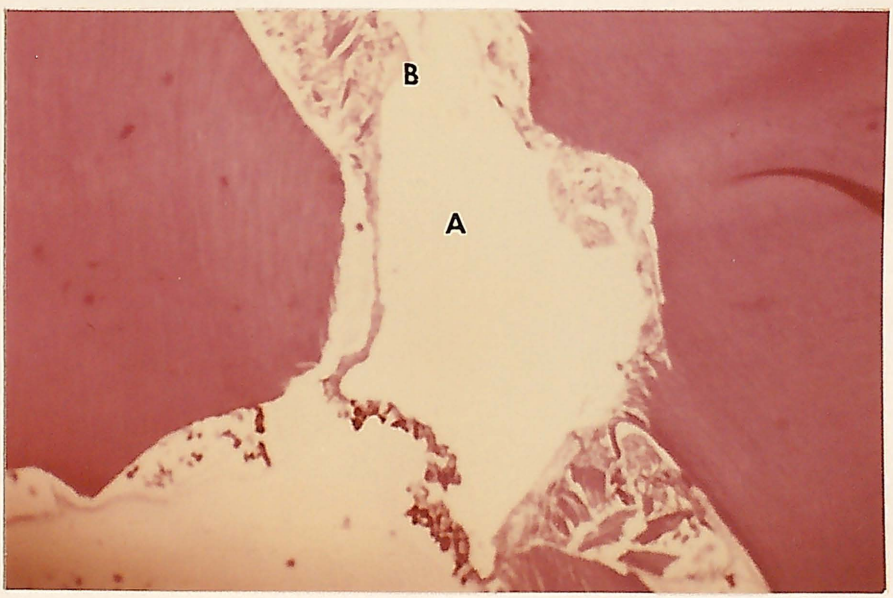


Figure 7. Unsatisfactory response in a maxillary left lateral incisor treated for 30 days with starch and water. Note the lack of reparative dentin.
A. Pulp exposure.
B. Necrotic coronal pulp.
(Hematoxylin and eosin stained, original magnification x 100).

Figure 8. Unsatisfactory response in the apical one-third of the tooth described in Figure 7. Note severe inflammatory response. (Hematoxylin and eosin stained, original magnification, x 1000 oil).


B
A

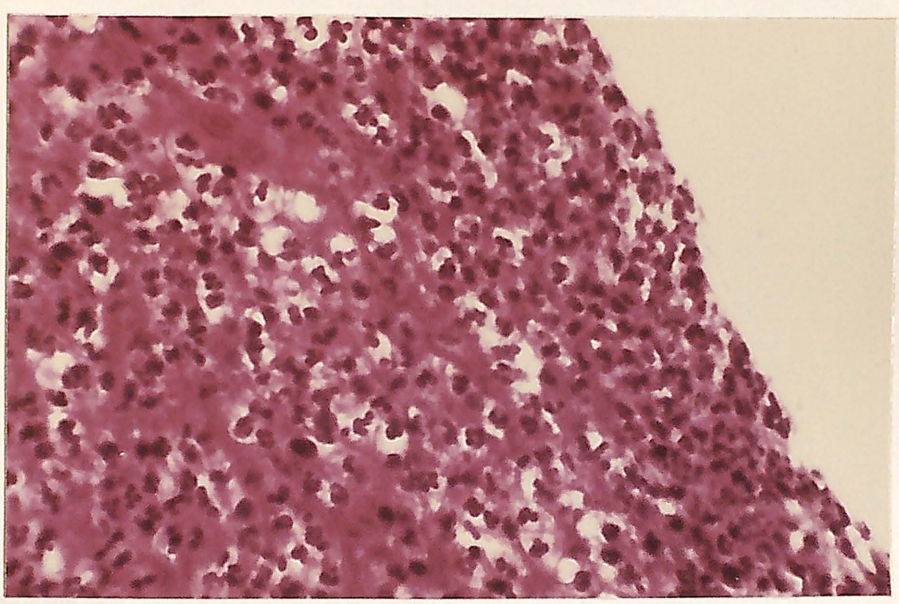
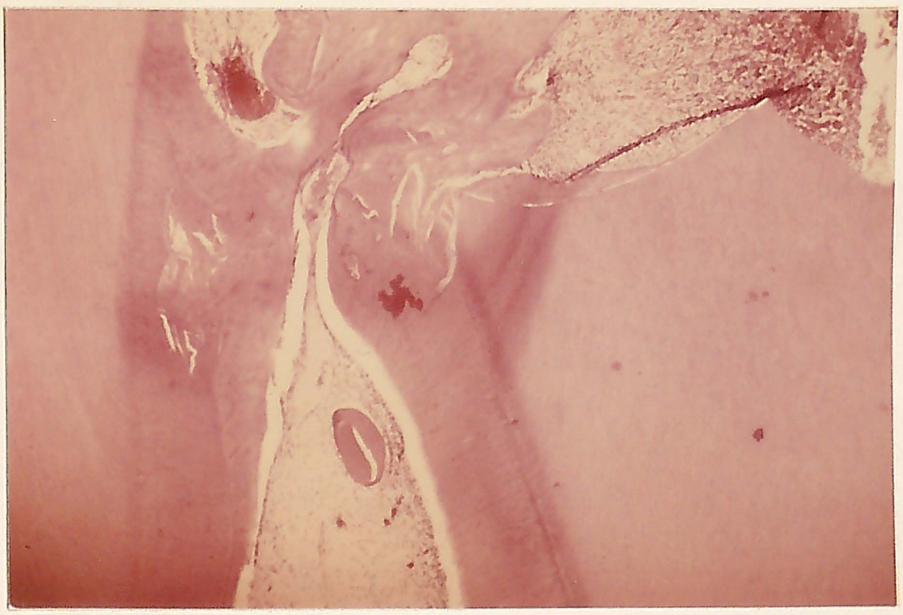


Figure 9. Mandibular right cuspid treated with vancomycin, starch and water. The absence of inflammatory cells and coalescence of reparative dentin around the chips indicates a satisfactory response. (Hematoxylin and eosin stained, original magnification x 40).

Figure 10. An unsatisfactory response in the coronal one-third of a maxillary central incisor which showed no reparative dentin. Note the lack of bacteria, but the presence of leukocytes and macrophages. (Gram stained, original magnification, x 1000 oil).



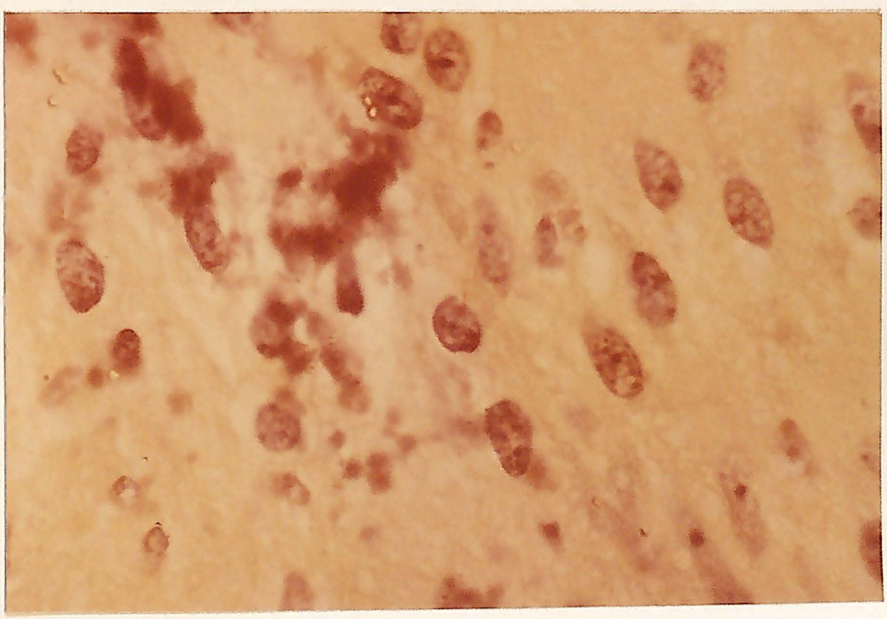
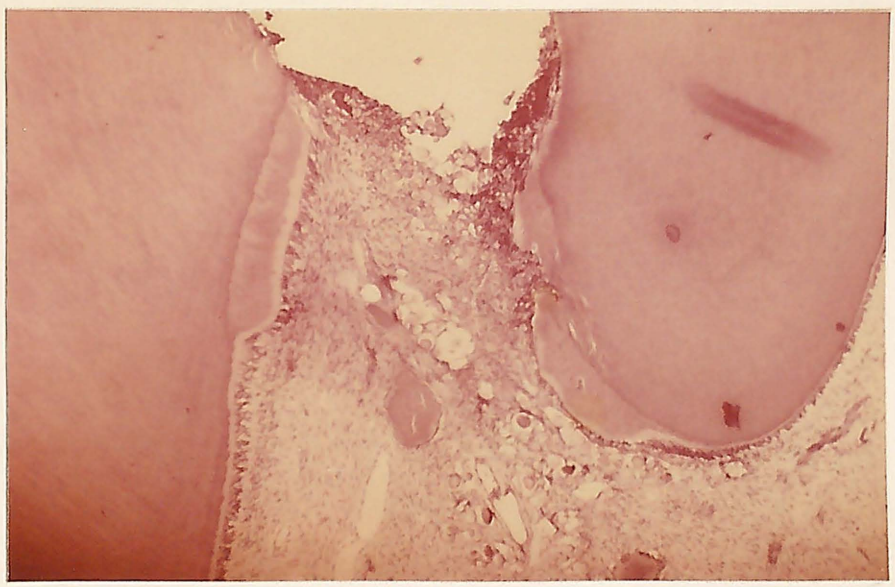


Figure 11. An unsatisfactory response to vancomycin, starch and water. Note the attempt at bridging and the numerous inflammatory cells. (Hematoxylin and eosin stained, original magnification x 40).

Figure 12. The coronal one-third of the unsatisfactory response in the tooth displayed in Figure 11. Note the heavy infiltration of dark staining inflammatory cells. (Hematoxylin and eosin stained, original magnification, x 1000 oil).



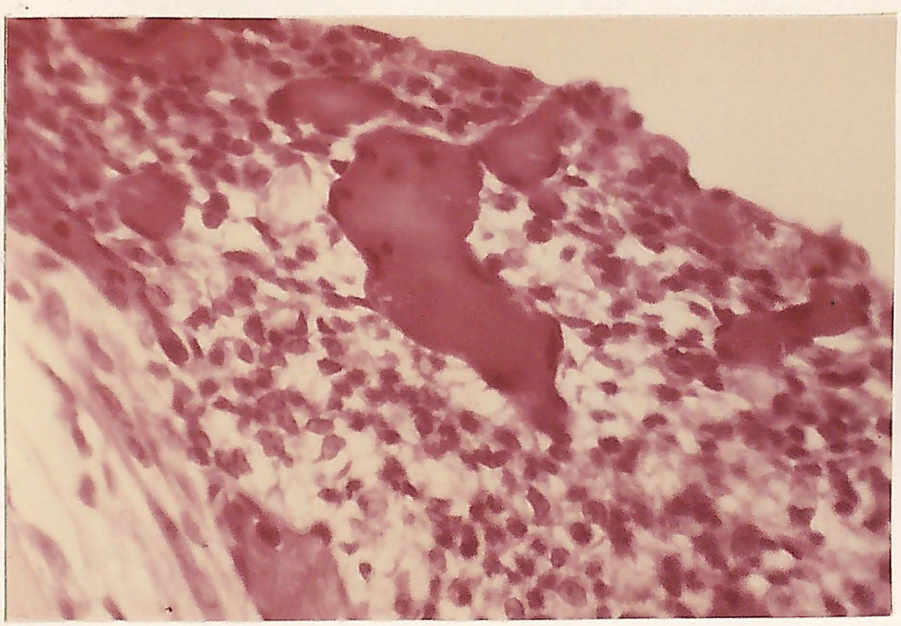


Figure 13. A satisfactory response of a maxillary first molar to calcium hydroxide, methyl cellulose and water at 90 days. Note the large number of dentin chips incorporated into the complete bridge. (Hematoxylin and eosin stained, original magnification x 100).

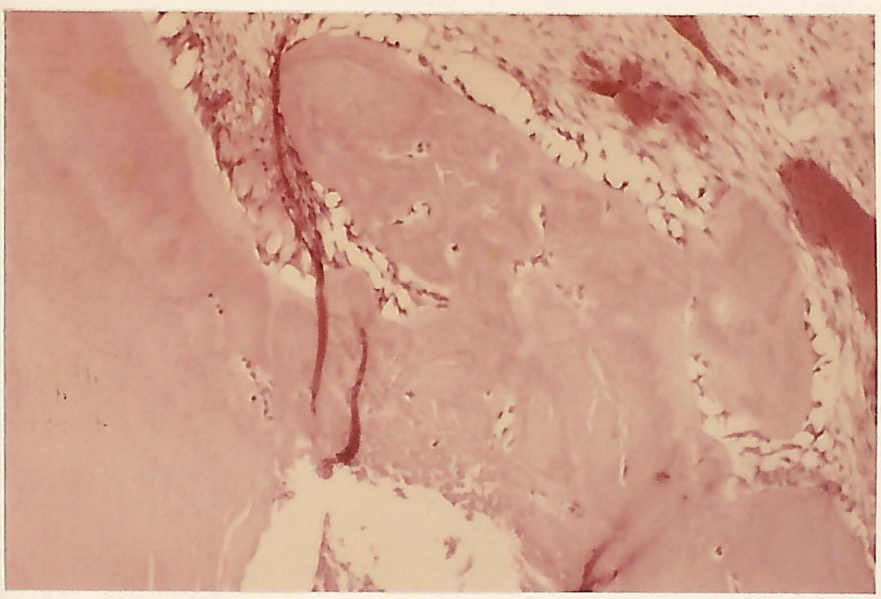


Figure 14. An unsatisfactory response in a mandibular central incisor when treated with calcium hydroxide, methyl cellulose and water. This amorphous, hematoxyphylic particulate material was surrounded by inflammatory cells (arrow). (Hematoxylin and eosin stained, original magnification x 100).

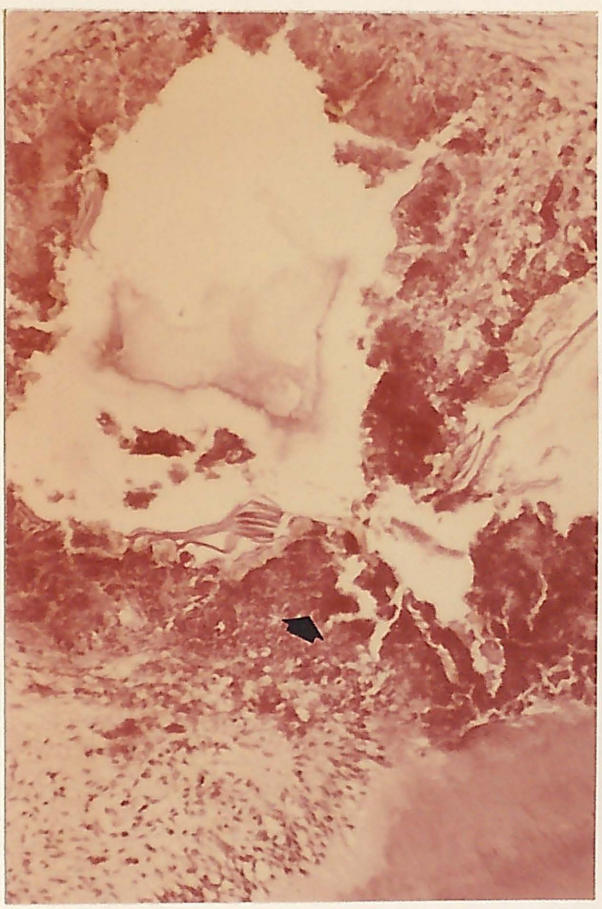


Figure 15. The amorphous hematoxyphylic particulate material described in Figure 14 is shown via oil emersion. Note the infiltrating inflammatory cells, but this pulp was considered reversible. (Hematoxylin and eosin stained, original magnification, x 1000 oil).

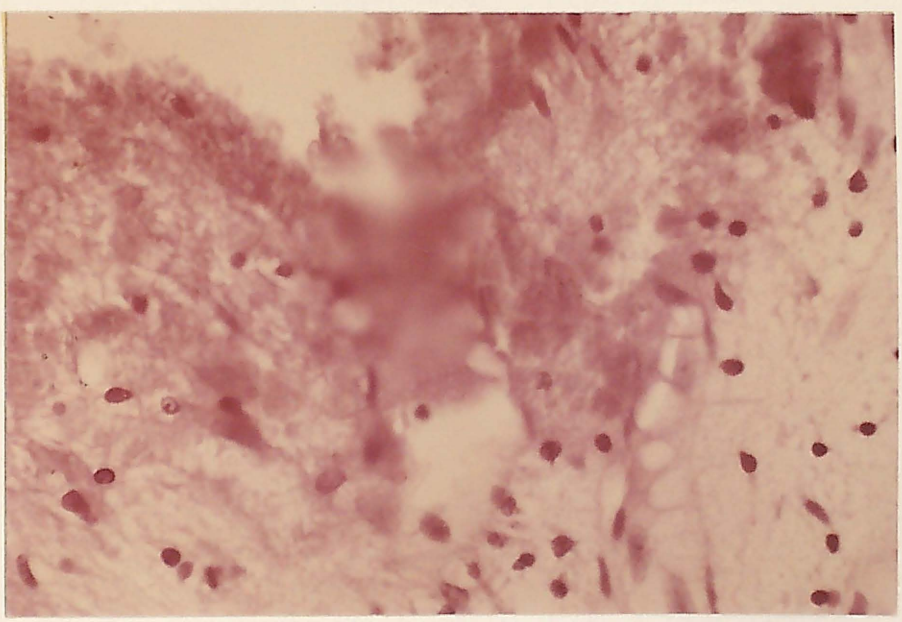


Figure 16. A complete dentin bridge in a maxillary left lateral incisor is the response after treatment for 30 days with vancomycin, calcium hydroxide, methyl cellulose and water.

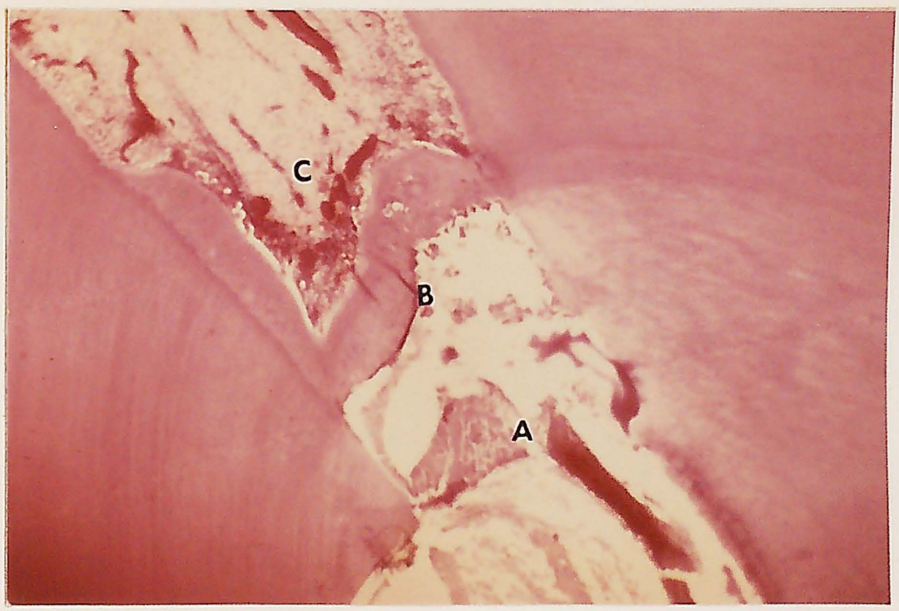
A. Exposure site.

B. Complete dentin bridge.

C. Normal pulp.

(Hematoxylin and eosin stained, original magnification x 40).

Figure 17. The complete dentin bridge demonstrated in Figure 16 is shown at a higher magnification. Note the reparative dentin along the pulpal wall in response to cavity preparation. (Hematoxylin and eosin stained, original magnification x 100).


C
B
A

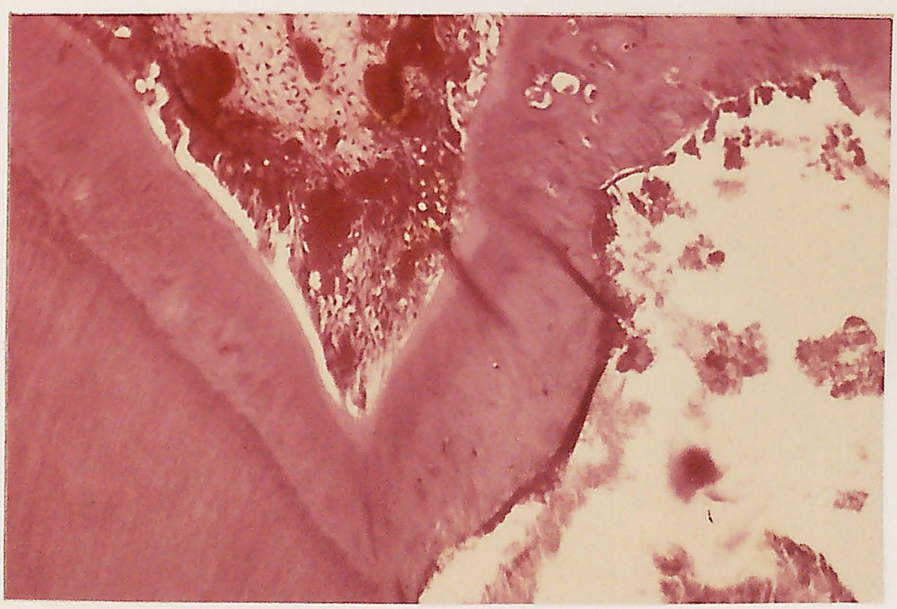
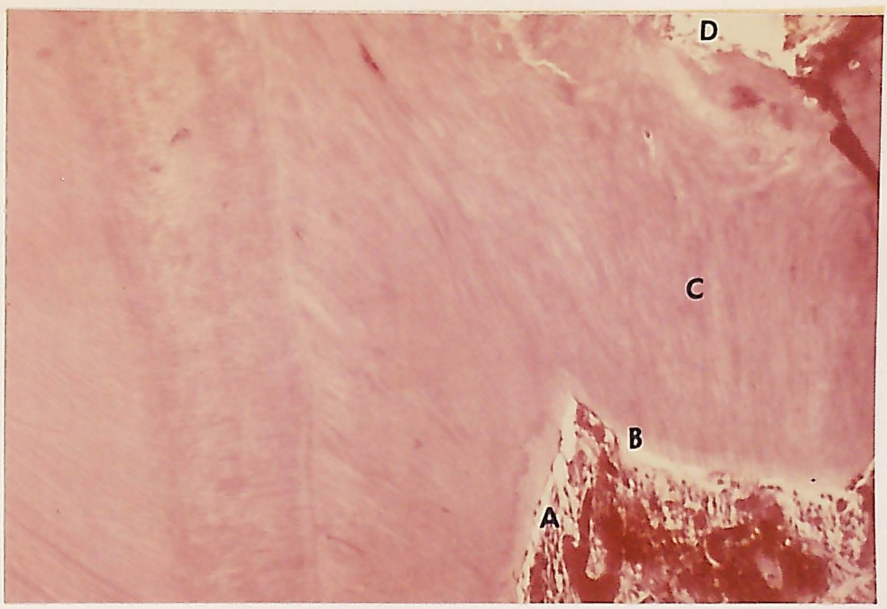


Figure 18. A dentin bridge displaying an odontoblastic layer (A), predentin layer (B), and a complete dentin bridge (C), across the exposure site (D). (Hematoxylin and eosin stained, original magnification x 450).

Figure 19. A satisfactory response and complete dentin bridge is demonstrated by a mandibular right lateral incisor after treatment with vancomycin, calcium hydroxide, methyl cellulose and water. This 90 day response displays a normal healthy pulp. (Hematoxylin and eosin stained, original magnification x 40).


D
C
B
A

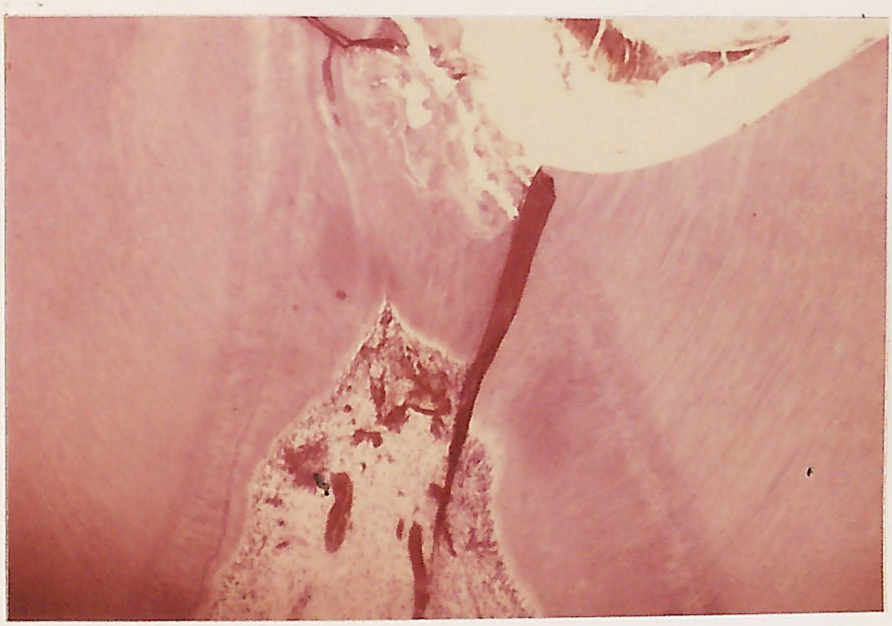


Figure 20. A mandibular left second molar with a complete dentin bridge and treated with calcium hydroxide, methyl cellulose and water displays no bacteria. (Brown and Brenn stained, original magnification x 100).

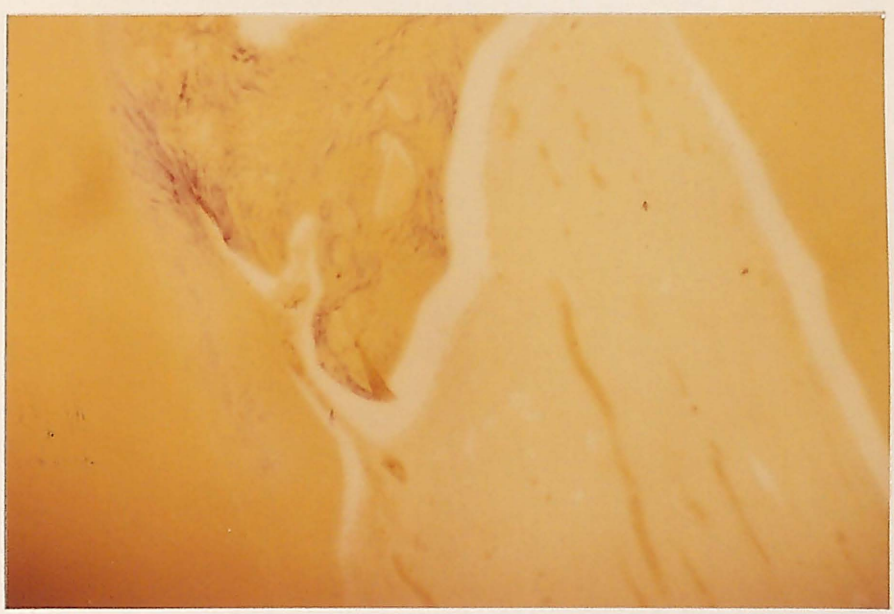


Figure 21. A satisfactory response with a complete dentin bridge was demonstrated by this maxillary right central treated with vancomycin, calcium hydroxide, methyl cellulose and water. Note the lack of bacteria and inflammatory cells. (Brown and Brenn stained, original magnification, x 1000 oil).

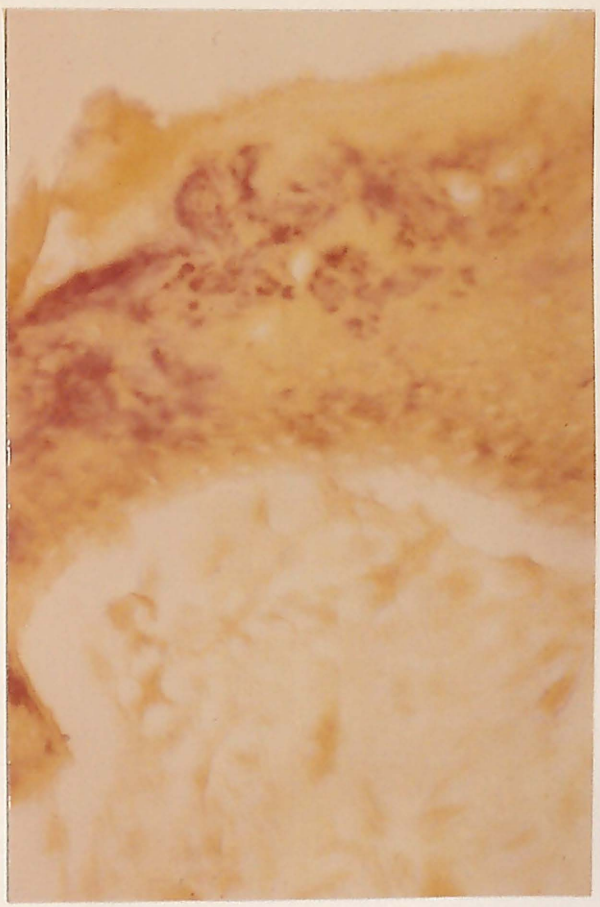
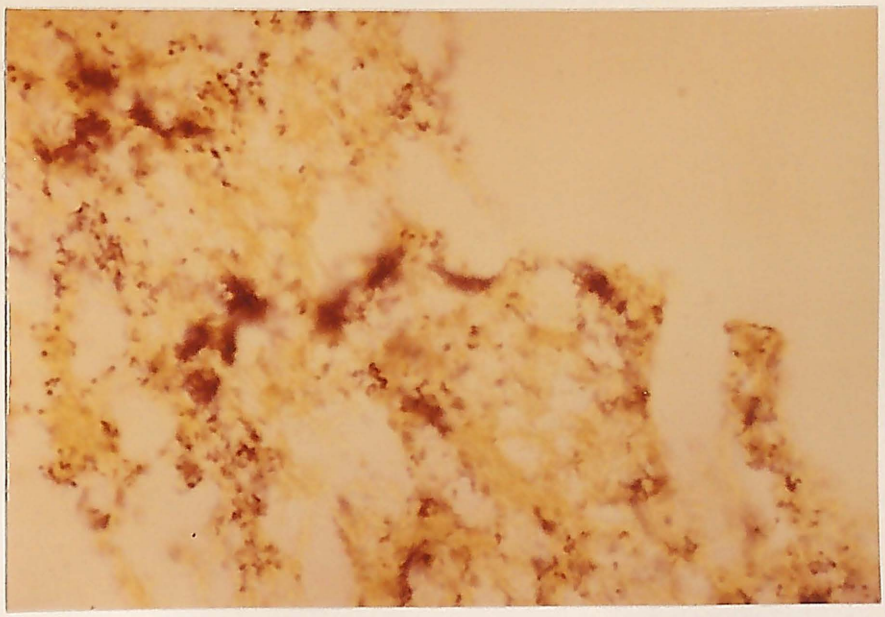


Figure 22. A mandibular central incisor responded unsatisfactorily to starch and water. Note the dark stained colonies of bacteria. (Brown and Brenn stained, original magnification, x 1000 oil).

Figure 23. The middle one-third of the pulp of a maxillary lateral incisor after treatment with starch and water for 90 days. Note the dark blue stained bacteria. (Brown and Brenn stained, original magnification x 40).



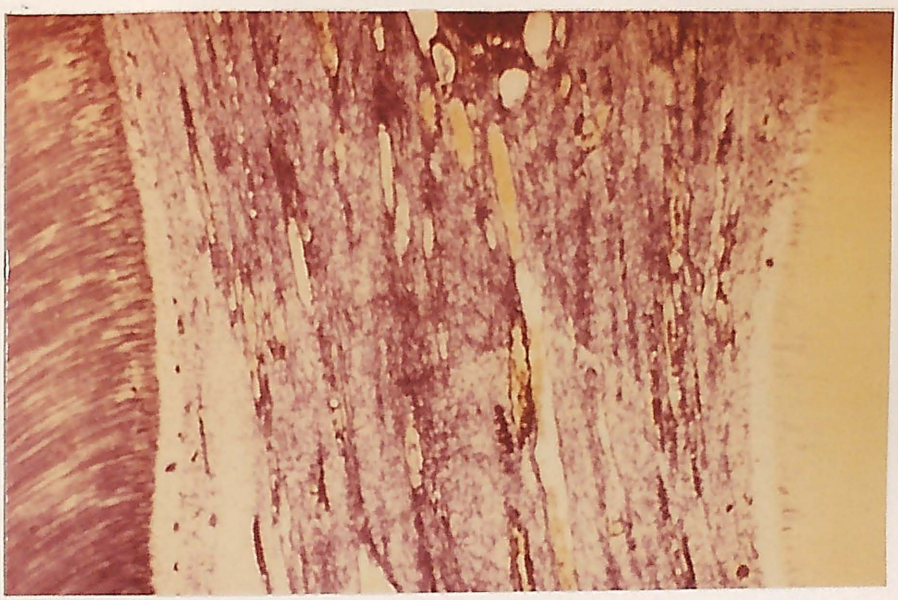


Figure 24. Dentin bridge in a tooth treated with vancomycin, calcium hydroxide, methyl cellulose and water exhibits red fluorescent marking. Note the bridge was completed when the dye was injected at 30 days.

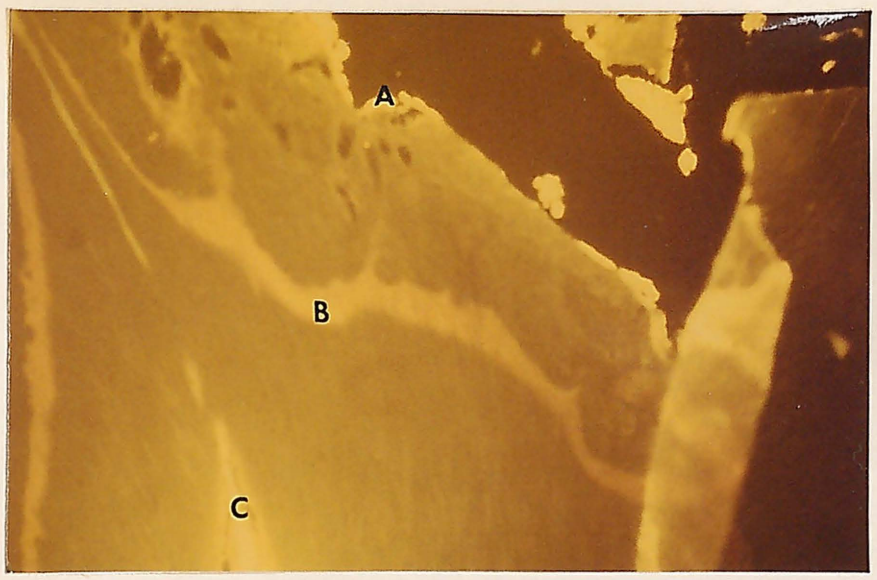
A. Exposure

B. Procion dye marking

C. Pulp

(Original magnification x 125).

Figure 25. Dentin bridge responded to vancomycin, calcium hydroxide, methyl cellulose and water, exhibits red marking. Note the reparative dentin deposited between the dye and the pulp. This represents 60 days of reparative dentin. Also note the dentin chips incorporated into the dentin bridge. (Original magnification x 125).


A
B
C

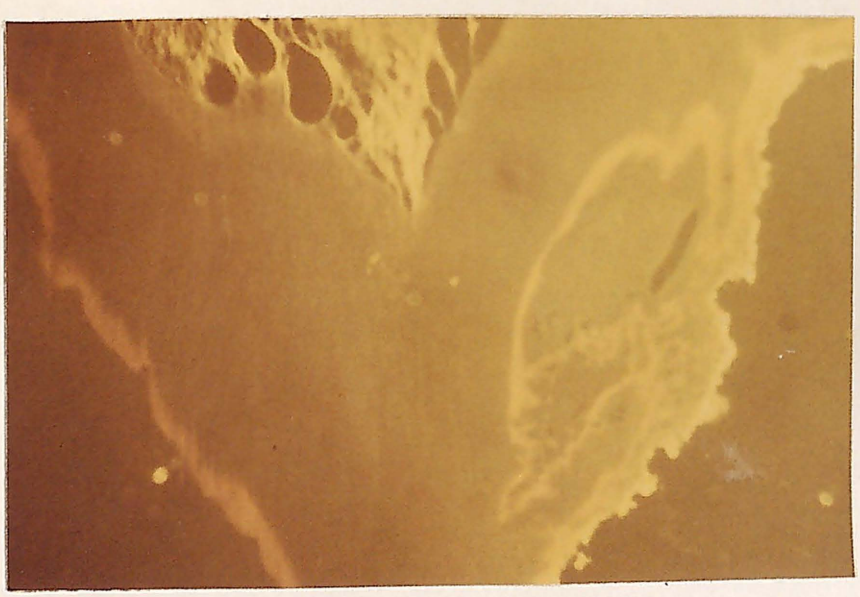


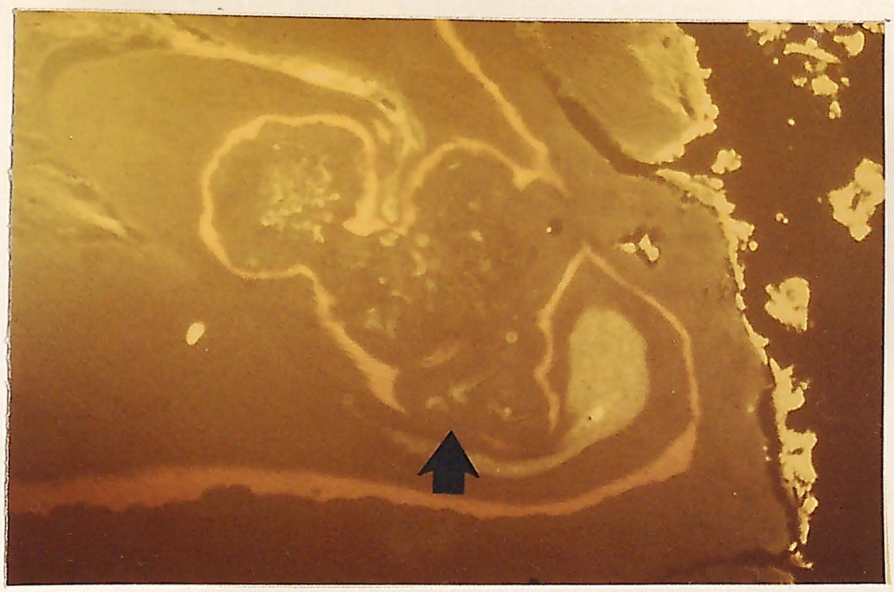
Figure 26. The break (arrow) in the red fluorescent line indicates the bridge was not completed at 30 days when treated with calcium hydroxide, methyl cellulose and water. Bridge was complete at 90 day extraction. (Original magnification x 125).

Figure 27. Unsatisfactory response to calcium hydroxide, methyl cellulose and water is demonstrated. There is no dentin bridge or red marking across exposure.

A. Exposure

B. Unsatisfactory pulp

(Original magnification x 125).



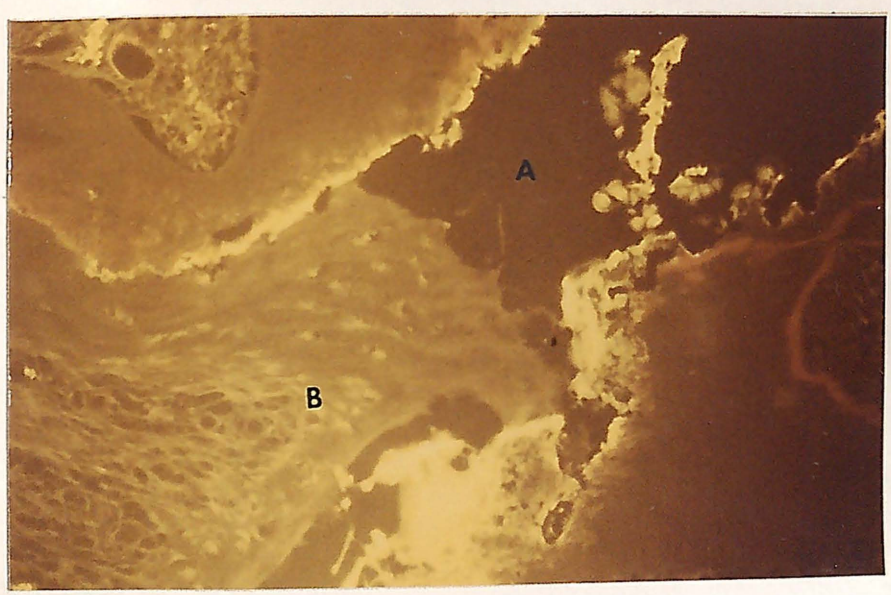
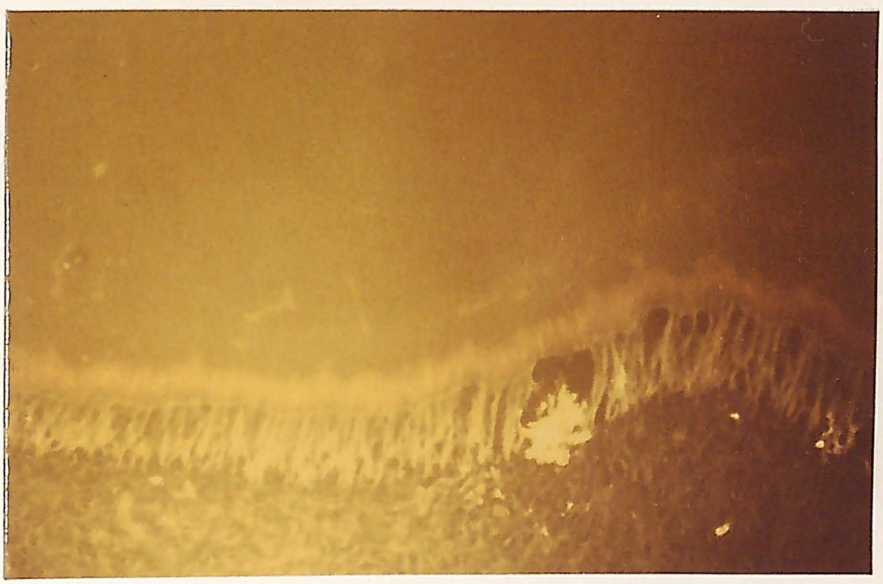

A
B

Figure 28. Red fluorescent marking at 30 days in this untreated tooth adjacent to the tooth in Figure 24. Note only a small amount of coronal dentin formation in 90 days in comparison to the reparative dentin formation in Figure 24. (Original magnification x 125).



DISCUSSION

This study demonstrated that the pulps of monkeys exposed to the oral environment for 48 hours became contaminated with primarily gram-positive organisms. That finding was consistent with the 24 hour exposure findings of Baker[37] and Eggers.[39] The role of bacteria in pulp pathosis has been identified by Kakehashi, Stanley and Fitzgerald.[63] They exposed the pulps of molars in 24 germ-free and 15 conventional rats and left them open to the oral environment from one to 42 days. After eight days or longer, the pulps of the conventional rats exhibited complete necrosis, while 18 of the 24 germ-free rat molars exhibited dentin bridging and little inflammation.

Kiryati[17] reported that the infection becomes more severe when pulps are left open for longer periods. Seltzer and Bender[64] made a similar observation. These results emphasize the important role that microorganisms play in pulpal pathosis, and there is mounting evidence that they are the dominant etiologic factor.

In this study, the role of bacteria assumes particular importance when one attempts to explain the marked difference in results between the capping agents. Many investigators[65-73] have proclaimed the effects of vancomycin on gram-positive bacteria. Several investi-

gators[65,66,67,68,72] have used vancomycin effectively when treating oral infections. The preliminary part of this study showed that vancomycin was very effective against the gram-positive microorganisms of the mouth.

This preliminary study also revealed the potency of calcium hydroxide as a bactericidal agent. All gram-positive and gram-negative organisms were sensitive to calcium hydroxide on agar plates.

It is apparent that if vancomycin is effective against gram-positive organisms, and calcium hydroxide against gram-negative, the two would complement each other nicely. Kutscher and Yigdall[53] found that the activity of certain antibiotics was decreased when the antibiotics are combined with other therapeutic agents (including calcium hydroxide). In this study, neither the vancomycin nor the calcium hydroxide lost its antibacterial effect when the two were mixed. In fact, there seemed to be a complementing effect since zones of inhibition on agar plates appeared larger when the two were mixed than when they were tested alone.

Local inflammatory reactions to vancomycin have been reported.[74] The subcutaneous implant method of screening pulp capping agents[56,57] was used in this

study to verify that finding. Although many sections revealed mild or mild to moderate inflammatory reactions, no specimen exhibited severe inflammation.

Satisfactory responses were observed in all infected pulps that were treated with the combination of vancomycin, calcium hydroxide, methylcellulose and water. Table VII compares with responses of the other medicaments. No inflammation was apparent and the pulps appeared normal. Fiore-Donno and Baume[35] stressed the importance of a solid dentin bridge in pulp healing to prevent reinfection. In this study, complete dentin bridges were observed in all teeth treated with the combination of vancomycin, calcium hydroxide, methyl cellulose and water. These bridges were horizontal to the pulp canals, complete in each serial section of the tooth, retracted away from the exposure site, and had a predentin layer as well as odontoblasts lining the pulpal border. Procion markings indicated that these bridges were complete at 30 days postoperatively, but the bridges thickened between 30 and 90 days (Table VIII).

In contrast, the calcium hydroxide mixed with methyl cellulose and water gave a more irregular bridge, which often was not completed at 90 days. This was emphasized by the Procion markings (Figures

24-27). In the one tooth giving an unsatisfactory response with this capping mixture, inflammation and bacteria were both present. The teeth which were treated with this mixture and gave only partial bridging revealed few inflammatory cells and no bacteria. However, gram-negative bacteria were difficult to see and may have been present. Macrophages contained dark staining intracellular material which appeared to be bacteria that were phagocytized. The prognosis of these pulps was very favorable and a 94.4 per cent success rate was observed.

Several investigators[14,17,37,63,75] had noted a relationship between the presence of inflammation and the amount of reparative dentin present. A similar observation was made in this study; inflammation seemed to inhibit reparative dentin formation, with the more severely inflamed pulps producing the least amount. Severely inflamed pulps usually were accompanied by large numbers of bacteria. However, those teeth treated with a combination of vancomycin and starch and deemed unsatisfactory, revealed few bacteria. This is probably due to the potency of the antibiotic, and further emphasizes that if inflammation occurs, reparative dentin may not form. Many of these pulps might have been reversible and might

have been bridged over if a longer period of time had been allotted. This was true in the study by Eggers.[39] He found a 92.9 per cent success rate when capping with vancomycin mixed with starch and hyaluronidase. When vancomycin and starch were used together, 71.5 per cent of the teeth gave a satisfactory response. This might indicate that the anti-inflammatory and "spreading" factors of hyaluronidase may help the pulpal response.

It is interesting that only 33.3 per cent of the teeth treated with vancomycin and starch in this study proved to be successful. The only alterations in methodology from that of Eggers[39] was the lack of a zinc oxide and eugenol base, and the 48 hour pulp exposure time, in comparison to 24 hours used by Eggers. The zinc oxide and eugenol base was not used because pulpal fluid often leaked through the starch containing capping agents. It was felt that if this occurred, eugenol might also leak through the capping compound and alter the pulp responses. Light condensation pressure was used to place the amalgam restorations, and this may have accounted for the three restorations that were lost before animal sacrifice. Copalite was applied only on the margins and not over the bases. Re-infection did not prove to be a problem with

the calcium hydroxide containing bases, but may have been a factor in the unsatisfactory responses to the starch containing agents. Walshe[75] experienced the problem of re-infection and recommended zinc oxide and eugenol bases covered by amalgam as a final restoration. This technique brought support from Eggers,[39] who re-emphasized the sealing properties of zinc oxide and eugenol.[76]

It has been suggested[39] that the successful response of starch alone as a capping agent, or in combination with hyaluronidase, may indicate that the animals have an inherent resistance to pulp infections, or that the molars did not become infected before the pulp-capping. Neither of these possibilities was applicable in this study, as only 11.2 per cent of the starch controls responded satisfactory and necrotic pulps treated in this manner. Again, the hyaluronidase used by Eggers may have caused the difference between this 11.2 per cent and the 42 per cent he reported using starch. The zinc oxide and eugenol bases may also have been a factor as well as the additional 24 hour exposure to the oral flora. Table IX displays the percentage comparison between the capping agents.

The oral flora of the monkey has been demonstrated to be similar to that of man.[77,78] It is speculated that the monkey will respond to antibiotic therapy in a manner similar to man.

Vancomycin is such a large molecular compound that it is not readily absorbed by the human body. Therefore, the risk of human sensitivity is decreased.

Dentin bridging is a continuous process which may take more than 90 days to complete. What will happen over a longer period is left for later research to determine.

SUMMARY AND CONCLUSIONS

This modified double-blind controlled study was undertaken to determine the effects of a potent antibiotic, vancomycin hydrochloride, in combination with calcium hydroxide as a pulp-capping agent in intentionally infected pulps of monkeys.

Preliminary investigations disclosed that the vancomycin containing capping agents were effective against gram-positive microorganisms. Subcutaneous implants studied histologically showed only a mild to moderate inflammatory response. Calcium hydroxide, either alone or in combination with vancomycin, was effective against gram-positive and gram-negative bacteria. The two agents seemed to complement each other well, both on the agar plates and in the subcutaneous implants. Pulps of four monkey teeth exposed to the oral environment for 48 hours were specially stained (Brown and Brenn) and the presence of many bacteria in the area of the exposures was shown.

In the principal portion of this study, the pulps of 74 permanent teeth in three monkeys were mechanically exposed to the oral environment for 48 hours. Each pulp was covered by one of the following pulp-capping materials:

1. Vancomycin, calcium hydroxide, methyl cellulose and water.
2. Vancomycin, starch and water.
3. Starch and water.
4. Calcium hydroxide, methyl cellulose and water.

The pulp-capping medicaments were sealed in the teeth with amalgam and no bases were placed.

The teeth were extracted before the animals were sacrificed two at 30 and one at 90 days. The teeth were decalcified, serially sectioned, and stained for histologic evaluation using hematoxylin and eosin.

The 90 day animal was injected with Procion brilliant red H-8BS dye as a hard tissue marking agent at 30 days postoperatively. This revealed the completeness of the dentin bridges at 30 days, as well as the amount of reparative dentin deposition between 30 and 90 days.

All the teeth treated with vancomycin, calcium hydroxide, methyl cellulose and water responded in a satisfactory manner with a lack of inflammatory cells and a complete dentin bridge with a normal pulp. This bridge was retracted from the exposure site, was reg-

ular in nature, showed a definite predentin layer, and was lined by odontoblasts.

The majority of the teeth treated with calcium hydroxide, methyl cellulose and water (94.4 per cent) revealed satisfactory results when examined histologically. Nine of the 18 teeth treated showed complete bridges, but the bridges were generally not as regular as those which resulted when the vancomycin and calcium hydroxide were used in combination. Procion dye markings revealed that with calcium hydroxide the bridges took longer to form and were more dependent upon dentin chips, than when the calcium hydroxide was mixed with vancomycin. Those teeth showing an unsatisfactory response to calcium hydroxide demonstrated only a few inflammatory cells and an apparent lack of bacteria.

One-third of the teeth responded satisfactorily when treated with vancomycin, starch and water. Another 33.3 per cent were unsatisfactory when viewed microscopically, and the remaining one-third were necrotic. Inflammatory cells were predominant in the unsatisfactory responses, but few bacteria were displayed. The necrotic pulps revealed large colonies of gram-positive bacteria.

Only 11.2 per cent of those teeth treated with starch and water responded satisfactorily. All others were unsatisfactory and many were necrotic. Apical granulation tissue was observed on three of these necrotic teeth.

From the results of this investigation, the following conclusions were made:

1. Vancomycin and calcium hydroxide are compatible when used in combination against gram-positive and gram-negative bacteria.
2. Vancomycin and calcium hydroxide in combination give a mild to moderate inflammatory response when implanted in subcutaneous connective tissue of rats.
3. Complete dentin bridging may be accomplished as early as 30 days post-operatively when vancomycin, calcium hydroxide, methyl cellulose and water are used as a pulp-capping agent.
4. Procion dye is an excellent marking agent to reveal completeness of dentin bridges.
5. Encouraging results indicate the need

for further investigations using pulp-capping agents containing vancomycin and calcium hydroxide; furthermore, clinical and longer studies may be indicated.

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ABSTRACT

TREATMENT OF INFECTED PULPS OF MONKEYS WITH VANCOMYCIN AND CALCIUM HYDROXIDE

By
Donald E. Gardner

This study was undertaken to investigate histologically the effect of a combination of a potent antibiotic and calcium hydroxide when used as a medication in direct pulp therapy.

The pulps of 74 teeth in one Macaca Speciosa monkey and two Macaca Nemestrina monkeys were exposed, and left open to the oral environment for 48 hours to insure contamination. These pulps received direct treatment with one of four experimental medications: 1) starch and water; 2) vancomycin, starch and water; 3) calcium hydroxide, methyl cellulose and water; and 4) vancomycin, calcium hydroxide, methyl cellulose and water. In 30 days the teeth were removed from two animals and at 90 days from the other for histologic evaluation.

A satisfactory response was observed in all the teeth treated with vancomycin, calcium hydroxide, methyl cellulose and water; in 94.4 per cent of the teeth treated with calcium hydroxide, methyl cellulose and water; in 33.3 per cent of those treated with vancomycin and starch; and in 11.2 per cent of those receiving starch and water. Complete bridging was seen in all teeth treated with vancomycin, calcium hydroxide, methyl cellulose and water. This was confirmed by the use of Procion brilliant red H-8BS dye and the study of serial sections.

Under the conditions of this investigation, vancomycin in combination with calcium hydroxide and methyl cellulose was effective in controlling infection and promoting reparative dentin formation in monkeys.