In vitro STUDIES OF THE PHARMACODYNAMICS OF THE ACTIVE COMPONENTS OF LEDERMIX PASTE, A CORTICOSTEROID-ANTIBIOTIC ROOT CANAL DRESSING MATERIAL

VOLUME I

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ABSTRACT

Ledermix is a compound therapeutic agent employed as a primary endodontic dressing. It contains, among other components, an antibiotic (demethylchlortetracycline) and a corticosteroid (triamcinolone). This study was undertaken to determine the *in vitro* release characteristics and dentine diffusion of the two active components in order to provide an indication of their availability to the periapical and periodontal tissues with time.

A new method has been devised using plastic root canal models and freshly-extracted human teeth filled with Ledermix paste containing 0.01 percent ^{3}H-triamcinolone and bathed in phosphate buffered saline (pH 7.4). The release and diffusion of demethylchlortetracycline and ^{3}H-triamcinolone were determined by using spectrophotometry and liquid scintillation spectrometry respectively at various time intervals up to 14 weeks. The concentrations of the two components within the coronal, mid-root and apical dentine were determined. Diffusion rates through coronal dentine to the pulp were also determined up to 8 days.

The plastic canal models showed that apical release is mainly dependent on foramen diameter size and only slightly dependent on paste volume. Peak release occurred in the first minute and then declined exponentially with time. There was little difference in the release from human teeth with an open apex compared with those with

an apical foramen which had been sealed, indicating that the main supply route to the periodontal tissues was via the dentinal tubules.

Removal of the canal smear layer by using E.D.T.A.C. as an irrigant significantly increased the rates of diffusion. Removal of the cementum had a similar effect, indicating that both the smear layer and cementum act as barriers to diffusion. Combining Ledermix paste with Pulpdent paste significantly slowed the release of the Ledermix components.

The mean concentrations of demeclocycline found within dentine indicate that its efficacy as an antibacterial agent within the dentinal tubules is questionable. Further research is indicated to determine whether other antibiotics may be more useful in these situations.

Data is not currently available on the concentration of triamcinolone required to achieve a local therapeutic effect, however clinical evidence exists which shows that the concentrations achieved by the use of Ledermix in endodontics are effective.

DECLARATION

This research report is submitted as a partial requirement for the Degree of Master of Dental Surgery in Endodontics at the University of Adelaide. Further requirements for the Degree were completed during 1984 and 1985.

This research report contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge, this report contains no material previously published or written by another person, except where due reference is made in the text of this report.

I consent to this research report being made available for photocopying and loan if it is accepted for the award of the degree.

PAUL V. ABBOTT

15th October 1985.

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CHAPTER 1

INTRODUCTION

1.1 GENERAL

Ledermix is a compound therapeutic agent used in several parts of the world in the treatment of pulpal and periapical diseases. Ledermix has two active components, triamcinolone (a corticosteroid) and demethylchlortetracycline (also known as demeclocycline, an antibiotic). It is commercially available in paste and cement forms. Despite the popularity of the agents in some countries, the use of corticosteroid-antibiotic compounds was described as "experimental or empirical" by the American Dental Association in their publication "Accepted Dental Therapeutics" (1979) and this opinion is still widely held.

Many studies have been conducted on the effects of Ledermix in the cement form as a pulp capping agent but few reports have been published on the use of the paste in either vital pulp therapy or root canal treatment. The majority of the research into corticosteroid-antibiotic compounds occurred in the 1960's and early 1970's, prior to the identification of the presence and role of anaerobic bacteria in the progression of pulpal and periapical diseases. As a result, the main areas of research were pulpal

reactions following pulp capping and the long-term effects of corticosteroids on pulp tissue. Little attention has been paid to the efficacy of the antibiotic component in the treatment of pulpal and periapical infections.

The review of the literature which follows (Chapter 2) covers aspects of the use of corticosteroids in dentistry, the development of the Ledermix formulations, an outline of the bacteria reported as being involved in endodontic infections, the susceptibility of these bacteria to antibiotic agents and the general principles of diffusion of molecules through dentine.

Research has demonstrated the presence of bacteria in the dentine, the periapical tissues and the periodontal tissues of non-vital teeth with associated periapical radiolucencies. As part of the treatment of these lesions, many dentists place Ledermix paste within the root canal, a procedure which appears to add to the efficacy of treatment (Schroeder 1962, Ehrmann 1965). The main aim of the present study was to investigate the release characteristics of the active components from Ledermix paste placed in root canals in an attempt to gain knowledge of its mode of action. Since bacteria may reside in the dentine of infected tooth roots, it was of interest to examine diffusion rates into and through root dentine as well as diffusion out of the root apex.

Ledermix paste may also be used as a cavity liner or temporary cement over an inflamed pulp. A second part of this study was therefore to investigate the availability of the components to the pulp tissues under these circumstances.

1.2 COMPONENTS OF LEDERMIX

Ledermix formulations are manufactured by Lederle Pharmaceuticals - a division of Cyanamid GMBH of Wolfratshausen. It is marketed as a kit containing:

Ledermix paste - 3 gm tube,

Ledermix cement - 2 gm bottle of cement powder,

- 2.5 ml bottle with dropper of hardener liquid "N" (normal setting time),
- 2.5 ml bottle with dropper of hardener liquid "F" (fast setting time).

The cement and paste are available separately, designated as Refill No.1 and No. 2 respectively. Refill No.1 contains 3 gm of powder and 2.5 ml of each liquid hardener. Refill No.2 contains a 5 gm tube of Ledermix paste.

The compositions of the Ledermix paste, cement powder and cement liquid are listed below. This information is taken from Ehrmann (1965), Allwright and Wong (1966) Lakshmanan (1972) and Lederle Pharmaceuticals (1981).

The chemical structures of Ledercort (the steroid, triamcinolone) and Ledermycin (the antibiotic, Demethylchlortetracycline or Demeclocycline) are given in Figures 1-1 and 1-2, from Schroeder (1965).

LEDERMIX PASTE: Triamcinolone acetonide 1.0 percent

Demethylchlortetracycline calcium 3.21 percent

in a water soluble cream consisting of :

Triethanolamine N.F.

Calcium chloride U.S.P.

Zinc oxide

Sodium sulphite (anhydrous)

Polyethylene glycol 4,000 U.S.P.

Distilled water.

LEDERMIX CEMENT:

Powder: Triamcinolone acetonide 0.67 percent

Demethylchlortetracycline HCl 2.0 percent

Zinc oxide U.S.P.

Canada balsam

Rosin N.F.

Calcium hydroxide U.S.P.

Liquid (hardener):

Eugenol U.S.P. 85 percent

Rectified turpentine oil N.F. 13 percent.

CHAPTER 2

A REVIEW OF THE LITERATURE

2.1 GENERAL HISTORY OF CORTICOSTEROIDS

The first person to recognise the physiological significance of the adrenal glands was Addison in 1855. At that time he described a syndrome resulting from the destructive disease of the glands. This syndrome is now known as Addison's Disease and is characterised by a marked loss of energy, hypotension, anorexia, vomiting and pigmentation of the skin and mucous membranes.

In 1856, Brown-Sequard conducted experiments on the effects of adrenalectomy and concluded from these that the adrenal glands are essential to life.

In 1896, Osler reported on a case of Addison's Disease that was greatly benefited by the use of a suprarenal extract from pigs (Gee, 1974).

The first people to isolate an adrenal cortical hormone were Kendall and asociates in 1936 (Gee, 1974). In 1937, Steiger and Reichstein made the first synthetic adrenocorticoid hormone. The hormone produced was desoxycorticosterone,also known as cortisone. By 1940, the Reichstein, Kendall and Wintersteiner groups had isolated 28 related crystalline steroids (Gee, 1974).

The next advance came when Hench et al (1949) used cortisone for the first time in chronic polyarthritis. They had observed that arthritic patients experienced temporary remission when they were either pregnant or jaundiced. They also tested the synthetic cortisone in a case of rheumatoid arthritis and produced a dramatic response. This was to be the start of a new era in the treatment of inflammatory and allergic diseases. According to Schroeder (1962), "today the substitution therapy of adrenal cortical insufficiency has given place to the pharmacotherapy of a large number of affections." Corticosteroids are used in many branches of medicine, and since 1952 have been used in dental surgery.

Synthetic analogues of cortisone were produced in 1953. The first were made by halogenation of either cortisone or hydrocortisone with chlorine or fluorine in the nine-alpha position. This increased the metabolic and therapeutic effects of the parent drug. In the oral cavity, nine-alpha-fluorohydrocortisone is of benefit because it is active locally and because of its increased potency and absorbability (Gee, 1974).

In 1955, prednisone and prednisolone were created by introducing a double bond between C1 and C2 in ring A of cortisone and cortisol respectively. This increased the anti-inflammatory properties of the parent hormones (Gee, 1974). Triamcinolone is a derivative of prednisolone and has similar anti-inflammatory properties but slight or absent mineralocorticoid effects (Cahn and Levy 1959, Zegarelli et al 1959, Fauci et al 1976) [See Appendix 3].

2.2 HISTORY OF CORTICOSTEROIDS IN DENTISTRY

a) TEMPORO-MANDIBULAR JOINT THERAPY

The earliest reports of cortisone being used in dentistry were by Spies et al (1952) and Strean (1952). These workers used intramuscular injections of cortisone to treat arthritis of the temporo-mandibular joint. Later that year, Ensign and Sigler (1952) were noted for administering hydrocortisone intra-articularly within the TMJ and reported good results.

Horton in 1953 injected no more than 0.55ml of a saline suspension of hydrocortisone acetate intra-articularly within the TMJ and noted that at this dosage, the side effects of systemic application (water retention, etc) were absent. Many other authors reported on similar uses of cortisone in TMJ diseases over the next few years. Perhaps one of the most significant findings was the rapid relief of symptoms, usually after only one injection. This property of the steroid drugs was most encouraging and was probably responsible, more than any other factor, for the intense interest and experimentation on the use of these drugs in dentistry.

An important point raised in 1954 by Henny was that the specific action of the hormone was directed solely against inflammation, and, that permanent success could only be expected if, at the same time, the causative factor (e.g. traumatic occlusion) was removed.

b) PERIODONTAL AND ORAL MUCOSAL LESIONS

At the same time that steroids were being used for TMJ disorders, interest in their use for periodontal and oral mucosal disorders was stimulated by Siegmund (1952). A large number of publications followed over the next few years.

Fisher (1956) experimented on 125 patients who had gingivitis. He treated them with either topical or systemic hydrocortisone and either with or without mechanical-dental treatment. He reported that hydrocortisone, whether given systemically or topically, undoubtedly reduced gingival inflammation, which was enhanced if mechanical-dental treatment was used. He also confirmed his results histologically.

Weisstein (1956) reported successful use of a fluorohydrocortisone acetate ointment (0.25 percent) for gingivitis, leukoplakia, pemphigus, herpes and aphthous ulcerations.

The various authors presented histological and clinical evidence for the corticosteroid therapy. Schroeder (1962) summarised these reports and emphasised that treatment with corticosteroids is not a causal therapy, but is merely adjunctive and therefore the causative factor must be removed by conventional means. He also stated that acute inflammation responded far more successfully than did chronic inflammation, and that the use of prednisolone enabled a shorter period of treatment than did hydrocortisone.

c) ENDODONTICS

i) Apical Periodontitis: The use of corticosteroid drugs in endodontics was initially reported by Wolfsohn in 1954. He treated

cases of acute apical periodontitis first by electro-sterilisation of the canal and then by flushing the canal with a hydrocortisone solution and was able to secure prompt relief of pain.

A few years later, Schroeder and Triadan (1961) found that even in the most severe cases of acute apical periodontitis it was not necessary to leave the root canal open for several days, as was the usual practice at the time. They treated these cases by preparing the canal, flushing it with a solution of 3 percent hydrogen peroxide and sodium chloride and then filling it with a corticosteroid-antibiotic preparation they had developed (see below). The canal was then sealed until the next visit and the patients reported that sensitivity to percussion and swelling were reduced dramatically. At the second visit, the exudate was remarkably reduced in amount and then standard root canal therapy was continued.

The literature has relatively few reports about the use of corticosteroids in periodontitic teeth. Most of these reports come from the 1960's and often only mention periodontitis as an indication for the use of corticosteroids. The reports have been written by: Schroeder (1962, 1965), Ehrmann (1964,1965,1972), Olsen (1966), Baume (1968), Schneider (1968), Laws (1969), Barker and Lockett (1971, 1972) and Erausquin (1972). These works were mainly clinical trials and the only authors to report histological findings were Schroeder (1965), Barker and Lockett (1972) and Erausquin (1972).

ii) Pulpitis: During the 1950's, dentists began to think that the corticosteroids could be the answer to their age-old problem of treating pulpitic teeth and at the same time maintaining their vitality. They believed the pulpal inflammatory processes contributed

towards a hastening of the pulp's destruction, because, in response to a harmful stimulus, the natural defense mechanisms of the healthy organ come into play with hyperemia, exudation, etc. It was thought that these very defense processes combined with the poor drainage of the pulp tissue and its surroundings of unyielding hard tissue on all sides and lack of collateral circulation lead to pulp destruction (e.g. Schroeder, 1962).

The rationale of treatment became one of removing the causative factor (e.g. carious dentine) to counteract the bacteria and to control the inflammation itself by using corticosteroids and thus prevent pulp death.

Rapoport and Abramson (1958) reported using hydrocortisone on exposed pulp. They applied it either as a powder or liquid directly onto the exposure site and then covered the wound with sterile cigarette paper or a small asbestos sheet and closed the cavity with rapid setting zinc oxide-eugenol cement followed by a zinc phosphate cement and then the final filling material. They compared a sterile technique with a non-sterile technique and found no significant difference between the success rates of 80 percent and 93 percent respectively. Their criteria for success was maintenance of vitality, however the follow-up period was short and only 60 teeth were tested. Their results did prove to be encouraging for other investigators.

Kiryati (1958) also reported the action of hydrocortisone alone and with antibiotics for pulp capping of infected rat pulps. He found that the combination of hydrocortisone, neomycin and bacitracin seemed to assist the survival of the pulps in 63 percent of cases, whereas hydrocortisone alone was only 22 percent successful.

Turell and Morales (1958) capped various animal's teeth and 16

healthy human teeth with a paste of 6 parts calcium chloride and 1 part cortisone acetate in anhydrous glycerine. The human teeth were extracted 25 to 45 days later for orthodontic reasons and their histological examination showed the pulps to be healthy and in a state of "increased readiness for defense". Some isolated cases were observed with hard substance formation.

The first people to use a corticosteroid-antibiotic combination in an attempt to reverse serous pulpitis in humans were Galluzzo and Bellomi in 1959. Their paste contained penicillin, streptomycin, tetracycline and prednisolone in a lanolin-vasoline ointment base. They observed 19 teeth for 6 months and reported positive results in all cases.

In 1960, Fry et al published their results of a study using the more potent corticosteroid, prednisolone. To this, they added camphorated parachlorophenol and metacresyl acetate (these two drugs were in common use at that time for the sterilisation of root canals and were considered to be only mildly irritating to living tissues). They used the paste on 34 teeth with exposed pulp or deep caries showing symptoms of pulpitis. They were able to report, after four months, positive vitality, freedom from pain and full functional capacity for all but one tooth, which was extracted.

Kozlov and Massler also reported in 1960 that cortisone and hydrocortisone were substances that had elicited a satisfactory reparative reaction of the rat molar pulp wounds after amputation.

In 1962, Vigg used "Terracortril" ointment, which contained 1 percent hydrocortisone and 3 percent oxytetracycline, as a pulp capping agent. This was in turn covered with calcium hydroxide in methyl cellulose, zinc oxide-eugenol cement, zinc phosphate cement

and a temporary restoration. He reported 14 successful cases out of 18 pulpitic teeth and a calcific bridge formed in all cases.

Schroeder and Triadan have done several clinical and histological trials (1961, 1962, 1963, 1968, 1972) and obtained a lot of material supporting the use of corticosteroid-antibiotic compounds in conservative pulp therapy. They initially experimented with hydrocortisone but after having obtained unsatisfactory results, they resorted to triamcinolone, which had "an essentially more intense action". It is one of the most potent of the corticosteroids and is commonly prescribed in both systemic and topical forms, such as Aristcort, Kenacomb, Kenacort, Kenalog, Kenalone and Ledercort (MIMS, 1982). They used a water soluble cream containing:

0.315 gm triamcinolone,

0.28 gm chloramphenicol, and

4 drops of 4 percent xylocaine,

in 1.5 gm of ointment base.

Their treatment protocol was to totally excavate the caries and apply the ointment on a pledget of cotton wool to the dentine overlying the pulp, or if the pulp was exposed, then it was applied directly onto the pulp, and then closed the cavity with zinc oxide-eugenol cement. At a second visit a week later, the pledget was removed and replaced with a hardening capping cement which also contained the active substances although in lower concentrations. They reported that in the 200 teeth tested, the pain disappeared in 2-3 hours even in cases of early suppurative pulpitis. The majority of these teeth were still vital after some months. In cases of purulent pulpitis, the pain ceased but there was no resolution until further treatment was completed.

In 1962, Schroeder then reported it was apparent that there was no need for the addition of a local anaesthetic to the capping agent. He stated that the corticosteroids were suitable for suppressing inflammation of the pulp and for providing immediate relief of symptoms, but that they do "paralyze the defense mechanisms" and could lead to accelerated suppuration of the pulp. Schroeder therefore suggested that the steroid component should be combined with a broad spectrum antibiotic and used in conjunction with the complete removal of the carious dentine. Schroeder also stated that the use of corticoids for capping the healthy pulp seems to be contra-indicated as "this brings the pulp from the normergic into the anergic condition which is likely to end in necrosis". An "anergic" state is one in which tissue has lost the capacity to respond with sensitivity or immunity to antigens.

Schroeder also considered the problem of what to cap the pulp with once it had been soothed by the use of corticosteroids. He was concerned that by using a material that continually releases the corticoid into the pulp, the formation of hard tissue may be hindered.

These findings by Schroeder led to a change of his formula of the capping paste and hardening cement. This material was then manufactured in Type A (paste) and Type B (cement) formulae by the Lederle Laboratories under the trade name of Ledermix. The formulae have not changed since then.

According to the manufacturer, Ledermix is indicated for use in:

a) Apical periodontitis - primary acute periodontitis and acute exacerbations of chronic periodontitis - place the paste in the root canal as a dressing agent.

b) Hypersensitive dentine - use the cement as a liner or mix the paste with a temporary luting agent.

c) Pulpitis (with exposed pulp) - dress with the paste for 2-3 days and then cap with the cement and complete the final restoration if symptoms were relieved and the pulp remained vital.

d) Pulpitis (no exposure) - indirectly cap the pulp with the cement and then complete the permanent restoration.

The formula of the paste was designed to:

a) reduce inflammation by the use of a corticosteroid,

b) control infection or spread of bacteria by the use of a broad spectrum antibiotic, and

c) the water soluble cream enables easy application and removal from the tooth and readily releases the active components.

The formula of the cement was designed to:

a) reduce inflammation and control infection as for the paste, but for a shorter period of time to avoid long-term action of the corticosteroid,

b) encourage calcific bridging of exposures by the use of calcium hydroxide,

c) provide some sedative action on the pulp by use of eugenol, and

d) provide a quick-setting hard base of zinc oxide-eugenol to seal the exposure and allow immediate permanent restoration.

The availability and release of the components was not investigated during the early use of the material. No published data could be found until 1981, when Hume and Kenney tested the release of triamcinolone from Ledermix cement. They found that 70 percent of it was released in the first 24 hours and most of the remainder during the following few days. They also showed that triamcinolone readily diffused through human dentine. This study confirmed the "hopes" of Schroeder, i.e. a quick-setting cement that would not continually release steroid and therefore not hinder hard-substance formation. The rationale of indirect capping with Ledermix cement to reduce inflammation and allow healing appears to be justified.

2.3 MICRO-ORGANISMS FOUND IN ROOT CANALS AND PERIAPICAL TISSUES

The microbial flora of root canals and periapical tissues is complex and much effort has been spent on its determination since the pioneering work of W.D. Miller in the last decade of the nineteenth century. Since the nature of the microbial flora is of some relevance to the present study an extensive review of the literature was completed by the author (Abbott,1984) and is summarised below.

Prior to 1966, many sampling and culture techniques were reported. These techniques were based mainly on aerobic growth conditions. In 1966, Möller published the results of an exhaustive study that demonstrated the presence of many anaerobic organisms. Möller developed a new technique that avoided oral contamination, inactivated any anti-microbial agents still present in the root canal and provided transport and culture media that allowed both aerobic and anaerobic bacteria to grow and be examined. Appendix 4 lists the formulae for Möller's transport media (VMG Media) and their properties and Appendix 5 lists the formulae for the culture media (HCMG base medium and supplements).

The development of this technique led to the initiation of new studies and conclusive evidence now exists which links specific pathogens to the progression of periapical tissue breakdown (Fabricius, 1982). Other studies have linked specific bacteria to situations of acute "flare-up" (Sundqvist 1976, Brook et al 1981).

Table 2-1 is a summary of the organisms reported as being present in infected root canals and periapical tissues by Dubos (1952), Möller (1966), Wittgow and Sabiston (1975), Sundqvist (1976), Dahlen and Hofstad (1977), Sundqvist and Johansson (1980), Sundqvist and Reuterving (1980), Sveen and Skaug (1980), Brook et al (1981), Von Konow et al (1981), Möller et al (1981), Dahlen et al (1982a,b), Fabricius (1982), Fabricius et al (1982a,b) and Robertson et al (1982).

2.4 SUSCEPTIBILITY OF THE PULPAL AND PERIAPICAL FLORA TO ANTIBIOTIC AGENTS.

The use of anti-microbial agents as an adjunct to the normal chemo-mechanical preparation of the root canal in endodontics is widespread. Antibiotics have been used either systemically or as intra-canal dressings.

a) SYSTEMIC ADMINISTRATION OF ANTIBIOTICS IN ENDODONTICS.

The use of systemic antibiotics in endodontics is usually restricted to situations where the symptoms are acute. Chronic infections do not normally require antibiotic therapy unless they have developed into an acute "flare-up" situation.

The choice of antibiotic is most important as the patient requires relief from symptoms and early treatment is necessary to avoid spread of the infection and further complications.

Hunt et al (1978) stated that penicillin and erythromycin are as effective against obligate anaerobes as they are against aerobes and facultative anaerobes, while Ernest et al (1977) found that clindamycin was the most effective antibiotic against the obligate anaerobes and ampicillin was the most effective against facultative anaerobes isolated from necrotic pulp chambers.

Morse (1981) found cephalexin, lincomycin, clindamycin, chloramphenicol and ampicillin to be effective. Chloramphenicol has been associated with serious toxic side effects (Woods, 1968) and lincomycin and clindamycin may cause gastro-intestinal effects that may occasionally be fatal (Morse, 1981). Miles (1984) stated that if

antibacterial spectrum was the only criterion, clindamycin would be the drug of choice for treating endodontic infections. Miles recommends the use of clindamycin as the drug of second choice behind penicillin V (or erythromycin in patients allergic to penicillin).

Metronidazole has been recommended for use against infections of anaerobic bacteria, especially the Bacteroides species (MIMS 1982, Mitchell 1984). Ingham et al (1977) carried out a controlled clinical trial comparing metronidazole and parenteral penicillin for the treatment of acute apical infections and concluded "that metronidazole, given orally, was at least as effective as parenteral penicillin" and "had the comparison been made with oral penicillin it is possible that metronidazole would have proved the superior agent".

Since most endodontic infections will be mixed infections, particularly of anaerobic bacteria, a combination of drugs may be required. Moule (1982) suggested a combination of amoxycillin and metronidazole for the treatment of dental infections. Metronidazole is known to react synergistically with penicillin (Bittner and Munteanu, 1976) and therefore this combination could prove very useful.

Akimoto et al (1985) were the first to study the concentration of an antibiotic (talampicillin) in human dental pulp following systemic administration by using extracted mandibular third molars. The mean peak concentration in the pulp and serum occurred at nearly identical times and higher values were obtained in teeth which had incomplete root formation. Since antibiotic concentration in tissue is related to tissue vascularity, the talampicillin concentration in pulp would be expected to decrease with advancing age. Akimoto et al (1985) concluded that sufficient concentrations of talampicillin were

found in oral tissue (gingiva, mandibular bone, dental folicle and dental pulp) to indicate that the drug may be useful in dental practice.

Similar studies are indicated to determine the concentration of other antibiotics in oral tissues (especially within the dental pulp, periodontal tissues, periapical abscesses, granulomata and cysts) following oral administration in order to determine their probable local efficacy.

b) THE USE OF ANTIBIOTICS AS ENDODONTIC MEDICAMENTS.

In order to treat infected pulp or periapical tissues it is necessary to reduce the endodontic microbial flora to as low a level as possible (Harty 1976, Morse 1976, Grossman 1978). This can be achieved by:

a) the use of aseptic procedures, e.g. rubber dam,

b) mechanical instrumentation,

c) irrigation,

d) use of systemic antibiotics,

e) use of antimicrobial intracanal medicaments,

f) proper obturation techniques, and

g) reducing presence of periodontal microbes.

(Morse, 1981).

An emphasis in recent years has been to reduce the concentration of irrigants and medicaments to prevent toxic tissue reactions and yet still to retain antimicrobial activity. Some of the functions of intracanal medicaments are:

a) antimicrobial activity,

b) anodyne effects (i.e. soothing effects),

c) reduction of exudate, and

d) psychosomatic effects.

(Morse, 1981).

A high incidence of culture reversals from negative to positive just prior to obturation has been reported by Seltzer (1971). Morse (1981) felt that the main cause of the culture reversals was leaking temporary restorations and he therefore felt that this was another reason for using an antimicrobial intracanal medicament. The efficacy of such a medicament would depend on how long it remained active, the time interval between appointments and whether or not there was a sufficient concentration of the antimicrobial agent in order to inhibit the bacteria.

Normal endodontic therapy involves mechanical debridement of the root canal associated with the use of irrigants to flush bacteria, organic material and other matter from the canal. This type of preparation does not clean the accessory or lateral canals if they are present. There are also reports of dentinal tubules containing bacteria (Von Waechter and Möse 1955, Hobson 1959), especially in close proximity to the root canal (Jolly and Sullivan, 1956). Therefore, antimicrobial intracanal medicaments are used as an adjunct to the principles of normal canal debridement and preparation in order to inhibit or destroy any residual bacteria that may be present.

Ledermix paste is an example of an intra-canal dressing material that contains an antibiotic agent, demeclocycline. The

popularity of this material is related to its clinical efficacy, especially in reducing pain, which is due to the actions of the corticosteroid component, triamcinolone (Schroeder 1962, Ehrmann 1965).

The filling of a prepared root canal with an anti-microbial medicament also allows diffusion of the medicament through either the dentinal tubules or the canal apex to the periapical tissues. This then exposes the bacteria present in the periapical tissues to the effects of the medicament and thus enhances repair of a periapical lesion by reducing the number of active bacteria. To date, no studies have been reported on the availability of antimicrobial agents from intracanal dressings to the periapical tissues by direct release through the apex or by dentine diffusion. The important factor is the available concentration of the agent in the tissues.

c) MINIMAL INHIBITORY CONCENTRATIONS.

The minimal inhibitory concentration (MIC) [see Appendix 1 for abbreviations] of an antibiotic is the lowest concentration of antibiotic that inhibits visible growth of the test organisms. The MIC can be read at various time intervals, e.g. 18 hours, 24 hours and 48 hours (Rotilie et al, 1975). The MIC can be read for inhibition of various percentages of the organisms, e.g. 50 percent, 90 percent, 100 percent or, can be given as a range of concentrations. The concentration is usually given as micrograms per millilitre.

The susceptibility of organisms to antimicrobial agents is, in most instances, predictable (Murray and Rosenblatt, 1977). Generally,

penicillin is effective against aerobes and anaerobes with the exception of Bacteroides fragilis and some strains of Fusobacterium and Clostridium. Penicillin-resistant strains of Bacteroides melaninogenicus have also been reported (Hackman and Wong 1976, Murray and Rosenblatt 1977). Variations of susceptibility within the group of Bacteroides fragilis have also been reported (Hansen, 1980).

Resistance to a particular drug has been defined by Brown and Waatti (1980) as being the presence of growth of the organisms in the presence of antibiotic concentration above a certain level. This level varies for each drug and they stated the following concentrations:

Penicillin	> 4.0 micrograms/ml
Tetracycline	> 4.0 micrograms/ml
Chloramphenicol	> 16 micrograms/ml
Clindamycin	> 8.0 micrograms/ml.

These authors reported that 97 percent of the Bacteroides fragilis strains tested were resistant to penicillin at a concentration greater than 4 micrograms/millilitre and 56 percent of B.melaninogenicus were resistant. They reported a variation among the various species, but a majority of the anaerobic clinical isolates were resistant to tetracyclines, while only a few were resistant to chloramphenicol. The B.fragilis and B.melaninogenicus had 4.4 percent and 3.2 percent resistant strains respectively to clindamycin. In view of the increasing frequency of the presence of these Bacteroides strains in endodontic infections, it is clear that the use of penicillin and tetracyclines may not be the most suitable method for treating these infections.

Table 2-2 is taken from the tests done by Sutter and Finegold (1976) using an agar dilution technique and lists the MIC's for the microbes commonly found in endodontic infections. The MIC's are for 100 percent inhibition of the tested organisms. Baker et al (1983) reported that the MIC's of oral isolates were similar to those for non-oral isolates of organisms of the same or closely related species.

The results of Sutter and Finegold (1976) demonstrate that penicillin V, ampicillin, amoxycillin, cephalothin, cefamandole, cefazaflur and tetracycline require very high concentrations in order to inhibit growth of Bacteroides fragilis, B.oralis and Fusobacterium necrophorum. Metronidazole was effective against most of the obligate anaerobes, however, not as effective against the facultative anaerobes. The drugs that required high concentrations for most of the listed organisms were tetracycline, doxycycline, cefazaflur and carbenicilin. The drugs that were effective against most organisms at concentrations of 32 micrograms/millilitre or less were: metronidazole, erythromycin, clindamycin, chloramphenicol, amoxycillin, ampicillin and penicillin V. This information suggests that a combination of antimicrobial agents may be the most effective treatment and agrees with the report by Moule (1982) discussed earlier. The use of metronidazole in combination with amoxycillin would ensure that all possible organisms are being treated and in particular tha Bacteroides group (e.g. B.melaninogenicus) which have been implicated in acute "flare-up" cases (Sundqvist, 1976).

Baker et al (1985) have also stated that combining two antibiotics in a topical preparation might give a broader spectrum of coverage at lower concentrations than using either antibiotic alone.

They suggest this does not imply synergism in the mode of action of the drugs, but is an overlapping of their susceptibility patterns and such drug combinations would need to be evaluated more fully.

Routine susceptibility testing of bacterial samples is impractical (Sutter and Finegold, 1976) because dental infections are polymicrobic and contain a large proportion of anaerobes and therefore relatively long periods of time for growth and isolation are required. It is essential to the patient's well-being that treatment commence as soon as possible to avoid further spread of the infection and a knowledge of the organisms likely to be involved and their susceptibility is essential in order to prescribe the appropriate antibiotic agent. However susceptibility testing would be useful in cases not responding to broad spectrum drugs with routine doses.

2.5 DIFFUSION OF MOLECULES ACROSS DENTINE.

Dentine can be regarded as a diffusion barrier consisting of a relatively impermeable mineralised matrix penetrated by numerous, uniform cylindrical water-filled tubules (Pashley et al, 1978a).

Garberoglio and Brännström (1976) used a scanning electron microscope to examine fractured coronal dentine of 30 intact human teeth in various age groups at various distances from the pulp. Close to the pulp they found approximately 45,000 tubules per square millimetre with a diameter of 2.5 micrometres. In the middle of the dentine, there were 29,500 tubules per square millimetre with a diameter of 1.2 micrometres. Peripherally, the corresponding values were 20,000/mm^2 and 0.9 micrometres. The tubule volume in coronal dentine was 10 percent. No significant difference was found between old and young teeth. These figures varied from other reports on the diameter of tubules (Meyer 1951, Bradford 1955, Ketterl 1961). These authors used decalcified fractured surfaces and reported diameters varying from 1 micrometre near the enamel up to 5 micrometres near the pulp.

There are indications that the peritubular dentine to a large extent is dissolved during decalcification in acid (Isokawa et al 1970, Garberoglio and Brännström 1972). The tubule diameter measured on decalcified sections thus gives an excessively high value (Bradford, 1955) and makes previous values based on such sections not valid (Garberoglio and Brännström 1976).

Several investigators have demonstrated fluid movement in dentine (Brännström 1960,1961,1962, Brännström and Åström 1964,

Brännström et al 1967,1968, Polhagen and Brännström 1971, Johnson et al 1973, Linden and Brännström 1976). These authors applied various stimuli to exposed dentine including hydrostatic pressure, an air stream, heat, cold, negative pressure and osmotic pressures. These stimuli caused pain and resulted in fluid flow through the dentinal tubules.

Anderson et al (1958), Anderson and Ronning (1962) and Anderson and Matthews (1967) provided further evidence of fluid movement in dentine when applications of various hyperosmotic solutions of $CaCl_2$, sucrose and other materials to exposed dentine caused pain. They also demonstrated in vitro that these solutions produced fluid movement through dentine (Anderson et al, 1967).

The movement of various substances from the blood circulation into tooth dentine has long been known (Fish 1926,1927a,b, Bodecker and Lefkowitz 1937, Ross 1941, Lefkowitz 1943, Kreudenstein 1958a,b) as has the permeability of dentine to radioactive tracers (Wainwright and Lemoine 1951, Amler and Beverlander 1951, Barber and Massler 1964). However these studies were qualitative rather than quantitative. Problems exist in trying to quantitate permeability characteristics in extracted human teeth due to the complexity of diffusion geometry of even simple cavities. Total dentinal surface area is hard to quantitate and keep constant because of the variability of enamel thickness and uneven contour of the dentine-enamel junction. The floor of the cavity can be made flat, but biological variations in the pulp horn and pulp chamber morphology make dentine thickness highly variable (Outhwaite et al, 1976).

The calculation of permeability coefficients requires precise

knowledge of surface area and thickness. Outhwaite et al (1976) therefore developed a technique using discs of dentine with known dimensions. These discs were cut from extracted human molars. Over a series of experiments this group was able to determine many of the factors affecting the permeability of materials through dentine.

The first report showed that the permeability coefficient depended on how near the pulp chamber the disc was prepared. Reduction in dentine thickness from the enamel side of the discs resulted in a larger increase in permeability than reduction from the pulp side. Increasing dentine temperature by $10^{o}C$ almost doubled permeability to radioactive iodide while post-extraction time had little effect on dentine permeability in vitro (Outhwaite et al, 1976).

In 1977, Pashley et al studied the relative rates of permeation of dentine of four radioactive-labelled substances: tritiated water, urea, iodine and pertechnetate. They also studied the effects of the presence of pulpal tissue, dentine thickness and cavity varnish on the rate of permeation. The four substances all have small molecular or ionic radii and are water soluble. The water and urea are uncharged while iodine and pertechnetate are negatively-charged ions. Pashley et al (1977) found the relative rates of permeation (i.e. $^{3}H_{2}O$ > ^{131}I > ^{99m}Tc > ^{14}C-urea) followed the sequence of molecular dimensions of these substances. They concluded that size may be more important than charge in determining the rate of permeation. The presence or absence of pulp tissue had only a minor effect on the kinetics of permeation and as the dentine was made thinner, there was a resulting increase in the rate of iodide permeation. The use of

cavity varnish resulted in a mean reduction of iodide permeation of 69 percent.

Pashley and Livingston (1978) further investigated the effect of increasing molecular radius on the rate of permeation of human dentine discs. They tested 10 substances and found a 19-fold increase in molecular radius from 1.9 Å ($^{3}H_{2}O$) to 37 Å (^{131}I-albumin) resulted in a 100-fold decrease in permeability coefficients. Acid-etching the dentine produced a 4-fold increase in $^{3}H_{2}O$ permeability and a 9-fold increase in ^{131}I-albumin permeability. They concluded that dentine

Reeder et al (1978) reported that when dentine was acid etched, permeability increased 32-fold due to removal of surface debris occluding the tubules. They also described a method which permits measurement of the ease with which fluid permeates dentine. This value, the hydraulic conductance of dentine, increases as surface area increases and/or as dentine thickness decreases.

Pashley et al (1978b) analysed fluid movement through dentine in terms of 3 resistances placed in series:

a) surface resistance due to the presence of debris occluding dentinal tubules,

b) an intratubular resistance due to mineralised nodules and internal irregularities within the tubules, and

c) a pulpal resistance due to the presence of odontoblast processes and cell bodies within the tubules. Surface resistance accounted for 86 percent of the total resistance.

Another report by Pashley et al (1978a) calculated from the data of Garberoglio and Brännström (1976) that the fractional tubular

surface area varies from 1 percent of the dentine surface at the dentino-enamel junction to 22 percent at the pulp surface. This explains the results obtained by Outhwaite et al (1976) when they reduced the dentine thickness from either side and found a larger increase in permeability when reduced from the enamel side.

Pashley et al (1978a) examined the effects of changes in the nature of dentine surfaces on the permeability coefficients of $^{3}H_{2}O$ and ^{131}I-albumin. Diffusional surface areas were calculated from these data. The dentine surface was altered from a highly-polished surface to a bur-roughened surface, to an acid-etched surface and to an oxalate-treated surface. The diffusional surface areas for $^{3}H_{2}O$ and ^{131}I-albumin are listed in Table 2-3. The increase in tubular surface area available for diffusion after acid-etching could be reversed by treating the surface with oxalate. This same paper reported that another technique based on measuring the volume of water occupying the tubules gave a mean surface area of 10.2-10.5 percent.

The dentine disc in a split chamber method was used by Michelich et al (1980) to show that bacteria can grow through, or be filtered by pressure through acid-etched dentine. Unetched dentine, while permitting fluid filtration, restricted bacterial penetration. Penetration of dentine tubules by bacteria reduced the rate of fluid filtration across dentine.

In order to determine the relationship between results obtained by <u>in vitro</u> permeability studies to the <u>in vivo</u> permeability characteristics, Pashley et al (1981) compared the <u>in vivo</u> and <u>in vitro</u> dog dentine permeability rates of ^{131}I. They demonstrated the rapidity of the systemic appearance of substances placed on intact

dentine. A comparison of the rate of permeation of ^{131}I made *in vivo* and *in vitro*, on the same teeth, indicated that the rates were very similar. The permeability increased as dentine thickness decreased and this indicated that the rate-limiting step was diffusion across the dentine and not the pulpal blood flow. The tracer was apparently removed from the pulp as rapidly as it appeared by the pulpal microcirculation *in vivo*.

Hume and Kenny (1981) used freshly-extracted human molar teeth to quantify the release of tritiated-triamcinolone from Ledermix cement. They demonstrated that triamcinolone will diffuse through dentine to the pulp when placed in a class 1 cavity. These authors compared the release of triamcinolone from small pellets of Ledermix cement placed into an aqueous medium with the release and diffusion through dentine. They concluded that the dentine provided only a minimal barrier to the diffusion of triamcinolone.

Pashley et al (1982) published a paper in which they concluded from their experiments that plasma, serum and whole (mixed) saliva are all capable of causing immediate reductions in dentine permeability. Individual plasma proteins and several different types of bacteria were also effective in reducing the permeability.

Hume (1984) described a technique for examining the *in vitro* diffusion of eugenol across dentine in freshly-extracted human molar teeth. He found that dentine acts as a barrier to eugenol release into the pulp and was able to quantify the eugenol concentration within different levels of the dentine. A definite concentration gradient occured through the dentine between the zinc oxide-eugenol material and the pulp. Further *in vivo* studies have shown similar concentrations of eugenol in samples of carious dentine collected

from teeth *in vivo* following treatment with zinc oxide-eugenol materials (Hume 1985, personal communication).

A report by Potts et al (1985) described an *in vivo* technique for measuring the movement of radioactive molecules across dentine in the dog. They reported that the movement of water depended on dentine thickness and that concentrations of alpha-aminoisobutyric acid were lower in dentinal fluid than in blood plasma. They stated that this was a reflection of its lower rate of diffusion compared to water and this was due to its higher molecular weight, as discussed by Merchant et al (1977) and Pashley and Livingston (1978).

The above studies demonstrate that molecules will diffuse through dentine. The rate of diffusion depends on a number of factors including: molecular size, the surface area available for diffusion, the patency of the dentinal tubules, the nature of the dentine surface, the thickness of the remaining dentine, the proximity of the surface to the pulp, and temperature. The presence of pulp tissue did not affect diffusion and the *in vitro* results were very similar to the *in vivo* dog experiments. Post-extraction time did not alter permeability.

CHAPTER 3

AIMS AND OBJECTIVES OF THE PRESENT STUDY

The aims of this study were to determine the *in vitro* release characteristics of the two active components of Ledermix paste, demeclocycline and triamcinolone, and, from this data, to obtain an indication of the probable availability of these components to the periodontal tissues *in vivo*.

Materials placed in the prepared root canal of a tooth can reach the periodontal tissues either by:

a) direct release through the apical foramen, or

b) diffusion through the dentinal tubules and any lateral canals that may be present.

This study was undertaken to examine these two release routes and the variations in their rates of release with time. Variations in the following conditions may also affect the rates of release of the two active components of Ledermix paste and therefore were examined:

a) Temperature

b) Age

c) Sex

d) Diameter of the apical foramen

e) Volume of paste placed in the canal

f) Different irrigants used during canal preparation

g) Presence or absence of cementum

h) Apical foramen sealed or unsealed

i) Ledermix paste mixed with a calcium hydroxide/methyl cellulose paste

j) Different surface area of dentine available for diffusion.

This study was also undertaken to determine the concentrations of the two components within different levels of the root dentine, both to learn more about the general characteristics of diffusion in root dentine and to gain an insight into the probable efficacy of the antibiotic component of Ledermix paste within the tissue.

Ledermix paste has also been recommended by the manufacturer for use as a temporary lining material in coronal cavities when pulpal inflammation is present. Therefore, the diffusion of the components of Ledermix paste through the coronal dentine from a Class 1 cavity into the pulp was also examined.

CHAPTER 4

MATERIALS AND METHODS

4.1 INTRODUCTION

The study was divided into 12 major sections. These will be described in turn. Appendix 1 lists the abbreviations used throughout this research report and Appendix 2 lists all the materials used and chemical formulae, where applicable.

Preliminary studies were undertaken to test methods of approach. Pilot experiments showed that spectrophotometric analysis could be used to determine the amount of demeclocycline present in an aqueous solution, however the spectrophotometer could not be used to quantify the amount of demeclocycline present within samples of dentine shavings because of similar absorption peaks from dentine components. Therefore tritiated-tetracycline (a molecule of similar size and, presumably, similar chemical reactivity to demeclocycline; tritiated-demeclocycline is not commercially available) was added to Ledermix paste and used in those sections of the work involving dentine samples.

Pilot experiments also indicated that release of triamcinolone could be quantified by adding a known amount of tritiated-triamcinolone to the Ledermix paste prior to insertion in the root

canal and using liquid scintillation spectrometry to count the radioactivity of the aqueous samples.

The first stage of the study was therefore to determine standard curves to convert the optical density or counts per minute readings to the number of nanomoles of the drug present in solution. Statistical analysis of the results obtained was carried out using the F-distribution and the student's t-test. The F-distribution was used to compare the variance between the samples and the student's t-test was used to determine whether there were any significant differences between the means of the samples at a significance level of 0.05 (Carlson 1973, Chao 1974).

4.2 SPECTROPHOTOMETRIC ANALYSIS OF DEMECLOCYCLINE

Demeclocycline HCl was purchased in powder form. This was used to prepare standard aqueous solutions of demeclocycline at the following molar concentrations: 10^{-7}M, 3 x 10^{-7}M, 10^{-6}M, 3 x 10^{-6}M, 10^{-5}M, 3 x 10^{-5}M and 10^{-4}M. 2.5 millilitres (mls) of the 10^{-4}M solution was placed into a cuvette in the spectrophotometer (see Appendix 2) and 2.5mls of distilled/deionised water placed in the control chamber cuvette. The spectrophotometer was used in the "Scan mode" to test all wavelengths of light from 270 Nanometres (NM) to 900 NM in order to find the wavelength at which peak absorbance of light by demeclocycline occurred. The wavelength thus obtained was 365.5 NM and this was subsequently used for all experiments. Subsequent readings were performed in the "Time Drive/Manual mode" of the spectrophotometer and three readings were obtained for each

sample. The mean of these three readings was calculated and used to determine the concentration present.

In order to quantify the presence of demeclocycline in solution, 2.5ml samples of each of the above standard solutions were tested to obtain an optical density (OD) reading. Mean OD's were used and a direct correlation was obtained between the logarithmic values of the concentrations and the OD's which enabled a formula to be devised to calculate the number of nanomoles of demeclocycline present in 2.5mls of solution from the OD reading. This formula was:

$$\log_{10}[\text{No.of nanomoles of demeclocycline in 2.5mls}] = \log_{10}[\text{OD}] + 2.22894.$$

(Note: all $\log_{10}$ conversions are hereafter expressed simply as log; i.e., only $\log_{10}$ was used.)

4.3 LIQUID SCINTILLATION SPECTROMETRIC ANALYSIS OF TRITIATED-TRIAMCINOLONE

Tritiated (^{3}H)-triamcinolone acetonide (see Appendix 2) was purchased with a radioactive concentration of 1 milliCurie/millilitre (1mCi/ml) and a specific activity of 46 mCi/mg. A total of 0.05434 gm of ^{3}H-triamcinolone acetonide was present in the package.

Ledermix paste consists of 1 percent, by weight, triamcinolone. Therefore, in order to make a paste of 0.01 percent ^{3}H-triamcinolone, the 0.05434 gm of ^{3}H-triamcinolone was added to 5.434 gm of Ledermix paste to give a total triamcinolone content of 1.01 percent. Samples of this labelled-Ledermix paste were then weighed in scintillation vials and 5mls of scintillation cocktail (see Appendix 2) added. A

Packard Tri-Carb Liquid Scintillation Spectrometer was used to determine the radioactivity in counts per minute (CPM). The relationship between the CPM and the total number of nanomoles of triamcinolone present in a 2.5ml solution was calculated and a conversion formula established. This formula was:

[No. of nanomoles of triamcinolone in 2.5mls]

$= (CPM \times 10^3) \times 2.6368$

This process had to be repeated when a second batch of ^{3}H-triamcinolone was purchased. At the same time, the Department of Dentistry purchased a new scintillation counter, a Beckman LS2800 System. This new machine was used for all experiments involving the second batch of ^{3}H-triamcinolone. A second formula was calculated for this combination:

[No. of nanomoles of triamcinolone in 2.5mls]

$= (CPM \times 10^3) \times 2.9267$

4.4 LIQUID-SCINTILLATION SPECTROMETRIC ANALYSIS OF TRITIATED-TETRACYCLINE

Tritiated (^{3}H)-tetracycline was purchased from New England Nuclear with a specific activity of 679.0mCi/mmol and a concentration of 0.25mCi in 0.16mg of tetracycline. The ^{3}H-tetracycline was dissolved in 200 microlitres of methanol and then added to 5mg of Ledermix paste to give a concentration of 0.0032 percent, by weight, of ^{3}H-tetracycline, which is the equivalent of 1/1000 the concentration of demeclocycline normally present in Ledermix paste. Standard solutions were prepared and the CPM determined using

the Beckman counter as for ^{3}H-triamcinolone. A conversion formula was calculated:

[No. of nanomoles tetracycline in 2.5mls]

$= (CPM \times 10^{3}) \times (8.681 \times 10^{-4})$.

4.5 PLASTIC ROOT CANAL MODEL EXPERIMENTS

In order to study the release characteristics of the components of Ledermix paste through the apical foramen, a plastic root canal analogue model was developed. This model consisted of plastic pipette tips which were hollow cone-shaped tubes. The apical foramen diameter and the tube length could be altered to study the effect of these variables. The effect of temperature was also examined in this section.

The tubes were initially measured using a micrometer (see Appendix 2) under 10x magnification to determine the tip diameter, base diameter and tube length. The volume was then calculated using the formula:

$$\text{Volume of Frustum of Cone} = \frac{\pi h}{3}(R^2+r^2+Rr),$$

where R = base radius, r = tip radius and h = tube length.

Three tip diameters were used (0.88mm, 1.40mm and 2.00mm) in conjunction with 3 different lengths, that enabled the tubes to be filled with paste volumes of: 0.026ml, 0.054ml and 0.085ml.

The experiment was divided into 9 parts according to the tip diameter and paste volume combination. Ten samples of each diameter/volume combination were tested and a mean result obtained.

The tubes were filled with Ledermix paste containing ^{3}H-triamcinolone. The wide end of each tube was sealed with yellow sticky wax and the tube waxed to the lid of a glass vial so that the apical tip was submerged in 2.5mls of phosphate-buffered saline (pbs), pH 7.4 (see Fig. 4-1). The vials of pbs had been incubated overnight at 37°C in all cases except the room temperature experiment.

At specified times, the tubes were gently removed from the vial and placed into a fresh solution of 2.5ml pbs. The times were: 0.3 min, 1.0 min, 4.0 mins, 14 mins, 44 mins, 2.5 hrs (150 mins), 8 hrs (480 mins), 1 day (1,440 mins), 3 days (4,320 mins), 10 days (14,400 mins), 33 days (47,520 mins), 14 weeks (141,120 mins).

The samples were analysed in the spectrophotometer as described above. A 1ml aliquot of the sample was then placed in a scintillation vial and 5mls of scintillation cocktail added. After shaking regularly over a 10 minute period, the CPM were determined by liquid scintillation spectrometry.

The OD and CPM were converted to the number of nanomoles of demeclocycline and triamcinolone, respectively, released from the paste into the 2.5ml solution of pbs. The rate of release was then calculated by dividing the number of nanomoles by the number of minutes in the time interval.

One experiment was done at room temperature and the remainder at 37°C.

4.6 DIFFUSION OF THE COMPONENTS ACROSS DENTINE

Freshly-extracted human teeth were obtained from the Oral Surgery Department of the Adelaide Dental Hospital for these experiments. The teeth were all extracted under general anaesthesia and placed in vials containing pbs, pH 7.4. The vials were stored at 4^{o}C until required. The patients' age and sex were noted.

Teeth with a single root and a single root canal were selected and the crowns separated from the roots just below the cemento-enamel junction with a high speed Jet #330 bur. This same bur was used to cut an access cavity to expose the root canal.

Pulp tissue was removed with a barbed broach and the canal instrumented with a size 15 Hedström file until the tip of the file was seen at the apical foramen. The canal working length was calculated to be 0.5mm less than the length obtained with the initial file. Hedström files were then used sequentially to clean and shape each canal until a size 45 file had reached the working length. The canal was flared during the preparation phases.

The canals were irrigated copiously with a 15 percent solution of E.D.T.A.C. (Von der Fehr and Nygaard-Östby, 1963) prior to filing and in between each file size from size 15 to size 35. Milton's solution, containing 1 percent sodium hypochlorite (NaOCl) was used after file sizes 35 and 40. E.D.T.A.C. solution was re-introduced into the canal for the final file (size 45) and was also used as a final flush.

Following completion of the canal preparation, a low speed bur (1/2 round) was used to cut a retrograde class 1 cavity at the apex

of the tooth and to provide a class 1 cavity at the coronal end of the root canal. The canal was then flushed with E.D.T.A.C. solution and dried with paper points. A light-cured composite resin material (VisarFil) [see Appendix 2] was used to seal the apical foramen and retrograde cavity prior to weighing the tooth using a Mettler balance. ^{3}H-triamcinolone-labelled Ledermix paste was gently spun into the root canal using a Neos spiral root filler in a low-speed handpiece and the tooth re-weighed. The weight of Ledermix paste placed in the canal was subsequently calculated.

The light-cured resin material was used to seal the coronal end of the root canal and the coronal root-face (see Fig. 4-2). The tooth was immediately suspended in 2.5mls of pbs with the coronal root end out of solution to avoid any leakage around the seal (see Fig. 4-3). The vial was sealed and incubated at 37^{o}C. A control tooth from each patient was prepared and sealed without any Ledermix paste. The pbs samples from the control were used as the standard solutions in the spectrophotometer to obtain a zero reading and as controls in the liquid scintillation counter to test for background counts.

At specified times the tooth was placed into a fresh sample of pbs. The following times were used: 1 hour (60 mins), 3 hours (120 mins), 8 hours (480 mins), 1 day (1440 mins), 3 days (4320 mins), 10 days (14,400 mins), 31 days (44,640 mins), 14 weeks (141,120 mins).

The pbs samples collected were analysed using the spectrophotometer to obtain an OD reading and 1ml aliquots were mixed with the scintillation cocktail and analysed by liquid scintillation spectrometry to obtain the CPM reading. The rates of release over each time interval were then calculated.

At the end of the experimental period, each tooth was cut in a

horizontal plane from both the apical and coronal ends until the interfaces between the composite resin and Ledermix materials were reached. The micrometer was used to examine the cross-sections and obtain measurements. At the apical end, the canal formed a circular cross-section whilst the coronal end formed an elliptical cross-section. Four measurements were therefore made and designated as:

r= radius of apical cross-section

a= semi-major axis length of coronal cross-section

b= semi-minor axis length of coronal cross-section

h= height (length) of root available for dentine diffusion

Assuming that a prepared root canal forms a regular frustum of an elliptoid cone, the following formula was used to calculate the lateral area (M) of the root canal:

Lateral area = 1/2 (sum of perimeters of bases) x (slant height)

i.e. $$M = \pi\left[\tfrac{3}{4}(a+b) - \tfrac{1}{2}\sqrt{ab} + r\right] \times \sqrt{\left[h^2 + (a-r)^2\right]}$$

(from Lange 1944, Pearson 1974).

This figure is an indication of the surface area of dentine available on the walls of the prepared root canal for diffusion.

In this section, 10 teeth from a 30 year old male, 5 teeth from a 50 year old male and 5 teeth from a 39 year old female were used to determine the rates of diffusion through dentine and to examine any differences between age, sex and surface area available for diffusion.

4.7 EFFECT OF THE APICAL FORAMEN

Sets of matched pairs of teeth were obtained for this experiment. The teeth were prepared in the same manner as previously described except that every second tooth did not have a retrograde apical seal placed (see Fig. 4-4). The apical foramina of these teeth were kept patent to the tip of a size 15 Hedström file.

The experimental procedure followed the same outline and time schedule as described previously and the lateral areas were calculated at the end of the experiment. The rates of release of the components were calculated.

Two teeth from a 15 year old female, 6 from a 30 year old male, 4 from a 65 year old male and 8 from a 54 year old male were included in this experiment.

4.8 EFFECT OF CHANGING IRRIGATING SOLUTIONS

Five teeth from a 47 year old male and five from a 26 year old male were prepared as previously described except that the canals were irrigated with Savlon in aqueous solution (1 per cent) between each file and as a final flush. All apices were sealed by a retrograde light-cured composite resin restoration and the experiment proceeded as previously described.

4.9 EFFECT OF CEMENTUM ON DIFFUSION

Results obtained from the first group of experiments indicated that both demeclocycline and triamcinolone were able to penetrate the cemental layer. This section was undertaken to examine whether there was any difference in the diffusion rates when the cementum was removed prior to placement of the root canal dressing.

The root canals were prepared in the manner described previously using the E.D.T.A.C./Milton's regime of irrigation. Once the canals were prepared, the cementum was removed with a high speed tapered fissure bur (#169L) used with a shaving action. The external root surface was then treated with 15 percent E.D.T.A.C. solution for 15 minutes to remove smear layer created by the bur, from the surface and from the dentinal tubules. The apical foramina were sealed and the experiment proceeded as described previously. Five teeth from a 36 year old male and five teeth from a 43 year old female were used.

4.10 EFFECT OF MIXING LEDERMIX PASTE WITH PULPDENT PASTE

Ledermix paste has been combined with Pulpdent paste (a calcium hydroxide/methyl cellulose paste - see Appendix 2) and used as a root canal dressing. This experiment was undertaken to examine whether any changes in release and diffusion rates occurred as a result of the mixture.

Seven teeth from a 24 year old male were prepared using Hedström files and the E.D.T.A.C./Milton's irrigating regime. The

apical foramina were not sealed in this experiment. The teeth were weighed prior to placing any medicament and after the addition of each medicament. The two pastes were placed into the canal separately using a Neos spiral root filler, which effectively mixed the two pastes within the canal. In five teeth Ledermix paste was placed in the canal first and in the other two teeth, Pulpdent paste was placed first.

4.11 DETERMINATION OF CONCENTRATIONS WITHIN ROOT DENTINE

Nine teeth from a 21 year old male were used to determine the concentration of triamcinolone within the root dentine and nine teeth from another 21 year old male were used for the demeclocycline concentration. As previously mentioned, it was necessary to use ^{3}H-tetracycline to obtain data that could be extrapolated to indicate the concentrations of demeclocycline within the root dentine.

All teeth were prepared and weighed as previously described. Hedström files and E.D.T.A.C. and Milton's solutions were used and the apices were sealed. Nine teeth from one patient were filled with Ledermix paste containing ^{3}H-triamcinolone and another 9 teeth filled with Ledermix paste containing ^{3}H-tetracycline. Following re-weighing and sealing of the coronal access cavity, the teeth were each submerged in 2.5mls of pbs and incubated at 37^{o}C for 1 week.

At the end of one week, the bathing solutions were analysed by liquid scintillation spectrometry to determine the concentration of either ^{3}H-triamcinolone and ^{3}H-tetracycline. These data were compared

to those obtained from previous experiments and were found to be similar.

The teeth were sectioned longitudinally by using a high speed tungsten carbide tapered fissure bur (#169L) to expose the root canal. All traces of Ledermix paste were flushed from the canal with pbs solution. The distance from the root canal to the external root surface was measured. Dentine was removed from the external surface by cutting with a slowly-rotating No.6 round bur until approximately one-third of the thickness of the root was removed. The distance from the root canal to this point was measured and a further sample of dentine obtained from the middle one-third of the root thickness. Similarly, a sample of dentine from the pulpal one-third was obtained. This procedure was performed at the mid-root level and at the apical one-third root level to examine the difference in diffusion rates (see Fig. 4-5).

The dentine powder samples were collected and weighed without drying, then mixed with 2.5mls of pbs solution. They were left standing overnight at 37^{o}C in a sealed vial. The next day, 1ml aliquots were taken and mixed with 5mls of scintillation cocktail and analysed by liquid scintillation spectrometry. The amounts of ^{3}H-triamcinolone and ^{3}H-tetracycline were thus determined and expressed as nanomoles of triamcinolone and demeclocycline respectively per milligram of dentine. These figures were then converted to micrograms/ milligram of dentine.

Several tooth roots were separated from their crowns with a high speed bur. The roots were weighed without drying and placed in an incubator at 37^{o}C. After 5 days drying, they were re-weighed. The water lost was found to be approximately 10 percent by weight. This

figure is in agreement with those of Garberoglio and Brännström (1976) and Pashley et al (1978a).

The concentrations of triamcinolone and demeclocycline within the dentine were then expressed as micrograms/millilitre of dentine fluid, on the assumptions that 10 percent of the dentine is made up of fluid contained within the dentinal tubules and that it is this fluid which contains the molecules as they diffuse through dentine.

4.12 DIFFUSION THROUGH CORONAL DENTINE

The method of Hume (1984) was used to measure the diffusion of the components of Ledermix paste through coronal dentine. ^{3}H-triamcinolone and ^{3}H-tetracycline labelled-Ledermix paste were used for these experiments.

Freshly-extracted, intact, human third molar teeth were removed from patients under general anaesthesia due to impaction. They were stored in pbs solution at $4^{o}C$ until required. A large occlusal cavity was cut in each tooth using a tungsten carbide Jet #330 bur at high speed with water spray. The roots were removed by horizontal section below the cemento-enamel junction and the mean thickness (over 10 points) of the dentine between pulp chamber roof and the cavity floor was determined. The mean thickness for all teeth used in this section was 1.81mm. A hemi-cylindrical plastic chamber was then waxed to the pulp side of each crown (see Figure 4-6). Pbs solution (2.5mls) was added to the chamber to bathe the dentine surface of the pulp chamber roof. The cavity was dried with cotton pellets and filled with the labelled-Ledermix paste. The crown and cavity were sealed to the

plastic chamber by a layer of wax. The specimens were then incubated at 37°C.

At designated times (see below), the pbs bathing solution was drained from the plastic chamber and replaced with fresh pbs solution. A 1ml aliquot was taken from the collected sample and placed into 5mls of scintillation cocktail and analysed by liquid scintillation spectrometry. Calculations were made to convert the CPM into the number of nanomoles of triamcinolone or demeclocycline that reached the pulp chamber and the rates of diffusion were determined. The sample times used in these experiments were: 1 hour, 2 hours, 4 hours, 8 hours, 1 day, 2 days, 4 days and 8 days.

Tritium-labelled triamcinolone was added to Ledermix paste (as previously described) and used in 10 teeth: 2 from a 25 year old female, 1 from a 17 year old male, 2 from a 17 year old female, 4 from a 24 year old male and 1 from a 28 year old female. One tooth from a 17 year old male was used as a control and had no materials placed into the occlusal cavity.

Tritium-labelled tetracycline was added to Ledermix paste (as previously described) and used in 10 teeth: 3 from a 23 year old female, 3 from a 17 year old male and 4 from an 18 year old male. One tooth from a 28 year old female was used as a control, with no materials placed in the occlusal cavity.

4.13 DETERMINATION OF CONCENTRATION WITHIN CORONAL DENTINE

Freshly-extracted human third molar teeth were used and the crowns prepared as previosly described (see section 4.12). The occlusal cavities were filled with labelled-Ledermix paste and sealed with wax. The specimens were incubated at 37°C for either 2 days or 8 days.

At the end of this period, the crowns were removed from the chambers and powdered dentine samples were prepared by cutting with a slowly-rotating No.6 round bur from the pulp chamber surface. The samples were collected from 3 levels within the dentine (see Fig. 4-7). They were weighed without drying, mixed with 2.5mls of pbs solution and left overnight at 37°C in sealed vials. The following day, 1ml aliquots were taken and added to 5mls of scintillation cocktail and analysed with liquid scintillation spectrometry. The concentrations of triamcinolone and demeclocycline within the coronal dentine at 2 days and 8 days were calculated in the same manner as for the root dentine experiments and expressed as micrograms/ millilitre.

Ledermix paste containing ^{3}H-triamcinolone was used in 2 teeth from an 18 year old male and 1 tooth from a 23 year old female over a 2 day interval. Five teeth from the previous experiment (see section 4.12) were used for the 8 day samples. These included: 2 teeth from a 25 year old female, 2 teeth from a 17 year old male and 1 tooth from a 17 year old female. The 17 year old male tooth used as a control for section 4.12 was also used as a control in this experiment.

Ledermix paste containing ^{3}H-tetracycline was used to determine the concentration of demeclocycline in the coronal dentine of one tooth from each of a 36 year old female, a 31 year old female and a 24 year old female after 2 days. Five teeth from the previous experiment were used for the 8 day samples. These included 3 teeth from a 23 year old female and two from a 27 year old male. The control tooth from a 28 year old male (see section 4.12) was also used as a control for this experiment.

CHAPTER 5

RESULTS

5.1 SPECTROPHOTOMETRIC ANALYSIS OF DEMECLOCYCLINE

A full scan of a 10^{-4} M solution of demeclocycline showed that peak absorbance occurred at a wavelength of 365.5 nanometres (NM) - see Figure 5-1. Table 5-1 and Figure 5-2 illustrate the relationship between the log(concentration demeclocycline) and log(optical density) at 365.5 NM. The logarithmic graph was linear, demonstrating an exponential relationship. The gradient of the line was 1.00522, the y intercept 4.169 and the correlation coefficient was 0.9999. These figures were then used to obtain the formula:

$$\log [x] = \log [y] + 2.22894$$

where x = No. of nanomoles of demeclocycline in 2.5mls of solution and y = Optical Density (OD) reading obtained. This formula was subsequently used to convert the OD reading for each sample to the number of nanomoles of demeclocycline present in the 2.5ml sample.

5.2 LIQUID SCINTILLATION SPECTROMETRIC ANALYSIS OF TRITIATED-TRIAMCINOLONE

Table 5-2 and Figure 5-3 show the relationship between the total number of nanomoles of triamcinolone plus ^{3}H-triamcinolone and the CPM obtained with the first batch of ^{3}H-triamcinolone and the old scintillation counter. The graph shows a linear relationship with a gradient of 0.9481, y intercept 4.1213 and a correlation coefficient of 0.9481. This data was used to formulate the following conversion equation (assuming that the intercept was zero):

$$a = (CPM \times 10^{3}) \times 2.6368$$

where a = total number of nanomoles of triamcinolone in 2.5ml of solution.

Table 5-3 and Figure 5-4 show the relationship obtained with the second batch of ^{3}H-triamcinolone and the new scintillation counter. The gradient obtained was 0.8542, the intercept was 6.3455 and the correlation coefficient was 0.9830. The conversion formula obtained was:

$$a = (CPM \times 10^{3}) \times 2.9267.$$

These formulae were subsequently used to convert the CPM readings for each sample to the total number of nanomoles of triamcinolone in each 2.5ml sample.

5.3 LIQUID SCINTILLATION SPECTROMETRIC ANALYSIS OF TRITIATED-TETRACYCLINE

Table 5-4 and Figure 5-5 illustrate the relationship between the number of nanomoles of ^{3}H-tetracycline and the CPM indicating a linear relationship. The gradient of this line was 2879.74, the intercept was 2.688 and the correlation coefficient was 0.9949. A conversion formula was obtained in the same manner as above:

$$a = (CPM \times 10^{3}) \times (8.681 \times 10^{-4})$$

to convert the CPM into the total number of nanomoles of ^{3}H-tetracycline (a) present in 2.5ml of solution.

5.4 PLASTIC ROOT CANAL MODEL EXPERIMENTS

The initial trial at room temperature ($22^{o}C$) showed a significantly slower rate of release of demeclocycline from the plastic tubes than at body temperature ($37^{o}C$) - see Table 5-5 and Figure 5-6. All subsequent experiments were therefore carried out at $37^{o}C$.

Nine sets of experiments were conducted using combinations of three tip diameters and three volumes. Ten samples were tested in each group and the mean rates of release of the two components were calculated at each time interval.

Tables 5-6 to 5-11 and Figures 5-7 to 5-12 show the relationships between the rates of release of demeclocycline via the apical foramen, the diameter of the apical foramen (i.e., the surface area of paste exposed to the bathing solution) and the volume of

paste in the model canal. The tables list the significance of the differences between the mean rates of release of the drug for each time interval at the 0.05 percent level of significance.

Tables 5-12 to 5-17 and Figures 5-13 to 5-18 demonstrate the same relationships for the release of triamcinolone via the apical foramen.

These results show that the size of the apical foramen was, in general, a significant factor determining the rates of release of both demeclocycline and triamcinolone. The volume of paste and consequently the number of nanomoles of each drug placed in the model canal did not appear to be as significant in determining the rates of release.

Table 5-18 lists the mean percent cumulative release per time interval for demeclocycline and triamcinolone.

5.5 DIFFUSION OF THE COMPONENTS ACROSS DENTINE

The mean rates of release of demeclocycline and triamcinolone across the root dentine of ten teeth from a 30 year old male were calculated and compared to those of five teeth from a 39 year old female and five teeth from a 50 year old male - see Tables 5-19 and 5-20 and Figures 5-19 and 5-20. There were no significant differences between the mean lateral internal surface areas of these three groups of teeth. Similarly, there were no significant differences between the mean numbers of nanomoles of each component placed in the root canals of these three groups.

In general, the results show that there was no significant difference in the rates of release of the two components based on age or sex of the patient. The exceptions were the first three samples obtained (i.e. at 1,3 and 8 hours) for demeclocycline when comparing the 30 y.o. male to both the 50 y.o. male and the 39 y.o. female. Triamcinolone had only one exception - sample 2 (3 hours) for the 30 y.o. male compared to the 39 y.o. female.

A comparison of the mean rates of release of demeclocycline and triamcinolone through teeth on the basis of lateral internal surface area of the canal walls was made. The mean release rates for teeth with lateral internal surface areas between 20.0mm^2 and 30.0mm^2 (mean 26.3mm^2) were calculated and compared to those of teeth with lateral internal surface areas between 40.0mm^2 and 50.0mm^2 (mean 45.6mm^2) and 60.0mm^2 to 70.0mm^2 (mean 63.4mm^2). The student t-test showed a significant difference between these means but not between the mean numbers of nanomoles of demeclocycline or triamcinolone placed. Table 5-21 and Figure 5-21 show that the increase in area of dentine available for release of demeclocycline did not have a significant effect on the rate of its total release.

Table 5-22 and Figure 5-22 compare the mean rates of release of triamcinolone based on lateral area. These figures indicate in general, that triamcinolone release was somewhat more dependent on the available surface area of dentine, however the dependence was not consistent.

5.6 EFFECT OF THE APICAL FORAMEN

The total number of nanomoles of demeclocycline and triamcinolone collected in each sample was calculated and a mean rate of release of each drug was determined for those teeth with an open apex (i.e. release via the apical foramen plus via dentine diffusion). Similarly the mean rates of release for teeth with sealed apices were calculated. Tables 5-23 and 5-24 and Figures 5-23 and 5-24 illustrate the relationship between these two groups.

There were no significant differences between either the mean lateral areas or the mean numbers of nanomoles of demeclocycline and triamcinolone in the two groups of teeth used in this experiment.

In general, the rates of release were slightly faster when the apices were left open, however, the differences between the mean rates of release of demeclocycline were not significant at any of the time intervals used. Only the first two time intervals showed a significant difference between the mean rates of release of triamcinolone.

It therefore appeared that the major route of supply of these drugs to the periodontal membrane is via diffusion through the dentinal tubules and cementum. This observation was confirmed by Tables 5-25 and 5-26 and Figures 5-25 and 5-26 which compare the mean percent cumulative release per time interval. The mean cumulative amount released via the apical foramen can be calculated by subtracting the figures for a "sealed apex" from those for an "open apex". These figures are similar, although smaller, to those obtained

for release via the apical foramen of the plastic tubes (see Table 5-18). The higher figure obtained with the plastic root canal experiments can be attributed to the larger apical foramina used in the plastic models.

There was no significant difference between the mean percent cumulative release for demeclocycline when teeth with open apices were compared to teeth with sealed apices. Triamcinolone, however, had significantly higher mean percent cumulative release at all time intervals under the same experimental conditions.

5.7 EFFECT OF CHANGING IRRIGATING SOLUTIONS

The mean rates of release across dentine for teeth treated with the E.D.T.A.C./Milton's irrigating regime are compared to those irrigated with Savlon in Tables 5-27 and 5-28 and Figures 5-27 and 5-28. The teeth treated with E.D.T.A.C./Milton's had a smaller mean lateral area (41.20mm^2) of dentine available for diffusion than did those treated with Savlon (48.73mm^2) however the difference was not significant. The average amount of demeclocycline (579.1 nanomoles) and triamcinolone (194.9 nanomoles) placed in the E.D.T.A.C./Milton's teeth was also less than in the Savlon teeth (694.8 and 257.7 nanomoles respectively). The difference between both of these means was significant at the 0.05 level.

Despite these variations, faster release rates were still recorded for the E.D.T.A.C./Milton's group. The differences between the mean rates of diffusion of demeclocycline were significant at all time intervals except the final one (14 weeks). Triamcinolone showed

a similar pattern but with the last two samples (4.5 weeks and 14 weeks) not being significantly different.

These results indicate that removal of the smear layer by using E.D.T.A.C. opens the dentinal tubules to allow molecules to diffuse across dentine faster. The effect of the smear layer appears to disappear with time and the relative release rates tend to equalise after approximately one to two months.

5.8 EFFECT OF CEMENTUM ON DIFFUSION

Removing the cementum from the root surfaces enabled both the demeclocycline and triamcinolone to diffuse through dentine at faster rates than if the cementum was not removed. These rates are compared in Tables 5-29 and 5-30 and in Figures 5-29 and 5-30. The differences between the mean rates of diffusion were significant at all time intervals for both demeclocycline and triamcinolone. There was no significant difference between the mean lateral areas of dentine and no significant difference in the mean numbers of nanomoles of demeclocycline or triamcinolone used in the two groups.

5.9 EFFECT OF MIXING LEDERMIX PASTE WITH PULPDENT PASTE

When an approximate 50:50 mix of Ledermix paste and Pulpdent paste was used within the root canals, the mean release rates of the components were generally slower than for teeth of a similar size

filled only with Ledermix. The apical foramina were left unsealed in this section and the differences between the mean lateral areas and the numbers of nanomoles of demeclocycline and triamcinolone were not significant.

Tables 5-31 and 5-32 and Figures 5-31 and 5-32 illustrate the relationship between these groups of teeth. The differences between the mean rates of release of demeclocycline were significant at all time intervals whereas, for triamcinolone, the differences were significant at only three intervals (i.e. 1 hour, 3 hours and 14 weeks).

Tables 5-33 and 5-34 and Figures 5-33 and 5-34 show the mean percent cumulative release of demeclocycline and triamcinolone respectively from a 50:50 mixture compared to that from Ledermix alone. The differences between the mean values were significant at all time intervals.

5.10 DETERMINATION OF CONCENTRATIONS WITHIN ROOT DENTINE

Tables 5-35 and 5-36 and Figures 5-35 and 5-36 show the calculated concentrations of demeclocycline and triamcinolone within the middle third and apical third root levels of the dentine after 7 days. Dentine samples were obtained for analysis from three points at approximately 0-0.5mm, 0.5-1.0mm and 1.0-1.5mm from the root canal. The demeclocycline concentrations were estimated by multiplying the ^{3}H-tetracycline concentrations by 1,000.

5.11 DIFFUSION THROUGH CORONAL DENTINE

The release rates of demeclocycline through coronal dentine were estimated from the data obtained for diffusion of ^{3}H-tetracycline (i.e. x 1000). These rates and the corresponding rates for triamcinolone are shown in Table 5-37 and Figure 5-37.

Dentine samples from the control teeth showed CPM equal to background counts when analysed in the liquid scintillation spectrometer.

5.12 DETERMINATION OF CONCENTRATIONS WITHIN CORONAL DENTINE

The samples were obtained at the following approximate distances from the pulp chamber: 0-0.5mm, 0.5-1.0mm and 1.0-1.8mm.

Tables 5-38 and 5-39 and Figures 5-38 and 5-39 list the concentrations of demeclocycline and triamcinolone at 2 days and 8 days.

CHAPTER 6

DISCUSSION

6.1 DETERMINATION OF CONCENTRATIONS OF COMPONENTS IN SOLUTION

Pilot experiments showed that the concentration of demeclocycline in an aqueous solution could be determined by using a spectrophotometer. Demeclocycline has peak absorbance at a wavelength of 365.5 NM. A standard curve was obtained and when the logarithmic values were used a straight line graph resulted. The correlation coefficient (0.9999) indicates a high degree of accuracy within the concentration range tested.

Further pilot experiments also indicated that the use of a spectrophotometer was not a reliable method for determining the amount of demeclocycline present within a sample of dentine powder. Spectrophotometric scans of dentine powder in pbs solution showed a high degree of variability in light absorbance, especially within the range of 330-390 NM. This variability was very probably due to the dentine components in solution. It was therefore decided to use a radioactive tracer for the sections of the study involving dentine samples. Since tritium-labelled demeclocycline was not available commercially, ^{3}H-tetracycline was chosen. Tetracycline has a similar

molecular shape and structure to demeclocycline. The molecular weight of tetracycline (444.4) is also similar to that of demeclocycline (464.9). According to reports by Pashley et al (1977) and Pashley and Livingston (1978), molecules of similar size should have similar diffusion characteristics. ^{3}H-tetracycline was added to Ledermix paste to give a concentration of 0.0032 percent by weight which was 1/1000 that of demeclocycline (3.21 percent) thus simplifying calculations.

Standard curves were established for ^{3}H-tetracycline and ^{3}H-triamcinolone concentrations and their respective CPM readings. Again, a high degree of correlation was found (0.9949, 0.9481 and 0.9830) for each set of standard solutions. A straight line graph resulted for each and enabled a direct conversion to the number of nanomoles of drug released and the rates of release. When devising the formulae, the total number of nanomoles (e.g.^{3}H-triamcinolone + triamcinolone) was used.

The statistical tests (F-distribution and student t-test) were chosen in order to: a) compare the variance between the two population means (F-distribution), and

b) determine whether the difference between the sample means was significant or not (student t-test) [Carlson 1973, Chao 1974]. In all comparisons of the variances, the value of the F ratio fell within the critical value at the 0.05 percent significance level for the appropriate degrees of freedom. This then enabled the student t-test to be used to make inferences about the differences between the means being compared. The 0.05 percent significance level was also chosen for the student t-test.

6.2 PLASTIC ROOT CANAL MODELS

Initial experiments were carried out at room temperature to test the model and the spectrophotometric analysis of demeclocycline. The results of these experiments were compared to those at 37°C. The differences between the mean values for demeclocycline release were significant at all time intervals. The rates of release at room temperature increased by between 1.8 and 100 times at 37°C. The greatest difference was noted in the first minute and then it steadily decreased to 1.8 times at 10 days and then increased to 10 times at 14 weeks.

As a general principle, increasing the temperature of a substance and its bathing solution will increase its solubility into the solution. Rate of diffusion is also temperature dependent. Outhwaite et al (1976) reported that a 10°C increase in the temperature of dentine almost doubled the permeability to radioactive iodide. Because of these considerations and the results noted, subsequent experiments were performed at 37°C.

The plastic root canal model was chosen for the initial part of this study in order to examine the probable release characteristics of the components of Ledermix paste through the apical foramen of teeth as distinct from release via dentinal tubules. It would appear from the present results that the rate of release via the apical foramen would be mainly dependent on its diameter. Although in the present experiments the rates of release did not increase in direct proportion with the increase in surface area of the tip, they did show that with a constant paste volume, increasing the tip diameter

can generally be expected to increase the rate of release. A more definite correlation might be obtained in the in vivo situation when fluid flow past the apex may increase the rates of release.

The true significance of the apical foramen diameter in the clinical situation is difficult to assess for a number of reasons. Generally, it is recommended not to pass root canal instruments through the apex in order to avoid irritation and trauma to the periapical tissues (Grossman, 1978) and to assist in creating an apical stop for the final root canal filling (Schilder, 1984). Other operators even advocate the formation of an apical dentine plug to seal the apical foramen (Oswald 1979, Oswald and Friedman 1980, Petersson et al 1982, Eldeeb et al 1984). An apical dentine plug may also be created unknowingly during canal preparation. The sizes of apical foramina vary greatly depending on the age of the tooth. In a mature tooth, the foramen can be expected to be within the range of 0.10mm to 0.3mm - producing areas of 0.03-0.28mm^2 which is at least one-third smaller than the smallest plastic canal model used.

Increasing the volume of Ledermix paste placed in the canal model and keeping the foramen diameter constant did not appear to significantly increase the rate of release via the apical foramen of either demeclocycline or triamcinolone. The experiment involving a 2.0mm foramen diameter had the greatest variation in rates of release between different volumes.

The effect of volume variation could be expected to be more significant when dentine diffusion of these substances is considered. In a natural root canal, the paste would have to fill the complete canal system and be in contact with all dentinal walls in order to gain the maximum diffusion. However, due to differences in root canal

morphology and root lengths it is very difficult to keep all other variables constant and vary the paste volume alone. This will be discussed further below.

6.3 DIFFUSION ACROSS DENTINE

Teeth used in this section of the study were collected from patients undergoing dental clearance procedures. They were collected and stored at 4°C in pbs solution and subsequently prepared in groups according to the experimental procedures outlined. For convenience and consistency, groups being compared were prepared and used at the same time. This occasionally resulted in teeth being stored up to several days prior to use. Outhwaite et al (1976) concluded from their studies that post-extraction time had no effect on dentine permeability over 3-4 weeks. They suggested that the dentinal tubules act as channels that remain relatively stable as a function of post-extraction time. It was therefore decided to store the teeth until sufficient numbers had been collected so that comparative groups could be tested under exactly the same conditions.

The results from this study demonstrated that age and sex did not significantly alter the diffusion of demeclocycline or triamcinolone across dentine. The number of tubules within the dentine of any tooth remains constant with increasing age. These tubules may become blocked by deposition of secondary dentine.

Carrigan et al (1984) claimed that the number of tubules did decrease with age. They used a scanning electron microscope to examine teeth in different age groups. However, the teeth used in

this study did not have any endodontic preparation of the canals prior to S.E.M. examination. Therefore, the presence of a layer of secondary dentine might have given an impression of less tubules. The deposition of secondary dentine is known to be a function of age of teeth (Nalbandian et al, 1960).

Endodontic preparation techniques such as those used in this study should remove any layers of secondary dentine or other diffuse calcifications which may be present on a canal wall, thus exposing the dentinal tubules to any medicaments or materials placed in the canal. The efficacy of instrumentation procedures in cleansing of the canal wall would be expected to be increased by the use of a chelating agent such as E.D.T.A.C. as an irrigant (von der Fehr and Nygaard-Östby, 1963).

The release rates for triamcinolone showed a general increase when the lateral area of the canal increased. The same effect was not as marked for demeclocycline. Increasing the surface area of dentine available for diffusion implies that more dentinal tubules are exposed to the medicament in the canal provided the medicament completely fills the canal and is in contact with the canal walls. The more significant increase in release rates for triamcinolone may be due to its smaller molecular weight (394.5) when compared to demeclocycline (464.9). As previously discussed, smaller molecules will have greater rates of diffusion than larger molecules (Pashley et al 1977, Pashley and Livingston 1978).

Tetracyclines have been reported to form complexes with bivalent and trivalent cations and to be deposited in teeth and bones during calcification (Walton and Thompson 1975, Meyers et al 1976, Cawson and Spector 1978, Montgomery, 1985). A recent study has

reported that tetracyclines form a relatively strong, reversible bond with the hard dental tissues and they exhibit a slow release over an extended period of time (Bjorvatn et al, 1985). It has not been reported whether this same reaction occurs with the endodontic use of any tetracycline drugs, however it is a likely reaction due to their high affinity for calcium ions. This may explain the less significant changes in release rates for demeclocycline under conditions that have caused a significant change in rates for triamcinolone, except that Ledermix paste consists of the calcium salt of demeclocycline (see Section 1.2). For the demeclocycline to bind with the tooth calcium, an exchange reaction must occur which would result in the release of a free calcium ion. As no studies have been reported on this reaction, a definite conclusion can not be reached.

In investigations of this nature the internal lateral surface area of a root canal can be increased in two ways, first by using a longer root and second by making the canal diameter larger at either end. It is difficult to determine whether the number of dentinal tubules exposed to the Ledermix paste increases every time the lateral surface area increases, since increasing the tooth length will increase the number of tubules available, but increasing the canal width will not. The present calculations were done on the assumption that the canals were prepared with a circular shape at the apex and an even taper with smooth walls to an elliptoid shape at the coronal end of the canal beneath the seal. The canals may not have strictly been this shape in all cases. Therefore, the figures calculated for internal lateral surface area can only be taken as an indication of the area of dentine available for diffusion and not an indication of the number of dentinal tubules exposed to the

medicament. This may account for the slightly inconsistent results obtained when comparing teeth with different lateral areas. It may be stated, however, that as a general principle increasing the surface area of dentine exposed to the medicament increased the total outflow of the components.

6.4 THE EFFECT OF THE APICAL FORAMEN

The results from the present study indicate that the major supply route for the components of Ledermix paste to the periodontal tissues is via the dentinal tubules. The mean percent cumulative release of demeclocycline from teeth with a sealed apex was not significantly different from teeth with an open apex. However the differences for triamcinolone were significant. This was very probably due to the Ledermix paste containing only $^1/_3$ as much triamcinolone as demeclocycline. The total release rates of these two drugs were similar, although demeclocycline was slightly faster at most time intervals (probably due to its higher concentration). The difference in their release rates was not of the same magnitude as the difference in concentrations (i.e. three times) and this was in agreement with the results obtained from the plastic canal model experiments on volume variations. Having a similar release rate implies that a similar amount of the material was being released per unit time thus leading to a higher percent cumulative release for triamcinolone.

Clinically, this information implies that the triamcinolone supply may be exhausted sooner than the demeclocycline. In the

current study, a mean cumulative release of 98 percent of the triamcinolone had occurred after 14 weeks when the apices were left open compared with 66 percent release of the demeclocycline. Based on this in vitro data, it would appear that long term dressings should be changed approximately every three months in order to maintain any therapeutic effects from the use of Ledermix paste as an endodontic dressing material.

6.5 THE EFFECT OF DIFFERENT IRRIGATING SOLUTIONS

Nygaard-Östby (1957) found a 15 percent solution of E.D.T.A. buffered to pH 7.3 with sodium hydroxide was effective in demineralising dentine. Its effect was self-limiting and it did not affect the periapical tissues. The mechanism of the antibacterial action of E.D.T.A.C. is to "starve" bacteria by chelating the metallic ions in their substrate (Cameron, 1984). Von der Fehr and Nygaard-Östby (1963) suggested adding 0.84gm of a quarternary ammonium bromide (Cetavlon or Cetrimide) to transform E.D.T.A. into E.D.T.A.C. Cetrimide reduces the surface tension (Goldberg and Abramovich, 1977) and fluid viscosity, thus enabling the chelating solution to be carried by endodontic instruments to the full negotiable length of the canal (Schilder and Yee, 1984).

Goldberg and Abramovich (1977) demonstrated that irrigation with E.D.T.A.C. solution resulted in the circumpulpal surface having a smooth texture and the dentinal tubules having a circular and irregular appearance. The diameters of the tubules were larger than in teeth not treated with E.D.T.A.C. Goldberg and Spielberg (1982)

showed that for maximum effect, E.D.T.A.C. should be in contact with the dentine for 15 minutes, although the effects were present to some extent after 5 minutes.

Sodium hypochlorite (NaOCl) has been used at concentrations of 5 percent (Grossman, 1943) and 1 percent (Schilder and Yee, 1984). Milton's solution contains 1 percent NaOCl. Essentially, a weak solution of NaOCl digests organic debris whilst having little effect on adjacent viable tissues and this is most desirable in endodontics (Schilder and Yee, 1984).

Heithersay (1984) reported that Savlon in aqueous solution (chlorhexidine gluconate [0.03 percent] and cetrimide [0.3 percent]) has been used as an endodontic irrigant since 1962. This solution has a strong antibacterial action and low toxicity, penetrates dentinal tubules and reduces surface tension which greatly assists drying of canals with paper points (Heithersay, 1984).

Goldman et al (1981) showed that the smear layer is primarily calcific in nature and is created by instrumentation. Only chelating agents such as E.D.T.A. have been shown to remove this layer (McComb and Smith 1975, McComb et al 1976, Goldberg and Abramovich 1977, Goldman et al 1981). Organic solvents such as NaOCl do not remove the smear layer (McComb and Smith 1975, Lester and Boyde 1977, Goldman et al 1981). No studies have been reported on the effect of Savlon on the smear layer, but since it does not contain any chelating agents it is not expected to remove the smear layer.

The combination of E.D.T.A.C. and Milton's in a manner such as that described in Section 4.6 allows for:

a) ease of preparation of the canals by the initial use of E.D.T.A.C. by chelation of calcium ions which helps to negotiate

narrow curved or calcified canals. It also aids in removal of dentinal debris and disinfection;

b) completion of disinfection and dissolution of organic debris by the use of NaOCl; and

c) removal of the smear layer and dentinal debris by the final use and flush of E.D.T.A.C. solution.

Removal of the smear layer leaves the dentinal tubules patent (Goldberg and Abramovich 1977, Goldberg and Spielberg 1982) and should thus increase the permeability of the tubules to intra-canal drugs (Hampson and Atkinson 1964, Stewart et al 1969, Cohen et al 1970).

The results of the present study give absolute confirmation of this probability. The rates of diffusion of demeclocycline and triamcinolone were significantly greater during the first 10 days in teeth where E.D.T.A.C. and Milton's were used as irrigants than in those teeth treated with Savlon. After 1 month, the diffusion of demeclocycline was still significantly faster in the former group, whereas for triamcinolone there was no significant difference. After 14 weeks the rates were not significantly different for either drug. These results were obtained despite the use (by chance) of a group of teeth with slightly smaller surface areas and which contained significantly less Ledermix paste than those irrigated with E.D.T.A.C. and Milton's.

The loss of apparent effect of smear layer removal after 1 month is probably due to the decreased amount of drug present in the canal after this time. It is also possible that in those teeth with an initially intact smear layer there may be some breakdown of the

layer with time due to the bathing action of fluid within the tubules, thus allowing a more equal rate of diffusion.

This study has confirmed that the presence of the smear layer does decrease the permeability of the dentine. An irrigation regime that removes this layer is therefore recommended to assist in canal preparation in order to obtain clean canal walls with maximum tubule exposure to the medicaments placed in the canal.

6.6 THE EFFECT OF CEMENTUM

The root surfaces of teeth may be denuded of cementum in several ways. Trauma can lead to several forms of external root resorption which all lead to loss of cementum. Surface resorption has been demonstrated as early as 1 week following replantation (Andreasen, 1980). This form of resorption generally repairs by the deposition of cementum after some weeks (Andreasen, 1985). Inflammatory root resorption has also been demonstrated as early as 1 week after replantation (Andreasen, 1980). This type of resorption is progressive if endodontic therapy is not instituted (Andreasen and Hjørting-Hansen, 1966a). The development of inflammatory resorption is dependent on at least four conditions. The first is injury to the periodontal ligament (e.g. luxation movements, removal of the periodontal ligament, drying of the root surface) leading to a resorption process similar to that in surface resorption (Andreasen, 1985). The second and third conditions for development of inflammatory resorption are that the initial resorption process exposes dentinal tubules and that these tubules communicate with

necrotic pulp tissue or leucocyte zone harbouring bacteria (Andreasen 1981a,1985). The final factor is age with inflammatory resorption being more frequent in immature and young mature teeth than in older mature teeth (Andreasen and Hjørting-Hansen, 1966a,b).

Replacement resorption (ankylosis) has been demonstrated histologically 2 weeks after replantation (Andreasen, 1980). Ankylosis represents a fusion of the alveolar bone with the root surface and in general will occur when the periodontal ligament is either removed before replantation (Andreasen, 1981b,c) or dried before replantation (Andreasen and Kristerson, 1981).

Other causes of root resorption have been reported and include orthodontic treatment (Philips 1955, De Shields 1969, Morse 1971, Sjölin and Zachrisson 1973), re-attachment procedures in periodontal therapy (Dragoo and Sullivan 1973, Adell 1974), bleaching of discoloured teeth (Harrington and Natkin 1979, Lado et al 1983, Cvek and Lindvall 1985), following conservative endodontic treatment (Strindberg 1956, Brynolf 1967, Seltzer et al 1967, Kerekes et al 1980) and following surgical endodontics (Andreasen and Rud 1972a,b, Rud et al 1972, Andreasen 1973).

The presence of bacteria in root canals has recently been related to root resorption (van Mullen et al 1980, Möller et al 1981, Pitt Ford 1982, Simon et al 1983) and specifically to inflammatory root resorption (Andreasen, 1981a). Andreasen (1985) stated that the cementum acted as an insulating layer against the penetration of bacterial products from the root canal and dentinal tubules into the periodontal ligament. If this barrier is removed experimentally, active inflammatory resorption develops in immature teeth, whereas in

mature teeth repair will take place with the formation of a new thin basophilic layer of cementum (Andreasen, 1973).

The present study confirms Andreasen's findings that the cementum acts in part as an insulating layer. However, the present results show that it is not a complete barrier to the movement of matter from a root canal to the periodontal tissues. This study has shown that the diffusion rates of demeclocycline and triamcinolone were significantly faster at all time intervals tested following mechanical removal of cementum and E.D.T.A.C. cleansing of the resultant dentine surface. While this procedure may result in a dentine surface different from that developed during external resorption, or that created by aggresive root planing, it is evident that the absence of cementum has a major effect on diffusion through the root. Therefore in teeth in which cementum has been lost, more of the root canal medicaments should reach the periodontal tissues per unit time thus increasing their therapeutic effects. Similarly if any toxic materials (bacteria, medicaments, etc) are present in a root canal, they will also reach the periodontal tissues at a greater rate and cause more damage to these tissues.

Removal or loss of cementum would also be expected to allow more diffusion of materials from the periodontal tissues into the pulp. This may lead to inflammation or necrosis of the pulp depending on the area of cementum lost, the initial status of the pulp and the materials available for diffusion (e.g. bacterial products from plaque, citric acid used in periodontal therapy, acidic foods).

6.7 LEDERMIX-PULPDENT COMBINATIONS

Heithersay (1977, 1984, 1985) has reported beneficial effects with the use of a 50:50 mixture of Ledermix paste and a calcium hydroxide [$Ca(OH)_2$] paste such as Pulpdent as a root canal dressing. The mixture can be used as a dressing after the second visit and/or following completion of the canal preparation procedures. In the treatment of extremely large periapical lesions a further application of the 50:50 mixture is as a long-term intra-canal dressing. The rationale for its use is that there is less sensitivity when a 50:50 mixture is used than when Pulpdent is used alone. Although there is considerable clinical evidence to support the use of a 50:50 mixture of Ledermix and Pulpdent there has been no research to date to support its use.

The current study compared the effects of mixed Ledermix/ Pulpdent pastes with Ledermix alone on the rates of release of the active components of Ledermix via both the apical foramen and the dentinal tubules. Demeclocycline release was significantly slower under the experimental conditions in the group of teeth containing the mixed pastes. The differences between the mean lateral areas of dentine and the number of nanomoles of demeclocycline placed in the two groups were not significant, indicating that the presence of the calcium hydroxide paste was the determining factor inhibiting the release of demeclocycline. This may be due to the affinity of demeclocycline for calcium ions as previously discussed. The saturation of the mixture with calcium ions from demeclocycline-

calcium, calcium hydroxide and the dentine calcium may create a situation conducive to interaction of the calcium ions and the demeclocycline. Although a similar total number of nanomoles of demeclocycline were present in the 50:50 mixture group as in the Ledermix only group, the concentration of demeclocycline in the canal was effectively halved, resulting in less of the demeclocycline molecules in contact with the dentinal walls and thus less available for diffusion through the tubules. However, this did not affect the release of triamcinolone from the mixture.

Although there was less demeclocycline reaching the periodontal tissues when Ledermix was mixed with Pulpdent, there was a higher concentration of demeclocycline maintained in the root canal itself simply because of the slower release. This higher residual concentration may be advantageous in the treatment of extremely large periapical lesions by the use of a long-term intra-canal dressing as advocated by Heithersay (1977, 1984, 1985) in that it would help to maintain sterility of the canal and the tooth.

The mean percent cumulative release of demeclocycline from the 50:50 mixture was significantly less than from Ledermix alone at all time intervals. These results suggest that less frequent changing of the dressing is required.

Triamcinolone reacted differently in the 50:50 mixtures. The differences in the rates of release were significantly slower at only the 1 hour, 3 hours and 14 weeks intervals. At the remainder of the intervals the differences between the mean rates of release from a 50:50 mixture were not significantly different from the mean rates of release from Ledermix alone. This variation is similar to that discussed earlier (Section 6.3). However, the mean percent cumulat-

ive release of triamcinolone was significantly slower from the 50:50 mixture at all time intervals tested. It can therefore be assumed that using a combined mixture will maintain the dressing's active components within the canal for a longer period of time than when Ledermix is used alone.

Under the conditions used in this experiment it was not possible to test whether there were any differences in pH changes throughout the dentine or periodontal tissues. The use of a phosphate-buffered saline (pbs) solution prevented the measurement of pH changes due to the buffering effect of the solution. Tronstad et al (1981) have studied the pH changes within the dental tissues following the use of calcium hydroxide in root canals. Further investigations are indicated to assess whether the 50:50 mixture creates any significant differences in the effect of the calcium hydroxide concentrations and pH changes within the tooth and surrounding tissues.

6.8 CONCENTRATIONS WITHIN ROOT DENTINE

Whittaker and Kneale (1979) studied the dentine-predentine junction of teeth and reported that there was a decreasing number of tubules from the crown of the tooth to the apex. They found no correlation between age and tubular diameter.

Carrigan et al (1984) found a decreased number of tubules as the location became more apical and the age increased. They also found that the number of tubules present in the coronal dentine and cervical and mid-root dentine were relatively similar (mean

251,287/mm^2). Their figure for the apical dentine was 49,140/mm^2. They attributed the lower figure for apical dentine to an increased formation of peritubular dentine in this region. They examined only the dentine-secondary dentine interface and not the peripheral dentine.

Since there are less tubules within the apical region of a tooth, the concentration of any root canal medicaments within this dentine would be expected to be lower than that of the cervical or mid-root dentine. The figures obtained in this study generally agreed with this for the dentine immediately adjacent to the root canal. Further peripherally the concentrations were similar to those in the mid-root dentine. The rate of movement of any molecule through a tubule is determined largely by the molecular size and tubule diameter. The rate is unlikely to be affected by the location in the root and therefore, once a molecule has entered the tubule, it will move steadily through to the peripheral areas. There are less tubules present in the apical dentine at the dentine-secondary dentine interface and these tubules will not diverge as much as in the mid-root dentine as they approach the cemento-dentinal junction. This is due to the root being thinner in the apical region. Most tooth roots are generally conical in shape and therefore the tubules will also be shorter near the apex.

In the present study the concentrations of demeclocycline and triamcinolone at the canal end of the tubules were lower in the apical dentine than in the mid-root dentine, as expected. The concentrations at the mid-tubule region and the peripheral region were similar.

No data has been published regarding the minimal inhibitory concentration of demeclocycline required for bacteria commonly found within root canals. However, assuming that demeclocycline has a similar range of activity as tetracycline, a concentration of 128 micrograms/ millilitre would be required to inhibit B. fragilis and B. oralis. A concentration of 64 micrograms/ml would be sufficient for inhibition of B. melaninogenicus, Peptococcus, Peptostreptococcus, Veillonella and Eubacterium. At 32 micrograms/ml, Streptococcus, Lactobacillus and Proprionibacterium would be inhibited while 16 micrograms/ml or less would be expected to inhibit other organisms present (see Table 2-2).

Ledermix paste containing 3.21 percent demeclocycline has a demeclocycline concentration of 50mg/ml. Therefore there is obviously sufficient demeclocycline present within a Ledermix paste-filled root canal to act as a bacteriostatic agent for the bacteria commonly found within the canal itself. Clinically, this may be of benefit when the canal has not been completely cleaned or prepared. However, normal endodontic preparation and irrigation should remove bacteria from the canal proper, although the presence of bacteria in canal ramifications and dentinal tubules is likely.

After one day the rate of release of demeclocycline was approximately 10 times the rate after one week. This difference can be used to estimate by extrapolation that the demeclocycline reached concentrations in the order of 200 micrograms/ml within the dentine adjacent to the root canal within the first day. By the end of one week the concentration had fallen to approximately 21 micrograms/ml. Using the same extrapolation, peak concentration peripherally would

be 17 micrograms/ml after one day, falling to 1.7 micrograms/ml after a week.

Hardnt (1963) and Shovelton (1964) reported that micro-organisms can be present within dentinal tubules of infected tooth roots. The efficacy of demeclocycline against these micro-organisms would depend on the type of organisms and whether they are close to the canal or well into the tubules near the cementum. If they survive the initial high concentrations of demeclocycline in the first few days, then the majority of commonly found endodontic bacteria would be able to survive the progressive decrease in demeclocycline concentrations.

There have been no reports to indicate what concentrations of any corticosteroid drugs are required in a "local" or topical situation in order to have a therapeutic effect. Therefore it is not possible to predict on the basis of the present experimental evidence whether or not there is a sufficient amount of triamcinolone reaching the periodontal tissues to influence biological processes. There is ample clinical evidence to support the claim that triamcinolone reduces pain and inflammation in periodontitic teeth (Schroeder 1962, 1965, Ehrmann 1964,1965,1972, Olsen 1966, Baume 1968, Schneider 1968, Laws 1969, Barker and Lockett 1971,1972, Erausquin 1972). The figures obtained in this section for the concentration of triamcinolone within the root dentine illustrate the concentration gradient established through the dentine and gives some insight into the diffusion characteristics of a medium-sized molecule through dentine without the effects of the calcium affinity that exists for demeclocycline. It is unlikely that any therapeutic effect is attained from the presence of triamcinolone within the dentine.

However the dentine acts as a slow release mechanism for triamcinolone to the periodontal tissues.

6.9 DIFFUSION AND CONCENTRATIONS IN CORONAL DENTINE

The manufacturers, Lederle Pharmaceuticals (1981), have recommended Ledermix paste as a direct and indirect pulp capping agent. They claim that the paste will reduce inflammation by the use of a corticosteroid and help control the spread of bacteria by the use of a broad spectrum antibiotic.

The use of Ledermix paste as a direct pulp capping agent exposes the medicament directly to the pulp. This would allow immediate release of the active components into the pulp tissue. De Deus and Han (1967) applied ^{3}H-cortisone to exposed hamster pulps and reported the presence of radioactive materials in the liver as early as 2 minutes after application. They were also detected in the submandibular lymph nodes after 5 minutes. Page et al (1973) applied ^{3}H-tetracycline to pulpotomised rat molar pulp stumps. They reported that radioactivity was detected in the general circulation within 10 minutes of application. This radioactivity was due to protein-bound, native, tritiated-antibiotic.

These studies illustrated the immediate release of the drugs from their vehicle and the rapid clearance from the pulp into the general circulation. This rapid clearance reduces the effective concentration available at the site of exposure and may reduce the efficacy of the medicament.

When used as an indirect pulp capping agent or as a lining material prior to restoration of a cavity, an intact layer of dentine is present between the Ledermix material and the pulp. The medicaments can only reach the pulp by diffusion through the dentinal tubules. This study measured the rates of diffusion through the coronal dentine by collecting and analysing a bathing solution at the pulpal surface.

In order to have some standardisation between teeth, the thickness of dentine between the cavity floor and pulp chamber was measured at ten points for each tooth. An attempt was made to keep the mean thicknesses equal (the mean thickness for all teeth was 1.81mm). By keeping the dentine thicknesses relatively similar, a similar number of dentinal tubules were available for diffusion of the components through the dentine. The cavity floors were not treated for removal of the smear layer created by the bur. From earlier results, the use of E.D.T.A.C. or an acid-etch solution would have removed this layer and allowed greater diffusion.This was not done, since in the clinical situation, if an operator was attempting to maintain pulp vitality, the application of an acid to dentine has been considered undesirable. In the light of recent studies by Hume and Wang (1985, personal communication), the application of an acidic solution to dentine is unlikely to harm the pulp due to the buffering effect of the dentine. E.D.T.A.C. is also unlikely to affect the pulp (Lindemann et al, 1985) and has recently been recommended as a cavity floor treatment prior to the use of some dentine bonding agents (e.g. Gluma).

In the present experiments both demeclocycline and triamcinolone reached peak diffusion rates after 2 hours. Demeclocycline

showed a progressive decrease after this time, whereas triamcinolone maintained its rate for the next 6 hours and then slowed progressively. When the concentrations of demeclocycline within the coronal dentine were calculated they showed that a concentration gradient exists across the dentine. Figures for the 2 hour concentrations can be extrapolated on the basis of the outflow data which showed that the rates of diffusion were ten times faster than at 2 days (see Table 5-37 and Figure 5-37). The high concentration of demeclocycline adjacent to the cavity floor (107 micrograms/ml) and at the mid-dentine level after 2 hours should inhibit any bacteria that would be present in this region. However the concentration directly adjacent to the pulp (4 micrograms/ml) is unlikely to inhibit many bacteria. The very small amount of demeclocycline reaching the pulp would be cleared very rapidly by the pulpal circulation (Page et al, 1973) and thus unlikely to have any anti-microbial effect within the pulp.

It can be estimated from the outflow data that the concentrations of triamcinolone within the coronal dentine would reach a peak at 2 hours and then be sustained for approximately 6 hours. Again, only minute amounts of the drug reach the pulp itself and would be cleared rapidly (De Deus and Han, 1967). Despite this rapid clearance, it appears that the agent has an effect clinically in reducing pulpal inflammation and pain. This phenomenon has been observed by many operators using corticosteroid-containing materials as either direct or indirect pulp capping agents (Schroeder and Triadan 1961,1962, Schroeder 1962,1963,1965,1968,1972, Vigg 1962, Ehrmann 1964,1965, Stanley et al 1965, Jokinen and Korte 1970, Clarke

1971, Shovelton et al 1971, Ulmansky et al 1971, Ivanov and Leibur 1974).

6.10 SYSTEMIC EFFECTS OF TRIAMCINOLONE

The maximum amount of Ledermix paste that was placed into the teeth used in this study was 19.2lmg. per root, i.e., a maximum amount of 0.192lmg of triamcinolone. If this amount of triamcinolone was placed into a molar tooth with four prepared root canals a total application of less than 0.8 mg occurs. Results from this study showed that approximately 30 percent (0.24 mg) of this triamcinolone would be released into the periodontal tissues during the first day. This figure is much less than the daily secretion of cortisol (20-30mg) and the secretion during "stress situations" (300-400mg/day) reported by Parnell (1964) and Walton and Thompson (1975). Although triamcinolone has been reported as being approximately 4 times more potent than cortisol (see Appendix 3, Table A.3-1)[Fauci et al, 1976], the amount present in a root canal system, even if it is all released simultaneously into the circulatory system, is unlikely to have any systemic effects.

Hume and Kenney (1981) estimated the maximum amount of steroid likely to be used clinically in Ledermix cement was 0.37mg, of which 0.26mg was released on the first day. They concluded that this amount was unlikely to cause any harmful local effects, let alone any systemic effects, especially when compared to endogenous steroid production.

Marshall and Walton (1984) showed that intramuscular injection of a steroid significantly reduced the incidence and severity of post-endodontic pain. The patient can be spared the extra trauma and possible side effects associated with the systemic administration of steroid drugs by the use of a paste such as Ledermix (i.e., by a topical application of a steroid to the area of inflammation). Moskow et al (1984) reported a significant decrease in the incidence of post-operative pain when a corticosteroid solution (dexamethasone) was used as the intracanal medicament.

It can be concluded, therefore, that the use of either Ledermix paste or cement is unlikely to cause any harmful systemic effects through the use of the corticosteroid, triamcinolone.

6.11 POSSIBLE ALTERNATIVES TO DEMECLOCYCLINE

As previously mentioned there have been no reports on the minimal inhibitory concentrations of demeclocycline necessary for the inhibition of bacteria commonly found in endodontic infections. In order to gain more accurate data on the efficacy of demeclocycline as an endodontic dressing, it would be desirable to investigate these MIC's.

Since the overall efficacy of demeclocycline was shown to be questionable (see Section 6.8), it is of interest to consider the possible alternatives for use within root canals during endodontic treatment.

Other antibiotics have been suggested for the systemic treatment of bacteria implicated in endodontic infections. These

include amoxycillin, erythromycin and metronidazole (Woods 1981, Von Konow 1981), metronidazole (Ingham et al 1977, Mitchell 1984), clindamycin (Ernest et al 1977, Miles 1984) and a combination of amoxycillin and metronidazole (Moule, 1982). An investigation of the efficacy of these antibiotics as root canal dressings would provide valuable information which may lead to the formulation of a new paste containing triamcinolone and an anti-microbial agent that is more specific for the bacteria involved and one that is more likely to achieve its MIC within the dentinal tubules and periodontal tissues. The diffusion patterns could be estimated from the molecular weights and sizes of these drugs. The molecular weights of the above antibiotics are:

metronidazole	171.2
amoxycillin	365.4
clindamycin	424.9
erythromycin	733.9

(from The Merck Index, 1984)

Metronidazole has a much smaller molecular weight than demeclocycline (464.9) and therefore would very probably have faster rates of diffusion through dentine. Amoxycillin should also have faster rates of diffusion. The absence of an affinity for calcium ions and a slightly smaller molecular weight would probably produce faster diffusion rates for clindamycin than those for demeclocycline. Erythromycin, with its high molecular weight would probably diffuse through dentine at a much slower rate than demeclocycline and therefore not produce concentrations within the tubules and periodontal tissues that would be inhibitory to micro-organisms.

Clindamycin appears to have the best range of activity against endodontic bacteria (Sutter and Finegold 1976, Miles 1984) and could

therefore be considered for use as a local medicament. Metronidazole may also be an excellent alternative, especially in cases involving anaerobic bacteria (for example, acute "flare-up" cases and intact teeth with necrotic pulps). Amoxycillin may be an effective medicament, however it is contra-indicated due to the potential development of amoxycillin-resistant strains of bacteria and the possible hypersensitivity reactions that occur in patients allergic to the penicillin group of antibiotics.

6.12 OTHER AREAS YET TO BE INVESTIGATED

The concentration of triamcinolone required for a "local" or a "topical" therapeutic effect to occur has yet to be determined and reported.

The rate of clearance of medicaments from the periapical region and periodontal membrane has been reported briefly by De Deus and Han (1967). They reported that the periapical vessels remained congested throughout their experiment and they found radioactive material present in the blood vessels up to 24 hours after application of ^{3}H-cortisone to exposed hamster pulps. In comparison, the blood vessels in the periodontal ligament remained relatively free of congestion and had few radioactive grains present. Pashley (1979) has discussed the rate of permeation of molecules through coronal dentine and the relationship of pulpal blood flow and rate of removal of these molecules from the pulpal tissues. Although these studies give some indication, they do not explain what happens when medicaments are placed within a root canal with no circulatory vessels to clear

the medicament. Clearance from the canal depends on diffusion through dentine or apical release prior to clearance by the circulatory system.

A recent report suggested the use of a tube of polymeric membranes filled with the medicament and inserted into the root canal to control the release of endodontic medicaments (Tronstad et al, 1985). This would appear to be unnecessarily complicated. The use of a paste containing the active component as described in this study provides a much simpler technique. Investigations with other pastes could be performed in the same manner as in this study.

Montgomery (1985) stated that research is currently underway to develop techniques for the local delivery of tetracycline to periodontal pockets to control periodontal infections without producing any systemic effects. The use of the root canal, filled with a paste containing tetracycline is a possibility. The major problem is in maintaining the outflow through the dentinal tubules such that a therapeutic concentration is continually attained within the periodontal pocket. Further reseach is indicated to develop this as a possible technique.

A further possibility exists in that the root canal could be used as a means of slow, controlled release of drugs required for systemic therapies. Further research is indicated to develop a means of controlling the release of the drug to deliver a constant dose and to gain information regarding when to replenish the supply within the root canal.

As previously discussed, further investigations are indicated to assess whether the 50:50 mixture of Ledermix and Pulpdent pastes creates any significant differences in the effect of the calcium

hydroxide concentrations and pH changes within the tooth and surrounding tissues. If the formulation of Ledermix is altered with another antibiotic replacing the demeclocycline, then the effect of mixing this new paste with Pulpdent should also be investigated.

CHAPTER 7

SUMMARY AND CONCLUSIONS

Ledermix paste is a compound therapeutic agent commonly used as a root canal dressing agent in some parts of the world. It enjoys popularity amongst the majority of Australian dentists as a primary endodontic medicament. The two active components of Ledermix paste are an antibiotic, demethylchlortetracycline (or demeclocycline) at a concentration of 3.21 percent, and a corticosteroid, triamcinolone at a concentration of 1.0 percent.

The use of corticosteroids in dentistry and especially in endodontics has been discussed. The inclusion of a corticosteroid in an endodontic medicament evolved as the pain-relieving potential of steroids was realised. Steroids have also been advocated for use as pulp capping agents, cavity liners and temporary cements in various forms. Schroeder was the first to use triamcinolone in dentistry due to its "more intense action" (i.e greater potency on a dose-related basis). He stated that it should be combined with a broad spectrum antibiotic since corticosteroids suppressed the body's defense mechanisms. Demeclocycline was chosen.

Many types of bacteria have been implicated in diseases of the pulp and periapical tissues. Their roles have been studied and there appears to be no doubt that they do play a major role in these

diseases. Specific bacteria have now been identified and studies have discussed their susceptibilities to anti-microbial agents.

Materials placed in contact with dentine (for example, within a coronal cavity or a root canal) can diffuse through the dentine. The dentinal tubules act as fluid-filled pathways for the movement of molecules either outwards from the pulpal surface of dentine or inwards towards the pulp. Pashley's group of workers in a number of papers have investigated the diffusion of molecules through dentine and found that diffusion rates are dependent on molecular size, temperature, nature of the dentine surface, location of the sample of dentine (in the disc method), surface area of dentine available for diffusion, patency of the tubules and thickness of the dentine.

When Ledermix is used as a root canal medicament, the active components may reach the periodontal tissues either via the apical foramen or by diffusion through dentine. The current study was undertaken to investigate these two pathways and the factors affecting them in an effort to gain an understanding of the availability and efficacy of this compound material within the tooth and its surrounding tissues up to a period of 14 weeks.

A plastic root canal model was developed to study apical foramen release of triamcinolone and demeclocycline. It was found that the rates of release depended mainly on the size of the apical foramen. The volume of paste placed in the canal had a minor although inconsistent effect on apical release. Although the sizes of apical foramina used in the plastic models were larger than most mature teeth, they gave some indication of the release dynamics at the apical foramen. However experiments with extracted human teeth showed that very little of the demeclocycline or triamcinolone was released

through the apex. The major supply route of these medicaments to the periodontal tissues appeared to be via diffusion through dentine.

Freshly-extracted human single-rooted teeth with a single root canal were used to study diffusion of the components through radicular dentine. The selected teeth had their crowns removed, root canals prepared, apices either sealed or left patent and were filled with Ledermix paste. The rates of release of each active component of Ledermix paste were determined at the following end times: 1 hour, 3 hours, 8 hours, 1 day, 3 days, 10 days, 1 month and 14 weeks. At the completion of the experiment the internal lateral surface areas of the canal walls were calculated.

The results showed that in the samples examined age and sex did not affect diffusion rates following root canal preparation. The area of dentine available for diffusion was however an important factor, with total release being greater when the dentine area was greater.

The nature of the dentine surface has been shown to be responsible for up to 86 percent of resistance to diffusion. A smear layer, which is primarily calcific in nature, is created by instrumenting dentine with endodontic files. The use of E.D.T.A.C. as an irrigating solution during instrumentation and as a final flush of the canal aids in removal of the smear layer. Diffusion of the active components of Ledermix paste was significantly faster after the use of E.D.T.A.C. than after the use of Savlon as the irrigant in this study.

Cementum acted as an incomplete barrier to diffusion in this study. Removal of the cementum and irrigation with E.D.T.A.C. created favourable conditions for a significant increase in diffusion rates. This may be an important factor in teeth with external root

resorption or teeth undergoing periodontal re-attachment procedures where medicaments from the root canal might be applied to the periodontal tissues via the dentine.

Ledermix paste has been advocated as a dressing in a 50:50 mixture with Pulpdent paste. This mixture allowed slower release and diffusion of the components to the periodontal tissues and may thus maintain the efficacy of the dressing over a longer period of time. This mixture has been found to be invaluable in the treatment of extremely large periapical lesions.

Diffusion of demeclocycline and triamcinolone through coronal dentine was quantified. The method used crowns from freshly-extracted human third molar teeth. Class 1 cavities were cut and filled with Ledermix paste following the sealing of the crown into a hemi-cylindrical plastic chamber. The pulpal surface was bathed with pbs solution and samples taken at end times of 1 hour, 2 hours, 4 hours, 8 hours, 1 day, 2 days, 4 days and 8 days. The results showed that demeclocycline and triamcinolone diffused through dentine with a peak rate of diffusion after 2 hours. The rate then decreased exponentially with time.

The concentrations of demeclocycline and triamcinolone within the dentine of the crown and the mid-root and apical-third levels of the root were determined. Concentration gradients were demonstrated for both of these materials. Demeclocycline reached an initial peak concentration within the first day which was sufficient to inhibit bacteria within the dentine immediately adjacent to the Ledermix paste. However, in the more peripheral areas of the radicular dentine and the more pulpal dentine of the crown, the concentrations were unlikely to inhibit the bacteria commonly found in endodontic

infections.It is unlikely that triamcinolone would have any effect within the dentine, however the dentine, or tooth, acts as a drug release system for triamcinolone either to the periodontal ligament (from the root canal) or to the pulp (from a coronal cavity).

In conclusion, the in vitro rates of release and diffusion of demeclocycline and triamcinolone from Ledermix paste through dentine have been determined. These rates are an indication of the probable in vivo availability of these components at the root apex and in the periodontal tissues when human teeth are treated with Ledermix paste. By applying the principles discussed, the amount of these drugs reaching the periodontal tissues from the root canal, or reaching the pulp from a coronal cavity, may be increased or decreased according to the operator's requirements.