STUDIES RELATED TO THE PREVENTION OF ROOT SURFACE CARIES.

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May 1989

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ACKNOWLEDGEMENTS.

I would like to thank Dr. R Strang for his useful guidance during this project and Professor K W Stephen for his extensive help during this thesis preparation.

Many thanks go to my colleague Dr. L Macpherson for her invaluable help during microbiological data analysis, to Dr. T W MacFarlane for his comments during the preparation of Chapter 6 and to Mr. D Mackenzie for his instructions regarding microbiological techniques.

Thanks are due to Shaikh W Marshall for providing materials required for this project, to Mrs F Damato, Mr D Weetman for their technical assistance and to Mr R Foye for his assistance with the polarised light microscope.

I am also thankful to Mr J Davis and his staff of the Dental Illustration Department for photographing the equipment, particularly for Miss A Hughes for her artwork.

I am also grateful to my M.Sc supervisor, Dr H W Noble for his kindness and constant regard for the well -being of my family.

My thanks and gratitude go to the University of Science and Technology, Irbid, Jordan, for their financial support during my studies in Glasgow.

Finally, my special thanks go to my lovely wife Nawal, brilliant son, Derar, and my gorgeous daughter Khawlah for their everlasting love, bearing my changing moods and making our stay in Glasgow most enjoyable. My sincere thanks also go to my parents in Jordan for keeping in touch and for their encouragement from that distance.

DECLARATION.

This thesis is my original work.

Z. S. M. E. Albashaireh

SUMMARY

Root surface caries has been regarded as one of the problems of the future and recently has been given more attention. However, in the absence of an ideal and durable conservative treatment, the ideal solution for the problem would be prevention of the disease. Identifying the organisms associated with the initial lesion and enhancing the salivary remineralising effect upon the subsurface lesion via fluoride administration might be effective methods of arresting the disease and reducing its prevalence.

In the experimental work reported in this thesis, an artificial caries system, capable of producing repeatedly root subsurface lesions with a hypermineralised surface overlying a lesion body, was developed. The remineralisation effect of a placebo and three different monofluorophosphate dentifrices on these lesions was investigated *in situ* using the single section technique and an intra-oral appliance. This system allowed the investigations to be carried out in the oral environment and eliminated the disadvantage of the control sample used by other techniques. Also the remineralisation effect of a placebo and three different monofluorophosphate solutions were investigated *in vitro*. A third study was concerned with identifying microorganisms associated with demineralisation of sound root surfaces and formation of the initial lesion. Microradiographic / microdensitometric

methods were used to assess the mineral changes in the lesions and demineralisation of the sound root surfaces.

The results of the *in situ* study indicated that significant remineralisation was attained when fluoridated compared to placebo dentifrices were used. In 66% of the subjects, maximum remineralisation was achieved when using 5000 ppm F^- dentifrice and in 33% of the subjects, maximum remineralisation occurred when 2500 ppm F^- was used. In addition, the results of the *in vitro* study were consistent with the results of the *in situ* study in that lesion remineralisation was significantly higher when treated with fluoridated than with placebo solutions.

Actinomyces spp. were significantly associated with root surface demineralisation after three weeks' exposure to natural plaque. To the contrary of some published results *Strep. mutans* and *Lactobacillus* spp. were not significantly associated with demineralisation. Applying sugar to the plaque seemed to have little effect on the plaque composition or on sound root surface demineralisation. These results supported the theory that root surface and coronal caries are of different origins.

In conclusion, tooth pastes with 1000 - 5000 ppm F^- used twice daily could contribute remarkably to the prevention of root caries. Of further interest in this field of research, will be to assess remineralisation when the subsurface lesion is exposed more frequently to fluoridated dentifrices or solutions. Also, large scale microbiological experiments should be carried out to establish the microbial aetiology of the disease.

CHAPTER ONE.
GENERAL INTRODUCTION.

1.1 THE POTENTIAL PROBLEM OF ROOT CARIES.

Dental caries is a multifactorial condition resulting from the interplay among four different factors: the host, microorganisms, dietary substrate and time (Newbrun *et al.*, 1984). It can be subdivided by location into:

A. Coronal Caries: lesions affecting the enamel and underlying dentine;

B. Root Caries: lesions affecting the cementum and dentine of the root.

Root caries is of frequent occurrence in many countries but, because of the problems posed by coronal caries, to date little attention has been paid to this condition. However, the past few decades have been times of changing circumstances and concepts in dentistry. Water fluoridation and fissure sealants have been introduced and new dental materials and techniques are being improved continuously. People's attitudes have changed to favour conservative treatment rather than extraction and, generally, higher oral hygiene standards are being achieved due to increased dental health education.

As a result, a higher proportion of teeth which previously would have been lost at an early age, will be retained in the mouth (Jordan & Sumney, 1973). These teeth

will then be subjected to the stresses and sequelae of chronic periodontal disease, the inevitable manifestation of which will be gingival tissue recession. This will result in the exposure of a more vulnerable surface of the tooth, the cementum, to the chemical attacks of the oral environment. In addition, the cementum is also subjected to physical attacks, as abrasion resulting from tooth brushing and scaling. This, in time, might expose another vulnerable dental tissue, the dentine, to the challenging chemical changes.

Either or both of these tissues may become involved in a type of carious attack designated by various terms, viz; cervical caries, radicular caries, root surface caries, senile caries or erosion. Regardless of the terminology employed, this can be one of the most frustrating conditions the dentist is called upon to treat. Root carious lesions are difficult to restore, especially when they extend below the gingival margin. A restoration in this area may be sensitive to thermal changes because of its proximity to the pulpal tissues. Moreover, decay recurrence is a frequent problem (Jordan & Sumney, 1973). Therefore, with more people retaining teeth for longer periods and in the absence of proper conservative treatment, the ideal solution would be to seek a preventive measure.

The potential for future problems resulting from root caries has been realised by the National Institute of Dental Research (NIDR) in the United States which invited applications in 1982 for research to

investigate the disease and methods for its control (Wagg, 1984).

In contrast to coronal caries, fewer reports are available in the literature concerning root caries. Hazen, Chilton and Mumma, (1973) reviewed the literature on dental caries and reported that many papers failed to deal with root caries. Indeed, two of the most recent textbooks of cariology only mention root caries on two pages in which the microorganisms associated with the disease were given (Nikiforuk, 1985; Thylstrup, 1986). However, a number of excellent literature reviews have been published, two of which have emphasised that root caries might be the dental problem of the future (Wagg, 1984; Katz, 1985), while two others stressed the need for early recognition and preventive measures such as topical fluorides (Mount, 1986; Nicholls, 1987). Nyvad & Fejerskov, (1982) described the clinical, histological and microbiological features of the disease and outlined the clinical implication of these features. The need for further investigations on the optimal fluoride concentration and the best application method were stressed.

The above publications reported few details about experimental methods or designs for studying remineralisation of the early root surface lesion. Many of the questions posed by the root caries problem might be solved by *in vitro* and *in situ* experiments as these techniques have been useful in studying remineralisation in enamel.

1.2. AIMS AND OBJECTIVES.

In view of this developing major public health problem this project had the following aims:

1. to establish an artificial caries system which can induce repeatedly standard subsurface root lesions;
2. to examine *in situ*, the remineralisation of such artificial lesions using dentifrices of different fluoride concentration;
3. to investigate *in vitro*, incipient lesion remineralisation using a pH-cycling experiment;
4. to carry out a demineralisation study on human root surfaces using natural plaque, and to correlate the presence of any microorganism with demineralisation extent (thus, studying the organisms associated with the initial lesion).

The following sections will summarise some of the available information and discuss methods of investigating and assessing the disease.

1.3 AETIOLOGY OF ROOT SURFACE CARIES.

As in the case of enamel caries, the aetiology of root surface caries is not entirely understood. Nonetheless acid production from dietary carbohydrates by cariogenic microorganisms is suspected as the major

mechanism in the root surface caries process (Banting & Ellen, 1976).

1.3.1 Associated aetiological factors.

Diet: There is little data available on the association between root surface caries and carbohydrate in the diet, but it was shown in the Vipeholm dental caries study (Gustafsson *et al.*, 1954) that cemental caries increased with increased daily intake of sugar, particularly when taken between meals.

Plaque microorganisms: That bacteria are essential to the caries process in roots is not in doubt (Banting & Ellen, 1976), but it has not yet been established which microorganisms are responsible for initiating the disease as an analysis of the plaque flora at different stages of lesion development has not been attempted.

Periodontal disease: Exposure of the cementum to the oral environment seems to be essential for root surface caries. The majority of reports on root caries have made reference to the presence of periodontal disease, either prior to or at the time of initiation of the lesion (Banting & Ellen, 1976).

Saliva: Reference has been made to the fact that changes in the saliva with age may render the teeth of older patients more prone to dental caries (Lyons, 1972). This might be attributed to the diminished cleansing, antibacteria or buffering functions of saliva in old people. Salivary flow rate is reduced in some physical conditions e.g Sjogren's syndrome, but, more importantly,

many drugs will produce a similar effect (Mount, 1986).

The tooth root: In discussing the root as the host to dental caries the following must be borne in mind:

(a) the relative roughness of the cemental surface due to presence of Sharpey's fibres might act as a predisposing factor for bacterial growth and retention on the root surface.

(b) the high organic content of the root tissues might retard demineralisation or enhance remineralisation, by acting as a framework on to which mineral will be deposited.

(c) the enamel and cementum meet at the amelo - cemental junction in only 5 - 10% of teeth. In the remainder they either overlap or do not meet (Ramsay & Ripa, 1969). Such anatomical variations may thus facilitate the caries process in that area by providing ready access to dentine.

Oral hygiene: Manual dexterity and motivation to maintain good oral hygiene decrease with increasing age (Schamschula *et al.*, 1974). Furthermore, as people get older they often experience some gingival recession and will be at risk of developing root caries (Newbrun, 1986).

1.4 EPIDEMIOLOGY OF ROOT SURFACE CARIES.

1.4.1 Introduction.

Little has been reported in the literatre about epidemiology of root caries. This situation could have arisen for the following reasons: (1) coronal caries was

considered the major cause of tooth loss in teenagers and young adults. (2) School children are the most readily accessible for clinical surveys, whereas representative populations of adults and the elderly are difficult to obtain (Nyvad & Fejerskov 1982). (3) Standardised methods have only recently been suggested for the assessment of root surface caries either at individual level or in population surveys (Newbrun *et al.*, 1984), and (4) Root caries occurs at an age when tooth loss has been accepted as the norm (Wagg 1984).

1.4.2 Root caries in ancient and primitive populations.

While coronal caries which is due to consumption of cariogenic foodstuffs, is more associated with industrialised societies, root surface lesions have been found to be more prevalent in ancient civilisations and primitive societies (Banting & Courtright, 1975). Hardwick, (1960) also noted a predominance of caries at the amelo-cemental junction in sixth century Anglo-Saxon skulls and it was a common finding in the ancient Egyptian skulls examined by Leigh, (1935) and in other Anglo-Saxon skulls examined by Corbett & Moore (1971).

In the new world, dentitions from several North American Indian tribes of the late pre-Columbian and early post-Columbian eras also showed the presence of root surface caries (Leigh 1925).

More recent studies performed on primitive societies by Schamschula *et al.*, (1974) in Papua New Guinea, and by Mehta & Shroff, (1965) in Australian

aborigines also revealed frequent root caries occurence. Indeed, Schamschula, Keyes and Hornabrook, (1972) reported that root surfaces had a caries prevalence higher than enamel.

1.4.3 Root caries prevalence.

As Nyvad & Fejerskov (1982), and Wagg (1984) pointed out, reports published on root caries prevalence measured diverse groups. Of these, two related to primitive aboriginal populations (Mehta & Shroff, 1965; Schamschula *et al.* 1972, 1974), two to institutionalised subjects (Gustaffson *et al.*, 1954; Banting, Ellen & Fillery, 1980), and two to military personnel (Sumney, Jordan & Englander, 1973; Lohse, Carter & Brunelle, 1977). Others related to patients with periodontal disease in the United States (Hix & O'Leary, 1976), Egypt (El Hadary *et al.*, 1975) and Sweden (Ravald & Hamp, 1981). If caries prevalence is expressed as the percentage of subjects having one or more root surface lesions, most of these studies have had a prevalence between 39 and 58 per cent, although in two, only 15% of subjects examined by Lohse *et al.* (1977) exhibited root caries, whereas Banting *et al.* (1980) observed the problem in 83%. Nonetheless this discrepancy might well be due to the fact that 85% of subjects observed by Lohse *et al.* (1977) were less than 49 years of age, while the latter study included only patients who had teeth at risk due to gingival recession.

There appears to be general agreement that root caries prevalence is age-related. Wagg, (1984) reported

that the proportion of the population experiencing root caries increased from approximately 1 : 10 for under 30s to approximately 2 : 3 for the over 60s. While it could be argued that gingival recession and root surface exposure might be parameters more directly associated with root caries than is age, there is little doubt that recession itself is strongly related to age (Hazen, Chilton & Rappaport, 1974; Wagg, 1984).

1.4.4 Root caries incidence.

Incidence rates for root caries are not as readily available and are difficult to compare because the populations observed were quite diverse and the method of reporting was not uniform. Gustafsson *et al.* (1954) showed that the incidence of cemental caries in the oldest age group was 0.51 lesion/person/ period. Ravald & Hamp (1981) found that 20 of 31 (62%) patients being treated for advanced periodontal disease developed root lesions over a four-year period (1976-1980) of observation but an attack rate was not reported. The same patients were evaluated over a second four-year (1980-1984) period (Ravald, Hamp, Birkhead, 1986). The results revealed that the patients who were caries-free in the first study remained so, but the rest of the group developed more lesions. Banting, Ellen & Fillery, (1985) found that 36% of 44 residents of a chronic hospital developed root lesions over a 34 month period. The incidence rate in this group was 1.9 lesions per 100 person-months at risk, or 0.25 lesions / person / year. Moreover, as these studies used populations

considered to be at high risk of the disease, the incidence rate in healthy persons might be expected to be lower.

1.4.5 Distribution of root caries within the mouth.

Root caries depends not only on gingival recession, but also on tooth type and surface aspects.

Mandibular teeth appear to be more frequently involved with root caries than do those of the maxilla (Sumney *et al.*, 1973; El Hadary *et al.*, 1975), although Hix & O'Leary, (1976) demonstrated an equal distribution of lesions between maxillary and mandibular teeth.

While the majority of reports agree that the posterior mandibular teeth are most affected by this problem (Sumney *et al.*, 1973; El Hadary *et al.*, 1975; Hix & O'Leary 1976; Katz *et al.*, 1982), Banting *et al.* (1980) stated that most caries occurred in the anterior teeth of both arches, although Katz *et al.*, (1982) reported that susceptibility decreased for mandibular premolars and incisors. In the maxillary arch, however, the anterior teeth have a higher root caries index than the mandibular teeth.

As Wagg (1984) has discussed, there is lack of unanimity among different investigators regarding the surfaces at risk of root caries. All clinical studies have reported lesions most frequently on buccal and lingual surfaces (Mehta & Shroff, 1965; Schamschula *et al.*, 1972; Sumney *et al.*, 1973; Hazen *et al.*, 1974), although the majority of investigators who studied

extracted teeth or fleshless skulls, found that approximal surfaces were most commonly involved (Hardwick, 1960; Moore & Corbett, 1971, 1973; Lunt, 1974; Westbrook *et al.*, 1974; and Banting & Courtright, 1975).

Katz *et al.* (1982) and Banting *et al.* (1985) compared the relative likelihood of different root surfaces becoming carious by tooth type, and reported that the buccal surface of mandibular molars was twice as likely to demonstrate root caries as the lingual or approximal surfaces, whereas the lingual surface of the maxillary molar was five times more susceptible than the buccal surface of the same tooth.

It would seem possible that lesions might have existed on proximal surfaces, but being inaccessible to probe and mirror, remained clinically undetected by investigators. Furthermore, radiographs cannot be relied upon since such lesions are not readily visible on X-ray film (Buchholz, 1965) and unequivocal differentiation of true lesions from artifactual cervical burnout is problematic. Moreover, one would expect less root caries on buccal and lingual aspects of teeth due to their accessibility to oral hygiene measures.

1.4.6 Characteristics of person, place and time.

Slightly higher prevalence rates for root caries have been shown for males than females (Vehkalahti *et al.*, 1983; Katz, Newitter & Clive, 1985).

Banting, (1986) compared the occurrence of root caries between two countries, Finland using Vehkalahti *et al.*, (1983) data and North America using Katz *et al.*, (1982) data. The comparison revealed that the occurrence

of root caries was much lower in Finland than in North America.

Few studies are available which consider the occupation and socioeconomic class for comparative analyses. However, Banting, (1986) compared published data on root caries susceptiblity of different occupations and socio - economic classes. Drug addicts (Hecht & Friedman, 1949), persons receiving periodontal treatment (Hix & O'leary, 1976), chronically-ill institutionalised, older adults (Banting *et al.*, 1980), primitive tribesmen (Schamschula *et al.*, 1974) and the elderly (Beck, Hunt & Kohout, 1985; Fejerskov *et al.*, 1985), all have elevated susceptibility when contrasted with other population groups such as insurance company employees (Katz *et al.*, 1982) military personnel (Lohse *et al.*, 1977) and a general population (Vehkalahti et al., 1983; Katz et al., 1985). These results could be due to the reduced ability and interest of the above special groups to maintain oral hygiene.

1.5 THE CLINICAL FEATURES OF ROOT SURFACE CARIES.

Clinically root surface caries has been identified as a yellow or light brown stained area/cavity on the exposed roots of teeth. The lesion is irregular in outline and usually shallow in depth, tending to spread laterally or vertically, rather than penetrate deep towards the pulp. Thus pain is not a common complaint (Banting &

Ellen, 1976; Nyvad & Fejerskov 1982). The lesions are commonly found at gingival crest level and are usually covered with thick white or yellow plaque which may fill the defect.

There are at least two phases of the root surface caries process. The acute, or active phase, which is characterised by a softened, lightly stained lesion, at or below the level of the free gingival margin, is without obvious cavitation. The chronic, or remissive phase lesion, appears more darkly stained, often almost black, and exhibits frank cavitation. On probing, it may be softer than the surrounding normal cementum, but often the consistency seems to be more leathery than the active lesion (Banting & Ellen, 1976; Nyvad & Fejerskov, 1982). Katz (1986) stated that a difference between an active and inactive root lesion was that the latter had a leathery feel upon moderate pressure probing. Unlike the initial enamel lesion, the early root lesion appears as one or more small, well-defined discoloured areas predominantly located along the amelo-cemental junction.

1.6 HISTOLOGY OF ROOT SURFACE CARIES.

1.6.1 Introduction.

After its exposure to the oral fluids, the cementum will become subjected to a series of demineralisation and remineralisation episodes. The result is either a hypermineralised surface which shows as a zone of high X-ray density along its outer layer

(Forsberg, Lagergren & Lonnerblad, 1960; Selvig & Zander, 1962), or a carious lesion.

The basic chemical and structural changes which occur during root caries have only recently been studied. In the following sections, the current knowledge of root caries histopathology will be described in relation to microradiographic, transmitted and polarised light microscopy.

1.6.2 Histopathology - the current knowledge.

Observations on sound root surfaces have confirmed the occurrence of a hypermineralised layer at the external surface of cementum exposed to the oral environment (Furseth & Johansen, 1968). However, carious cementum has shown subsurface demineralisation and the frequent occurrence of a densely mineralised surface layer wider than that observed in exposed, non-carious cementum. A densely mineralised layer was frequently found at the cemento-dentinal junction. In the central portion of the lesion, two patterns were observed: a uniform demineralised area or areas of marked demineralisation, alternating with areas of high mineral content, giving the tissue a brush-like appearance. It was believed that the various changes observed resulted from the repeated processes of de- and remineralisation.

Westbrook *et al.* (1974) evaluated 103 teeth exhibiting root caries, as to morphologic distribution and histopathologic classification. They reported that approximately 70% demonstrated microscopically a carious

progression characterised by dentinal tubular matrix destruction due to invasion of dentine by microorganisms. Microradiographs revealed a moderate degree of surface hypermineralisation similar to that described by Furseth & Johansen, (1968).

Hals & Selvig (1977) found that the microradiographic image of early root caries lesions corresponded to previous descriptions by Furseth & Johansen (1968), with an outer 10-13 μm wide layer showing a varying degree of radiopacity. The underlying cementum was radiolucent except for a narrow radiopaque zone in the region of the cemento - dentinal junction. A zone of peripheral dentine also appeared radiolucent. In several instances, the radiolucent zone in cementum and dentine extended up to 300 μm from the root surface. Nyvad & Fejerskov, (1982) reported similar results with a radiolucent zone deep to an outer 10 - 30 μm wide surface zone. More recently, Mellberg (1986) examined 64 teeth exhibiting root caries and reported that 22% had a hypermineralised surface zone but about 50% had more than 90% of the normal amount of mineral. Most of the surface layers were less than 40 μm thick but few were as thick as 100 μm.

Under the intact microradiographic surface zone demineralisation spreads into the underlying dentine as evidenced by a distinct radiolucency extending several hundred micrometres beneath the root surface (Selvig, 1969). Frequently, the dentinal tubules are hypermineralised deep to the advancing lesion and as the

cariogenic attack progresses into dentine, it demineralises the more highly mineralised peritubular dentine more so than intertubular dentine (Selvig, 1968), thus widening the tubules and increasing permeability. Dentinal lesions, on exposed dentine after loss of cementum, may also develop a hypermineralised surface layer (Selvig & Zander, 1962; Westbrook *et al.*, 1974; and Takuma, Ogiwara & Suzuki, 1975).

The microscopic picture of root surface caries is consistent with the microradiographic one. According to Wefel, Clarkson and Heilman (1985), the histological features are similar when seen under the polarised or transmitted light microscope. With the latter, the body of the lesion appears to be a yellowish, darker area when compared with the surrounding dentine. The course of the dentinal tubules appears to be disrupted at the deepest aspect of the lesion and are less prominent within the body of the lesion. These features are observed with water imbibition but may become more distinctive when imbibed in quinoline, which distinguishes clearly between normal dentine and demineralised areas. While transmitted light microscopy revealed a structureless area at the deepest aspect of the lesion body, polarised light may show an actual reversal in the birefringence sign of this area's tissue, known as "phenol reacton" (Wefel *et al.*, 1985). This phenomenon occurs when the collagen fibres lose some of their mineral phase and the oriented adsorption of phenol molecules reverse the sign of birefringence from positive to negative.

In the same study (Wefel *et al.*, 1985), it was reported that, of 50 lesions viewed in transmitted light, a lesion body occurred in 100% of the cases, dentinal tubular reactions were seen in 32% of the cases, reparative dentine in 15%, a structureless area in 77%, and phenol reaction in 16%.

Observations at the ultrastructural level were consistent with microradiographic studies reported by Furseth & Johansen, (1968). Unexposed sound cementum from the cervical portion of roots displayed variation in electron density within limited areas. The crystals of the unexposed sound cementum are plate-like in shape. The densely mineralised surface layer of non-carious cementum which had been exposed to the oral fluids, revealed some different structural characteristics. Here, crystal width was smaller than that of unexposed cementum, while the thickness and length generally exceeded that of the plate-like crystals. These crystals were referred to as tablet-shaped (Furseth & Johansen, 1970). The selected-area diffraction pattern showed the crystals were hydroxyapatite (Furseth, 1971)

The ultrastructural observations of Furseth & Johansen (1970) on the highly mineralised surface layer of carious cementum, revealed masses of attached bacteria. They were also seen in lacuna-like spaces on the cementum surface and penetration of the microorganisms into the cementum tissue was further confirmed by Furseth, (1971). The underlying tissue generally had a high mineral content but spaces devoid of crystals, and also enlarged spaces

between crystals, were observed. These crystals were similar to the tablet-shaped variety observed in the surface layer of exposed, non-carious cementum i.e hydroxyapatite. The microradiographic demineralisation area revealed an uneven distribution of mineral when studied under the electron microscope. The brush like-appearance areas were found to be spikes of cementum with a high mineral content alternating with bacteria filled areas(Furseth & Johansen, 1970; Furseth, 1971).

1.6.3 Discussion.

Since root dentine is continuous with that of the crown, most investigators have felt that once caries involves root dentine, it is an identical process to coronal dentinal caries (Pindborg, 1970). However, as there are more dentinal tubules per unit area in the crown than in the root portion of teeth (Sicher and Bhaskar, 1972), one could postulate that significant differences in the rate of progression, and amount of dentinal sclerosis, might be present. Wagg, (1984) attributed the slow dentinal caries progression, as compared to that in enamel, to the high number of dentinal tubules.

The hypermineralised surface zone covering early lesions seems to be a consistent finding in exposed root surfaces, whereas it is not present in non-exposed tissue. These observations imply the precipitation of dissolved mineral in available spaces (or microchannels), formed as a result of demineralisation (Furseth & Johansen, 1970; Furseth, 1971). Thus the exchange of mineral may be quite

extensive at the cementum-saliva interface, and topical fluoride treatment may enhance this process, as shown experimentally *in vivo* by Furseth (1970).

Acquisition of a hypermineralised layer is not limited to cementum alone. Dentine, when exposed to oral fluids, could also acquire a hypermineralised surface layer, as was observed in ground dentine facets (Mjor, 1967), and also in crown dentine exposed by abrasion (Bergman & Engfeldt, 1954). It was considered most likely that these zones were the result of inorganic elemental deposits derived from salivary fluids. This concept is supported by the fact that saliva is supersaturated with regard to calcium and phosphate (Brudevold, Gron & MacCann, 1965), favouring mineral deposition on tooth surfaces.

The finding that a densely mineralised surface layer of carious cementum was wider than the corresponding layer in the sound exposed tissue, indicated that further mineral deposition, or a remineralisation process, had taken place during lesion development. The source of minerals could be the oral fluids, ions diffusing out from the carious lesion, or both.

The most consistent observations, as expected, were (1) a radiolucent lesion body, followed by (2) a structureless area (deep to, and surrounding the body) which was radiolucent to a lesser degree and, (3) partially radiopaque surface layers.

One notable difference between cemental and enamel caries appears to be the frequent occurrence of bacterial

invasion in the former case (Furseth & Johnsen, 1970; Furseth, 1971; Nyvad & Fejersov, 1982), whereas this does not occur in enamel caries until the surface has disintegrated.

1.7. THE MICROBIOLOGY OF ROOT SURFACE CARIES.

1.7.1 Introduction.

Only a few microbiological investigations of human root surface caries have been reported in the literature (Sumney & Jordan, 1974; Syed *et al.*, 1975; Hill *et al.*, (1977).

Both human (Banting & Ellen, 1976) and animal studies (Keyes and Jordan. 1964) have shown that organisms involved in the initiation and progression of coronal and root surface caries appear to differ.

Much data has accumulated over the past decade which suggest that *Streptococcus mutans* and *Lactobacilli* are strongly implicated in the aetiology of coronal caries (Loesche *et al.* 1975; Mikkelsen & Poulson, 1976; & Duchin & Van Houte, 1978), but the situation regarding root caries is more confused.

1.7.2 Root surface caries in experimental animals.

Root caries was successfully initiated in experimental animals fed a carbohydrate-containing diet (Dale, Lazansky & Keyes, 1944; Mitchel, 1950; Keyes & Jordan, 1964), and hamster periodontal disease was thought to be induced by a Gram positive, catalase-positive

branching filamentous organism (Jordan & Keyes, 1964; 1965). Similar human filamentous organisms were classified as *Actinomyces viscosus* (Gerencser & Slack, 1969) which was isolated from gingival crevice plaque and thought to be responsible for erosion of cementum.

Human isolates of *Actinomyces naeslundii* and *A. viscosus* induced periodontal pathology and root surface caries in hamsters and gnotobiotic rats (Socransky, Hubersak & Propas, 1970, Jordan, Keyes & Bellack, 1972). Strains resembling *Streptococcus salivarius* induced both enamel and cemental lesions in rats. Also, gnotobiotic rats, monoinfected with *Strep. mutans*, and fed vitamin-supplemented diet 585, developed fissure, approximal and root surface caries (Gibbons *et al.*, 1966). More recently, Firestone *et al.* (1987) induced root caries in surfaces exposed by gingivectomy and inoculated with *Strep. mutans* and *A. viscosus*. However, further experiments with gnotobiotic rats infected with various species of *Lactobacilli* did not record the presence of root surface lesions (Rosen, Lenny & O'Malley, 1968; Fitzgerald *et al.*, 1981).

1.7.3 Root surface caries in humans.

Root caries lesions in extracted human teeth were sampled for the presence of filamentous bacteria. Strains of *Rothia dentocariosa*, *A. viscosus*, *Actinomyces naeslundii*, *Actinomyces odontolyticus* and *Actinomyces eriksonii* were identified (Jordan & Hamond, 1972). *A. viscosus* and *A. naeslundii* isolates obtained from these

teeth induced severe periodontal pathology, with root surface caries, in monoinfected gnotobiotic rats. Sumney & Jordan (1974), in their attempt to characterise bacteria isolated from human root surface carious lesions, found that *Strep. mutans* was the predominant microorganism. A filamentous organism appeared typical of the genus *Actinomyces*, while other streptococcal forms were *Streptococcus sanguis* strains, *Streptococcus mitis*, and some with the characteristics of *Enterococci*. Plaque samples, obtained from surfaces exhibiting typical root caries, could be divided into two groups on the basis of the presence or absence of *Strep. mutans* (Syed *et al.*, 1975). In Group I plaques, *Strep. mutans* comprised 30% of the total cultivable flora and *Strep. sanguis* was not found. In contrast in Group II plaques, *Strep. mutans* was not detected and *Strep. sanguis* formed 48% of the total flora. In both Groups I and II, *A. viscosus* was the dominant organism in all plaque samples, accounting for 47% and 41% respectively.

The above reports included no comparative data on plaque from non-carious root surfaces. However, Ellen, Fillery and Banting, (1980) sampled approximately 150 sound root surfaces in 44 subjects prone to root caries in search for *Strep. mutans*. Here it was reported that the total cultivable plaque isolates (colony forming units) identified as *Streptococci* were much lower than that reported for established lesions, accounting for 55% of the flora. Also, intact root surfaces of 44 chronically hospitalised subjects with 154 exposed caries-free

surfaces were sampled periodically over 32 months to count bacterial species believed to be associated with root caries (Ellen, Banting & Fillery, 1985b). It was revealed that *A. viscosus* was isolated from every subject and *Strep. sanguis* and *veillonella* were detected in over 90% of subjects. *Strep. mutans, A. naeslundii* and *Lactobacilli* were detected in approximately 60%, 70%, and 70% of the subjects respectively. It was concluded that, with the high degree of variability in the data, speculation about the proportion of any particular species which would be necessary for root caries, would be invalid.

Billings, Brown and Kaster, (1985) acquired plaque from six adults undergoing treatment strategies for root surface caries namely: self - applied 1% sodium fluoride gel in combination with smoothing away of shallow caries and the restoration of cavitated lesions with glass ionomer cement. Samples were taken prior to the clinical examination and at 6 and 12 months after therapy. Pre - treatment examinations showed *Strep. mutans* levels on sound root surfaces to be lower than those on lesions where they made up 87% of total *Streptococci. A. viscosus* was found to be the predominant organism on sound root surfaces and was relatively higher than those at lesion sites. Furthermore, when plaque samples were collected from 150 root surfaces with gingival recession, seven times over a 32 month period, it was revealed that both *Strep. mutans* and *Lactobacilli* were isolated more frequently from surfaces which become carious, than from those remaining caries-free. It was noted that isolation

frequencies were also higher for caries-free surfaces in caries-active subjects, than for caries-free surfaces in caries-inactive subjects (Ellen *et al.* 1985a). *A. viscosus* was present in all subjects, and in almost all samples, rendering its detection virtually useless for risk assessment.

Bacteria have also been collected from deeper parts of carious root lesions (Sumney & Jordan, 1974), where there was a predominance of microorganisms resembling the genus *Arthrobacter* which conflicts with observations on the advancing front of coronal caries, where *Lactobacilli* were often found to predominate (Edwardson, 1974).

1.7.4 Discussion.

Neither human nor animal studies have agreed on the microorganisms responsible for root caries. Most of these were cross - sectional in design and changes in the microflora could not be monitored to determine whether specific bacteria became more plentiful, or whether a characteristic mix of bacteria colonised a root surface prior to the initiation of clinically detectable caries.

Both *A. viscosus* and *Strep. mutans* have been shown, although not established, to be strongly associated with root caries. Further details relating to microbiological studies on root surfaces will be presented in Chapter Six.

1.8 THE PREVENTIVE APPROACH TO DENTAL CARIES.

1.8.1 Introduction.

Dental caries is a biosocial disease, the causes of which are rooted in the culture, technology and economy of a society. The lack of a major public response to reduce caries by minimising sugar intake can be attributed to two reasons namely: (1) generally, people like to do, and particularly eat, as they please, (2) the loss of teeth and their replacement after a certain age has become socially acceptable.

Attempts to control the disease by increasing dental manpower have merely resulted in more restorations being placed without diminshing the caries prevalence (Brown, 1982). One means of reducing caries is by use of systemic and/or topical fluoride therapy. However such reductions, although significant are greater on smooth than on occlusal surfaces (Backer Dirks, 1974). Accordingly, the ideal approach to reduce or prevent dental caries would be to consider the following:

1. The promotion of artificial/ non-fermentable sweeteners to replace, or reduce sucrose consumption;
2. The promotion of effective oral hygiene to ensure removal of cariogenic plaque;
3. At a family level, the use of non-sweet snacks;
4. The wide use of fissure sealants;
5. Fluoridation of communal water supplies;

or

6. The use of a systemic fluoride supplement;

and

7. Topical fluoride application.

However, it is outwith the scope of this introduction to discuss the first four points, but the next section will present an historical background of the fluoride story. Systemic and topical fluoride uses will also be discussed.

1.8.2 Fluoride and teeth.

The association of fluoride with dental tissues has a long history that dates back almost 200 years. According to Ostrom, (1980) the earliest report was credited to Morozza who, in 1802, detected fluoride in the teeth of a fossilized mastodon. In 1823, Berzelius analysed water and detected trace levels of fluoride that varied from 0 - 3 ppm. Magitot, in 1866, noted that certain teeth decalcified more rapidly than others and attributed his findings to the varied fluoride content of the teeth. As a result, fluoride lozenges (pastilles) were then introduced and used in Europe.

The forerunner to the finding that fluoride and dental caries were related was the discovery by Eager (1902) of a dental condition, known as "Denti Chiaie", in persons living in Naples, Italy. This was characterised by white flecks and brown stains of the teeth which Eager suggested may be due to an agent in the drinking water. McKay (1916a) soon after his arrival in Colorado Springs,

USA, noticed that many of his patients had an apparently permanent stain on their teeth known to the local inhabitants as "Colorado Stain", which he termed "mottled enamel". Mottled enamel was studied histologically by Black & McKay, (1916) and Black's interest and authority raised the study of the problem from that of a local curiosity to one which merited the concern of all dental research.

Evidence on the association between mottled enamel and a causative agent in water came from Britton, South Dakota, U.S.A, in which after the change of water supply in 1898, it was noticed that all children who had passed through childhood before 1898 had normal teeth while those who had grown up in Britton since 1898 exhibited mottling (McKay, 1918). Similar evidence was reported in the town of Bauxite where the first domestic water supply came from shallow wells and springs, but in 1909 a new source of water was obtained from a well. It was discovered that no mottling occurred in people who grew up on Bauxite water prior to 1909, but all native children who used the new water supply after that date had mottled teeth (Kempf & McKay, 1930). Further evidence came from Oakley, Idaho, U.S.A where mottling was no longer seen on children's teeth seven years after the water source was changed (McKay, 1933).

Thereafter, Churchill, (1931) who chemically analysed the water from Bauxite, established that the likely aetiological agent of enamel mottling was fluoride (13.7 ppm) this subsequently being shown to be present in

water samples from all of McKay's original survey areas. Thus the term "fluorosis" was given to the condition.

Thereafter, the U.S. Public Health Service assigned Dean to study mottled enamel and find its extent and geographical distribution - the "Shoe Leather Epidemiology survey" (Dean, 1933).

Here, observations on 5,824 children in 22 cities of 10 states in the U.S.A revealed the following :

1. Mottling was wide - spread where the concentration of fluoride was 3 ppm or more.
2. At 4 ppm, the degree of fluorosis was so severe that teeth showed signs of discrete pitting.
3. At 2.5 - 3.0 ppm fluoride, the affected teeth had a dull, chalky appearance and, post-eruptively, took on a characteristic brown stain which increased with age.
4. In areas where the water supply contained 1.0 ppm or less, there was little clinically significant mottling of teeth.

Prior to the finding that excessive levels of fluoride in drinking water caused enamel mottling, the question of a relationship between fluorosis and dental caries was discussed in a number of reports (McKay & Black, 1916; Bunting *et al.*, 1928). The preliminary evidence indicated that caries prevalence in endemic areas was less than in control communities. This then raised the question as to whether or not fluoride was also responsible for the reduced caries rates, and Ainsworth, (1933) made a significant contribution by

producing statistical data showing that caries experience in endemic areas was lower than the average. The general acceptance of the fluoride - caries relationship was strengthened further by the demonstration that experimental caries in rats was significantly reduced by adding fluoride to their diets or water (Cox *et al.*, 1939).

As a result of the above, an epidemiological study was set up to determine what fluoride concentration would be low enough to eliminate the fluorosis complication yet inhibit, or reduce, dental caries (Dean, 1945). The study of 7,257 children, aged 12-14 years, in 21 cities of four U.S states, revealed that children drinking water supplies containing little or no fluoride had a mean DMF index in the range of 6-10, while those drinking water with 1 ppm fluoride had an index value between 2-3. Therefore fluoride did not completely inhibit dental caries but a difference of about 60% was observed, with no significant fluorosis.

The following conclusions were drawn from the data of Dean's study, along with a survey of an additional 36 cities (Striffler, 1958):

1. A striking reduction in caries with no fluorosis of aesthetic significance was associated with drinking water containing fluoride at approximately 1 ppm;

2. A slight additional benefit accrued when the concentration of fluoride in water exceeded this level;

3. This benefit was less, although detectable, when the fluoride concentration was as low as 0.5 ppm;

and

4. Fluoride must be ingested during the pre-eruptive stage of dental development for maximum benefit (although this is now questioned).

In the light of encouraging epidemiological data from populations living in naturally fluoridated areas, the logical recommendation was to artificially fluoridate communal water supplies where the fluoride level was below the optimal 1 ppm F^- concentration.

Thereafter, results obtained from long - term (10 year) investigations of controlled communal water fluoridatiion indicated that the benefits were equivalent to those observed in naturally fluoridated areas and signalled the wide - spread introduction of water fluoridation as a public health measure that would significantly reduce caries (Nikiforuk, 1985).

1.8.3 Benefits of continued exposure to fluoride.

Klein, (1946) examined children of Japanese ancestry who had been transferred from a community containing 0.1 ppm fluoride or less, to Arizona where the water contained 3 ppm fluoride. While teeth which were in the process of eruption received maximum benefit from fluoride, it was also found that teeth exposed to fluoride shortly after eruption were also protected. Hence, the topical benefit of fluorides was suggested and the race for effective topical fluoride agents began.

Observations on the caries history of migrant children from South Dakota, where the water naturally contained 1 ppm F^-, but who moved into an area where the

water supply contained only 0.2 ppm F^-, indicated a progressive loss of the cariostatic effect of fluoride (Russell, 1949). This study therefore established the important principle that the cariostatic action of fluoride - containing water was dependent upon continued exposure.

Epidemiological data showed that the caries - reducing effect of fluoride extended into adult life. Russell and Elvove, (1951) found that the average DMF index in adult natives of Colorado Springs (water with 2.5 ppm F^-) was approximately 60% lower than in Boulder (water with 0.1 ppm F^-) for life-time residents of 20 - 44 years of age. Furthermore, persons in non-fluoridated Boulder had lost four times as many teeth as persons of the same age living in Colorado Springs.

1.8.4 Systemic fluoride administration.

People who reside in areas where communal water supplies are not fluoridated, either naturally or artificially should have alternative fluoride sources. Fortunately, fluoride can be delivered to the body systemically via water, food, beverages etc., or topically by applying materials containing fluoride, e.g gels, dentifrices or mouthrinses.

While fluoride is acquired during the pre-eruptive development of teeth, a significant amount of enamel mineral, including fluoride, is also acquired as a result of posteruptive maturation (Sakae & Hirai, 1982).

Drops and tablets.

The most practical non-communal methods of systemic fluoride administration are in the form of drops or tablets. Fluoride drops are particulary suitable for children under 2 years of age, and in the U.K most commercially available tablets contain either 0.25 mg, 0.5 mg, or 1.0 mg F^-. Tablets should be of moderate size, to encourage slow dissolution and have a flavour mild enough to promote this intraoral function without producing high salivary outflow to dilute the topical benefit obtainable prior to swallowing (McCall, Stephen, and McNee 1981).

Fluoride supplement salt.

A study with fluoride supplemented salt (90 mg/Kg F^-) which started in 1955 produced 25% caries reduction (Marthaler *et al.*, 1978). However, a major clinical trial began in Hungary in 1966, using 250 mg/Kg F^- domestic salt, and resulted in a caries reduction of approximately 50% (Toth, 1976), although 60% was achieved by a later increase to 350 mg/Kg (Toth,1984).

Milk supplemented fluoride.

Theoretically milk is a reasonable vehicle for fluoride since it is a food which is used universally by infants, pregnant women and children during the period of dental development.

A significant caries reduction of 70% was reported for children who consumed fluoridated milk by Rusoff,

(1975). In a double-blind study, a 43% DMFS caries reduction resulted from the daily ingestion at school of 200 ml of milk fluoridated with 7 ppm F^- (1.5 mg NaF added to 200 ml of milk) for 5 years, (Stephen *et al.*, 1981). While the use of fluoridated milk as topical alternative to water fluoridation, would eliminate any question of compulsory fluoride ingestion, the multiple sources of preparation in any country, coupled with fluoride's capacity to bind to calcium over a period of time, would suggest it is far from the ideal F^- vehicle.

1.8.5 Topical application of fluoride.

Without doubt water fluoridation is the most cost - effective public measure for reducing dental caries. However, water fluoridation is not popular amongst all people, therefore another logical extension, in view of the pronounced topical benefits of water fluoridation (*vide supra*) was to apply fluoride - containing materials topically on the teeth.

Fluoride materials can be either self - or professionally - applied on the teeth.

Professionally applied fluorides.

The first of the professionally - applied fluorides was the topical solution, a 2% NaF preparation being applied on the teeth for 3 - 4 minutes and repeated three times at weekly intervals. The procedure, known as the Knutson technique, was recommended at 3, 7, 10 and 13 years of age to coincide with eruption of various groups

of teeth. By so doing, a caries reduction of 40% was achieved (Knutson, 1948).

Some clinical trials showed superior caries reductions from one or two annual applications of 8% SnF_2 (at low pH), than from three or four applications of NaF, with benefits between 21 and 69% having been recorded (Ripa, 1981).

Acidulated phosphate fluoride solutions were then formulated as it was thought that the fluoride uptake of enamel might be enhanced if the pH was low. Brudevold *et al.*, (1963) developed a preparation based on acidification of NaF solutions with phosphoric acid, and commercial APF preparations have been widely used since then with clinical studies demonstrating caries reductions which generally ranged from 20 - 40 %.

Gels, with a pH of 4.3 - 5.5, have also been produced commercially as they have the advantage of adhering to teeth for a considerable time. Furthermore, when trays are used, more than one tooth may be treated. However, there is little evidence that gels are superior to solutions, although daily applications at school of 0.5% fluoride as APF gel for two years, resulted in an outstanding caries reduction of 80% (Englander *et al.*, 1969).

Acidulated phosphate fluoride gels containing 0.5% fluoride ion are effective for short term home therapy of a few weeks, while neutral 1% NaF gives excellent results if used for longer term therapy (Wei & Wefel, 1982).

Several materials have been developed which are

relatively simple to apply and permit an extended time of about 12 - 48 hours for fluoride fixation. Duraphat (ICN Pharmaceuticals, Eschwege, FRG) and Fluor Protector are commercially available materials to serve the above purpose. The majority of the clinical studies indicated that the preventive effect of Duraphat is similar to those obtained using other topical fluoride preparations (Murray, Winter & Hurst, 1977)

The controlled release of fluoride into the oral cavity could play a major role in dental caries prevention as caries was inhibited on all tooth surfaces in rats wearing devices releasing 0.15 mg F^- daily, with reductions ranging from 42% on the sulcal surfaces to 75% on smooth surfaces (Mirth *et al.*, 1982, 1983).

Self - applied topical fluoride agents.

Such agents are especially recommended for the following:

1. Patients with rampant caries.
2. Individuals with root caries.
3. Patients undergoing medical or surgical treatment that may induce a high risk to caries.

Fluoride Dentifrices.

Dentifrices are used in conjunction with tooth brushing, either individually, or as a part of an oral hygiene programme. Initially, dentifrices were merely introduced for use with a toothbrush for the purpose of cleansing and polishing the teeth, and as

potential breath fresheners. However, they have become excellent vehicles for the delivery of therapeutic agents, particulary fluoride, in the fight against dental caries.

With respect to fluorides, the following are the main different fluoride compounds which are added to a dentifrice for the purposes of caries prevention.

(a) Sodium fluoride.

This was the first fluoride compound ever to be added as an active ingredient to a dentifrice. Unfortunately, the formulation was far from successful in its early days due to the interaction between its abrasive (calcium carbonate or orthophosphate) and NaF, forming CaF_2. Later, acrylic particles were substituted to overcome this problem and more recently silica systems have been employed.

Caries inhibition by sodium fluoride dentifrices is directly related to fluoride concentration within the range 250 - 1000 ppm F^- (Reed, 1973) and these, unlike stannous fluoride toothpastes, do not stain the teeth, are stable and have neutral pH.

(b) Stannous Fluoride.

Stannous fluoride solutions produced greater reductions in enamel solubility than did other fluoride salts (Muhler, 1950). Therefore, stannous fluoride solution (8%) and dentifrices (0.4%) were introduced to the market. However, this dentifrice formulation despite its caries - inhibition qualities has now been

removed from the market as it stains teeth, has unacceptable metallic taste and was relatively unstable.

(c) Sodium monofluorophosphate.

Sodium monofluorophosphate (MFP; Na_2PO_4F): is produced commercially by heating sodium metaphosphate, orthophosphate, condensed phosphates and sodium fluoride.

The chemical properties of this compound are: (1) it interacts with dental enamel; (2) it decreases dental enamel solubility in acid; (3) it has anticariogenic activity; and (4) it is compatible with conventional abrasives such as dicalcium phosphate hydrate and calcium carbonate.

The caries prevention action of MFP is as yet not fully established. Ingram (1977) suggested that the MFP anion (PO_3F^{--}) has anti-cariogenic properties of its own and may exchange with phosphate groups in apatite crystals, and that this reaction is not competitive with fluoride. Others, however, have suggested that its action is linked to its slow hydrolysis whereby small concentrations of fluoride ions are released. It has been suggested that the hydrolysis action is very slow and would take many years to hydrolyse half a sample (White, 1983). However, it has been reported that plaque suspensions rapidly hydrolyse MFP (Jackson, 1982). Sodium fluoride is present as an impurity in MFP and might also exert anti-caries action as it has been shown that NaF plus MFP is more effective than either compound alone (Hodge *et al.*, 1980).

The reduction in the caries rate after using MFP was greater in persons living in non-fluoridated areas (34%) than in persons living in fluoridated areas (17%) and, as with water fluoridation, approximal surfaces of the posterior teeth benefitted most (Andlaw & Tucker, 1975).

Dentifrices containing MFP do not stain teeth, have a pH close to neutral, are stable and appear to have no contraindications nor side effects. The abrasive material used in recent MFP formulations has been aluminium oxide trihydrate, thus eliminating any possibility of orthophosphate ions being present, which could inhibit MFP uptake by enamel, as was the case when sodium metaphosphate was used (Andlaw & Tucker, 1975).

(d) Amine fluoride.

Amine fluorides were first tested in Switzerland where it has been shown that they have had antibacterial, physicochemical and anti - solubilising properties (Muhlemann, Schmid & Konig, 1957; Muhlemann *et al.*, 1960). These dentifrices, marketed in only a few European countries, demonstrated caries reduction effect significantly higher than MFP (Cahen *et al.*, 1982).

Fluoride mouthrinses.

It is known that fluoride incorporation in enamel increases when enamel is exposed to a saliva-fluoride solution. Based on this observation, fluoride-containing mouthrinses were extensively studied by Ericsson, (1961),

some of which demonstrated significant benefit.

Because fluoride mouthrinsing is a simple, inexpensive and practical procedure, it has become one of the main caries-preventive measures to be employed for use with school children.

As a result, an important observation has been that the benefits from long-term usage of mouthrinses duplicated those of communal water fluoridation programmes (Birkeland *et al.*, 1977; Ripa *et al.*, 1983). This further emphasises the topical importance of fluoride from both communal water and mouthrinses.

Although many regimens and concentrations have been suggested, a 0.2% sodium fluoride solution (1000 ppm F^-) is commonly used on fortnightly or weekly basis, while 0.05% solution (250 ppm F^-) is used daily. Such a solution has to be employed frequently over a prolonged period to achieve maximum results. Fluoride solutions are not designed for children under the age of 4 years because of fluoride ingestion risk. In communities who ingest fluoridated water and brush their teeth with fluoridated toothpaste, it is questionable if a further topical fluoride would produce significant benefits which outweigh the cost and effort involved. Although evidence supports the proposition that additional benefits can be obtained from multiple programmes in both fluoridated and non-fluoridated areas, it is important that, before embarking on multiple fluoride usage, the cost-benefit of such an expensive programme should be carefully assessed.

1.9 THE CARIOSTATIC MECHANISM OF FLUORIDE.

Although the precise and complete mechanism of fluoride's action is not fully understood, there is sufficient evidence to indicate that fluoride exerts several effects on the calcium - phosphate system and, in addition, on the metabolism of dental plaque (Nikiforuk, 1985).

The caries-preventive mechanism of fluoride has been explained by three main theories (Thylstrup & Fejerskov, 1986). The first theory was based on epidemiological studies which showed that the caries prevalence among subjects living in a fluoridated area, especially during the tooth mineralisation period, was lower than that of children in non - fluoridated areas. It implied that caries - resistance, once obtained, would last for a life - time and that fluoride provided during mineralisation of the teeth was significantly more effective than when given later, since the post-eruptive uptake of sound enamel was minimal even with extensive topical therapy. However, the concept of life - long caries resistance was questioned since it was observed that the low caries experience of those born and raised in communities with fluoridated water eventually discontinued after they moved to non-fluoridated areas (Russell & Hamilton, 1961; Lemke, Doherty & Arra, 1970).

Fluoride (systemic) incorporation into the enamel during development was the rationale behind many dental preventive measures, with the assumption being that the

fluoridated hydroxyapatite formed was more resistant to acid dissolution (Isaac *et al.*, 1958a). This concept was based on the findings that teeth formed in communities with 1.0 - 1.5 ppm F^- in the water supplies have about 50% less caries as compared to those formed in non-fluoridated areas. Also, it was observed, such teeth had an increased fluoride content in the surface enamel (Brudevold, Gardner & Smith, 1956; Isaac *et al.*, 1958b). Therefore, it was natural to consider this situation a cause and effect relationship. However, *in vitro* studies have demonstrated that no correlation existed between enamel bound fluoride and demineralisation (Mellberg, 1977; Kidd *et al.*, 1980). Thus, it is difficult to explain the 50% caries reduction observed in fluoridated areas on the above basis.

Much emphasis has been put on the acid resistance of hydroxyapatite, but it is noteworthy that complete substitution of hydroxyl ions in human enamel corresponds to a fluoride content of 3.7%. However, even in severely fluorosed human enamel, such a complete substitution is never achieved, as less than 25% of the hydroxyl ions in the outer enamel were replaced by fluoride (Richard, Ferjerskov & Larsen, 1979), whereas in human enamel formed in areas with 1 - 1.5% ppm F^- in the water, the substitution amounts to only 10%.

It is generally accepted that the length of the pre-eruptive maturation phase influences the amount of fluoride acquired. Thus, deciduous teeth from a fluoridated area contain much less fluoride in the surface

enamel than permanent teeth from the same area, and even less than permanent teeth developed in a low fluoride area (Mellberg, 1977). Consequently, it is important to consider that the effect of systemic fluoride on caries prevalence in the primary dentition, is of similar magnitude to that in the permanent dentition, irrespective of the amount of fluoride incorporated during tooth development (Fejerskov, Thylstrup & Larsen, 1981).

The above considerations thus raised doubt as to the importance in the caries prevention concept, of pre-eruptive fluoride incorporation in enamel.

The second theory claims that fluoride has to be present in the aqueous phase around the teeth, i.e in the saliva, the plaque fluid and the aqueous phase of enamel pores for effective caries-inhibition. This theory is based on the inhibitory effect of fluoride on dental tissue demineralisation and because of fluoride clearance from oral fluids, it implies a more or less continuous availability of fluoride. Hence, any discontinuation of fluoride may allow demineralisation to override the remineralisation process, with the appearance of a resultant carious lesion.

Immediately after mineral dissolution during an acid attack, a process of remineralisation (healing) takes over to compensate the mineral loss and reverse any early lesion formation. Although supersaturated with respect to hydroxyapatite, saliva probably contributes little to white spot remineralisation. Fluoride's efficacy as a remineralising and early lesion reversal agent has been

documented clinically (Grondahl, 1979) and demonstrated experimentally *in vivo* (Von der Fehr *et al.*, 1970). This concept was supported further by *in situ* and *in vitro* studies which have also demonstrated the fluoride remineralisation effect (Silverstone *et al.*, 1981; Featherstone *et al.*; 1982 Strang *et al.*, 1987). Lesions remineralised via fluoride deposition, have been shown to be less soluble, and therfore more caries - resistant (Feagin *et al.*, 1971).

During an acid attack, the remineralisation process functions at the earliest stage of caries formation. Fluoride causes mineral dissolution to cease as the pH begins to rise and results in the formation of larger, less soluble crystals, which contain more fluoride and less carbonate. The final result is partial remineralisation of the etched area which can be further remineralised by subsequent exposure to fluoride (Weatherell *et al.*, 1971). The entire body of the subsurface carious lesion does not have to be remineralised for it to become protected as this can be achieved if the surface zone alone is remineralised (Silverstone *et al.*, 1981). Therefore, the presence of a natural caries white spot does not mean that the area is undergoing a carious attack as it could represent an arrested caries area which might also appear as brown spot due to organic stains absorption.

The third theory is based on the antimicrobial action of fluoride, which is thought to produce disturbance of bacterial colonisation, growth and

fermentation.

It has been shown that while a low fluoride concentration of 10 to 25 ppm in the tooth's fluid environment may stimulate *Strep. mutans* growth, at a 50 ppm F^- concentration it is markedly reduced and at 100 ppm F^-, is completely inhibited (Brown & Konig, 1977).

It is thought that fluoride can inhibit bacterial enzymatic processes as *in vitro* studies (Hamilton, 1977) have shown that enolase and phosphatases, enzymes involved in carbohydrate metabolism, are inhibited by fluoride.

When plaque is acidified, either by adding acid *in vitro* or, as a result of bacterial metabolism *in vivo*, fluoride ions are liberated from bound forms and about 30% or more of the total may be free i.e ionised (Birkeland & Charlton, 1976). It seems likely, therefore, that bound plaque fluoride acts as reservoir for the ionised form and favours remineralisation, as well as inhibition of bacteria. It has also been reported that the pH at which much of the F^- ions are released (pH 5) is also the same as that at which bacteria become most sensitive to fluoride (Birkeland & Charlton, 1976).

In addition to the above theories, fluoride might affect the morphology of the tooth as diameter and cusp depths of teeth were noticed to be smaller if fluoride was present during tooth development. Also subjects living in fluoridated areas have been reported to have shallower fissures in their teeth and fewer carious lesions (Assenden & Peebles, 1974). Such shallow fissures present more self - cleansing areas and would thus accumulate

less cariogenic plaque with obvious clinical benefit.

1.10 PREVENTION AND ROOT SURFACE CARIES.

1.10.1 Introduction.

Fluoride concentrations in the outer layer of root surfaces have been shown to increase with increasing fluoride levels in water supplies and with advancing age (Brudevold, Steadman & Smith, 1960; Yoon *et al.*, 1960; Nakata, Stepnick & Zipkin, 1972; Stepnick, Nakata & Zipkin, 1975; Banting & Stamn, 1979). This suggests that root surfaces may benefit from two possible sources of fluoride i.e systemic, by incorporating fluoride in the root tissue during development and topical, in case of root exposure, by the frequent availability of fluoride to root surfaces. Root surface resistance to acid attacks could be improved by enhancing the root fluoride uptake. The following sections will discuss the available evidence on reducing root surface caries by introducing fluoride systemically or topically.

1.10.2 Evidence from epidemiological surveys and animal studies.

Epidemiological data have shown a low root caries prevalence in life-long residents in fluoridated areas. Stamn & Banting, (1980) compared root caries in 502 life - long residents of the naturally fluoridated (1.6 ppm) community of Stratford, with that in 465 adults from the matched but non-fluoridated community of Woodstock. Their comparison showed that the Stratford sample had a mean of

0.48 root surfaces decayed and a further 0.16 filled, against 0.99 and 0.37 respectively for Woodstock. However, no comparative data are available on correlation between optimal water fluoridation (1 ppm) and root caries prevalence.

Root caries in rats has been shown to be significantly reduced by administration of fluoride in the drinking water and the reduction was positively correlated with fluoride concentration in water (Rotilie, McDaniel & Rosen, 1977; Rosen, Beck & Beck, 1985).

1.10.3 Evidence from clinical studies.

A noteworthy clinical study was conducted by Johansen *et al.*, (1987) to monitor remineralisation and prevention of carious lesions in elderly patients. The experimental regimen was designed to increase tooth resistance by self-administration of a sodium fluoride gel, to decrease the acid attack rate by brushing and effective tooth flossing, and to enhance the intraoral maintenance processes by rinsing the teeth with a remineralising solution (5mM Ca^{++}, 3mM PO_4^{3-} and 5 ppm F^-) and by stimulating salivary secretion. Lesion remineralisation was assessed clinically depending on increased surface hardness, altered lesion colour and lack of lesion progression in subsequent examination. The post treatment data showed marked success in arresting the lesions in existence at the start of the study. A higher proportion of root surface lesions than coronal lesions were remineralised.

1.10.4 Evidence from in vitro experiments.

In vitro studies have shown that fluoride in an acidified buffer could inhibit mineral dissolution from root surfaces exposed to acid and result in a hypermineralised surface layer formation (Phankosol *et al.*, 1985; Wefel *et al.*, 1986). Increased fluoride uptake by roots resulted in root surfaces which were less soluble in acids (Shannon, Buchanan & Mahan, 1976). This suggests that hypermineralised root surfaces, or remineralised subsurface lesions, might resist the carious challenge better than normal tissue. Also, this might explain why teeth remain caries-free for some time even after discontinuation of fluoride therapy (Leske *et al.*, 1985).

Stannous fluoride mouthrinses were reported to be significantly more effective than NaF mouthrinses in reducing both crown and root solubility (Shannon, 1980). Tveit (1980) reported that electron probe microanalysis of twelve root surfaces which were treated with the fluoride varnish "Fluor Protector" for 48 hours revealed that all surfaces acquired fluoride concentrations greater than 1500 ppm; and values up to 25000 ppm were recorded. Hals *et al.*, (1981) compared fluoride uptake from TiF_4 (Titanium fluoride), acidulated fluoride (APF) and NaF solutions and showed that uptake from the TiF_4 was greater than that from the NaF solution.

Tveit & Halse, (1982) treated sectioned root dentine surfaces with either an NaF solution or a fluoride varnish and then exposed them to 1 M acetate

buffer (pH 5.8). The varnish treated group specimens showed a demineralised zone which was significantly less deep than those observed in the NaF and control groups. Mellberg & Sanchez (1986) stated that monofluorophosphate slurries were significantly more effective than placebo slurries.

1.10.5. Evidence from in situ experiment.

Teranaka & Koulourides, (1987) reported that a 100 ppm fluoride mouthrinse contributed to increased fluoride incorporation into artificially created lesions *in situ*. In addition, acid resistance at the outer layer of specimens exposed to the fluoridated mouthrinse improved compared to those exposed to the placebo mouthrinse.

1.10.6 Discussion.

It is thus apparent (*vide supra*) that fluoride has a role in reducing root caries, but it is not established, which fluoride agent, and at what concentration it would be best employed for better disease prevention. Currently there is little evidence that the fluoride concentrations available in commercial toothpastes have contributed to reducing root caries prevalence. This situation might reflect the need for higher fluoride concentration if this disease is to be effectively contained.

1.11 MODELS FOR CREATING ROOT SURFACE CARIES.

1.11.1 Introduction.

In order to investigate the problem of root surface caries and to study treatment effects, a characteristic lesion has to be produced. A possible source is the natural carious lesion but such a lesion is not suitable due to the following reasons: (1) the history of the lesion is unknown; (2) the lesion may be both active and arrested at the same time (Massler, 1967) and (3) clinically, the lesion is identified by its brown stain, but an early lesion might not be identified as easily making it extremely difficult to obtain.

Therefore, it was necessary to establish a system which could produce a standard, reproducible artificial lesion in root surfaces. Such systems have included the use of cariogenic microorganisms, acid gels and buffered solutions.

1.11.2 Cariogenic microorganisms.

Root caries-like lesions were consistently produced *in vitro* using *Strep. mutans* FAI cultured in thioglycollate broth containing dextrose and gelatin (Clarkson, Wefel & Miller, 1984). These workers claimed that their lesions had some of the characteristics described for natural root caries. Their *in vitro* lesion had a completely demineralised layer below a less radiolucent surface zone of the lesion, and further experiments with *Lactobacillus casei* and *Strep. mutans* E49

have produced similar enamel and root caries lesions (Clarkson *et al.*, 1984). In a different *in vitro* model, not designed for microscopic investigation, sound root surfaces were attacked by a mixture of *Strep. mutans*, *Actinomyces viscosus* and *Actinomyces naeslundii* and observed visually and by probing. It was revealed that the lesion was leathery on touch and resembled the clinical carious lesion (Katz, Park & Palenik, 1987). In a more recent study, Kaufman, Pollock & Gwinnet, (1988) produced *in vitro* root caries by incubating exposed root surfaces to *Strep. mutans*, *Lactobacillus casei* and *A. viscosus*, and reported that the root surface pH at the end of induction averaged 4.43, 5.00, and 5.20 respectively for the three microorganisms used. The lesion depths measured on microradiographs averaged 131, 83, 34 μm for *Step. mutans*, *Lactobacilli casei* and *A. viscosus* respectively.

The claimed advantages of such a model are appealing. It can be used to compare the cariogenic potential of different bacterial strains or a mixed bacterial population, to assess the cariogenicity of food and to investigate the aetiology and prevention of coronal and root caries. Clarkson *et al.*, (1984) admitted rightly, that their systems suffered from two major disadvantages namely: it was not a well-defined system; and the use of batch culture might not totally represent the *in vivo* situation.

1.11.3 Acidified gel model.

In this model, windows of a known surface area are made on the root surfaces using an acid resistant nail varnish. Crude gels were used to create artificial lesions in root surfaces (Phankosol *et al.*, 1985a, 1985b; Wefel *et al.*, 1987) and adjusted to the required pH (ranging between 4 - 5.5) by the addition 0.1 M lactic acid. Some gels contained carboxymethyl cellulose (Al-Joburi & Koulourides, 1984; Arends *et al.*, 1987), hydroxyethyl cellulose or 25% gelatin (Mellberg & Sanchez, 1986). To eliminate the disadvantages of impurities in crude gels, dialysed gels were used and reconstituted by the addition of calcium, phosphate and fluoride (Feagin, 1984; Wefel, Clarkson & Heilman, 1987). The exposure period of the root surfaces to the acidified gel varied from 4 days (Mellberg & Sanchez, 1986) to 4 weeks (Phankosol *et al.*, 1985a, 1985b). Investigators have claimed that their systems have successfully created lesions in the root surfaces which have the characteristic features of the natural lesions. However, the successful attempt of Phankosol *et al.*, (1985a, 1985b) to create lesions in the root surfaces suffered from the following drawbacks: (1) impurities in the undialysed gels might have been important in the successful lesion formation. Among these impurities were calcium, phosphate and fluoride (Pearce, 1983). The reported laminations could have resulted from a substantial amount of fluoride in the system; (2) the windows on both enamel and root surfaces were exposed

simultanuously to the acid attack which might have resulted in the acquisition by the roots of the mineral effluxing from the enamel; (3) roots which had been exposed to the oral environment were used. Such teeth might have acquired a hypermineralised surface while exposed to the oral fluids, and (4) the period required to form a lesion is too long as a source of experimental material in de - remineralisation experiments.

It has to be emphasised that systems suitable for creation of lesions in enamel are not necessarily suitable for creating lesion in root surfaces. This might be ascribed to the high content of organic material in the root as compared to that in enamel. Phankosol *et al.*, (1985b) showed that root surface lesions were as twice deep as enamel lesions when created by the same system, and exposed to the same gel, for the same period.

1.11.4 Buffered solutions.

This is a pure demineralising system used to study demineralisation in root surfaces or to form a subsurface lesion (Hoppenbrouwers, Driessens & Borggreven, 1986, 1987; Heilman, Clarkson & Wefel, 1987; Featherstone, McIntyre & Fu, 1987; Wefel *et al.*, 1987). The pH of the buffer solution should be slightly higher than that used for enamel because of the higher demineralisation rate of the root. Buffer solutions may be constituted with the addition of calcium, phosphate and with (Heilman *et al.*, 1987; Wefel *et al.*, 1987), or without (Hoppenbrouwers *et al.*, 1986; Featherstone *et al.*, 1987) fluoride.

This system has been used extensively and successfully to create enamel lesions (Featherstone, Duncan & Cutress, 1978; Van Dijk, Borggreven & Driessens, 1979) and these investigations highlighted the important role of calcium and phosphate in maintaining or building the surface zone.

Fluoride's availability in this system seemed to be essential to create a characteristically natural lesion in root surfaces. Heilman *et al.*, (1987) created subsurface lesions only when fluoride was available for a week in the system. Hoppenbrouwers *et al.*, (1987) studied human root demineralisation by exposing them to a buffer solution at pH 5.5 which contained different concentrations of fluoride. They reported that the fluoride helped to maintain a surface zone whereas solutions without fluoride failed to produce a microradiographically distinct surface zone. Polarised light microscopic studies have shown that a surface zone could be preserved by the addition of methane hydroxy diphosphonate (MHDP) to the system (Featherstone *et al.*, 1987)

Buffer solutions have the advantages of ideal pH control, short period to form a lesion, and are impurity-free as compared to the gel system. For this project a buffer solution system was developed which could repeatedly produce subsurface lesions (*vide* Chapter 3).

1.12 ASSESSMENT OF LESION DEMINERALISATION AND REMINERALISATION.

1.12.1 Introduction.

Different investigators have shown that lesion demineralisation and remineralisation can be assessed by a variety of techniques. Among these are microradiography (Groeneveld & Arends, 1975), birefringence measurements (Silverstone, 1973), microhardness measurements perpendicular to the anatomical surface which was exposed to demineralising solution (Koulourides *et al.*, 1974), microhardness measurements on cross - sectional (cut) surfaces (Featherstone *et al.*, 1981) and measurements of backscattered light intensity (ten Bosch *et al.*, 1980).

1.12.2 Microradiography and microdensitometry.

Microradiography (so named by Goby in 1913) was introduced to dentistry by Bodecker & Applebaum (1931) in their pathological studies on dental caries.

Microradiographic and microdensitometric techniques are based on the fact that the absorption of X-rays by a calcified tissue depends on the mineral content of that tissue. Contact microradiography differs from normal dental radiography in that relatively thin tooth sections (approximately 150 μm) are placed in contact with a high resolution X-ray plate or film, and exposed to a monochromatic X-ray source. Microdensitometry is used to quantify the greyness of such an exposed X-ray plate. The technique consists basically of measuring the amount

of visible light that is transmitted through an X-ray plate. The quantitative assessment of mineral content using these techniques was first reported by Angmar *et al.*, (1963) who developed an equation for calculating the mineral content of sound enamel from enamel microdensitometric measurements and a reference aluminium stepwedge (see Section 2.5.1). This was extended to assess enamel mineral loss after demineralisation (Bergman & Lind, 1966) and, more recently, has been employed to monitor mineral loss progression (Featherstone *et al.*, 1983) and mineral changes in incipient enamel lesions following remineralisation (Strang *et al.*, 1987).

Quantitative microradiography is an accurate, repeatable and convenient method for the determination of changes in mineral content of a mineralised tissue (Goto, 1965). It obviated the drawbacks of polarised light microscopy (*vide infra*) and is almost completely independent of the physical state of both mineral salts and apatite orientation. After its success in assessing the de- and remineralisation of dental enamel, it has become increasingly popular in similar root caries studies (Mellberg & Sanchez, 1986). As this was the method used to determine mineral content in the studies reported in this thesis, further details on the technique and its theory are presented in Chapter 2.

1.12.3. Polarised light microscopy

Light can be thought of as waves of different frequencies vibrating in all possible planes. If the light is restricted to a single plane it is "plane polarised". The rays may then behave differently in their orientation with respect to the structure of the material. Examples of such birefringent structures are enamel, dentine and cementum. The mineral of these structures has the property of resolving a beam of plane polarised light into two rays which travel at different velocities. Such a structure has two refractive indices, related to the two planes of transmission within the crystals (Schmidt & Keil, 1971).

In an enamel prism, the slower ray is found to vibrate at right angles to the length of the prism. Therefore the sign of birefringence is described as negative (intrinsic birefringence) with respect to prism length, the opposite to that found with protein fibres (Silverstone, 1973).

Dentine contains three birefringent components, the positive collagen fibrils, the negative crystallites of the mineral and the positive Tomes' fibres (Schmidt & Keil, 1971), with the last having no significant effect upon the total birefringence. Collagen birefringence is closely associated with that of the mineral component since the crystallites are orientated along the collagen fibres in such a way that the optical axes of the two components (of opposite sign) coincide. The strength and sign of the birefringence of dentine in its natural state is determined by the collagen components. In intact ground

sections, mineral salts will only be perceived if they are spherically (in globules) arranged, or if the collagen has either disappeared during development, as in durodentine, or through fossilisation.

The cementum possesses a weaker birefringece than dentine and enamel. The sign in radial and transverse ground sections is opposite to that of the adjacent dentine.

As soon as the carious process has reached the dentine, the progressive decalcification results in a greater contribution to the birefringence. Therefore, the partially decalcified regions would exhibit stronger birefringence (positive) than healthy dentine due to the removal of mineral which is of negative sign.

The use of polarised light microscopy has several advantages. As a descriptive technique the demarcation between cementum and dentine is quite obvious as the tissues have opposite signs of birefringence with respect to the cemento-dentinal junction (Wefel *et al.*, 1985).

Unfortunately, in comparison to the use of polarised light to study subsurface enamel lesions, no change in the sign of dentine birefringence is observed in the body when imbibed in water. When sections are imbibed in quinoline, a structureless area may show an actual reversal in the birefringence sign, know as "phenol reaction" (Wefel *et al.*, 1985). (For further details on the microscopic appearance of the subsurface carious lesion see Section 1.6).

Polarised light microscopy has been used to

quantify the mineral loss in the enamel subsurface lesion by measuring the observed birefringence after imbibing the section in a known refractive index material (Silverstone, 1973). However, the author is unaware of any similar attempt in the case of root caries.

Polarised light, as a sole routine quantitative method of lesion assessment, is limited since not only is it time-consuming, it underestimates mineral loss and needs to be calibrated against microradiography (Shellis & Poole, 1985).

1.12.4 Microhardness.

Microhardness measurements involve indenting the hard tissue of the tooth, with the assumption being made that resistance to indentation will increase with increasing content of mineral. The methods used commonly for hardness evaluation include Vicker and Knoop. Measurements are made using a microscope since the indentations are too small to be seen by the naked eye.

Changes in enamel hardness have been measured in two different ways namely: (1) the indentor load is perpendicular to the anatomical enamel surface; (2) the indentor load is parallel to the anatomical surface i.e perpendicular to the cut surface. The first technique has the claimed advantage of being non destructive and enabling a longitudinal study of the same specimen (Featherstone, 1983). Arends *et al.*, (1979) have shown a correlation between the indentation length and the lesion depth for lesions created with acidified solutions or

gelatin gels, but such a correlation did not hold after mineralisation. A disadvantage of the technique where indentations are made on the outer enamel surface, is that it does not give details of hardness changes below the surface nor in different regions of the lesion.

In experiments on cross sections (cut surfaces) changes during de- and remineralisation were compared but without knowing what the changes imply quantitatively in terms of mineral loss (Featherstone, Cutress & Rodgers, 1981, Featherstone, Rodgers & Smith, 1982). A relationship between microhardness on the cross-sectional surface and microradiography-assessed mineral content has been demonstrated (Featherstone *et al.*, 1983; ten Cate *et al.*, 1985).

Few investigations have been carried out to study the microhardness of the root tissues (Rautiola & Craig, 1961; Jones & Boyed, 1987). Rautiola *et al.*, (1961) showed that there was no difference between the microhardness of exposed and unexposed cementum which implied that the amount of mineral in both was the same. This was in direct contradiction with microradiographic data which showed that the cementum exposed to the oral fluids was more mineralised than unexposed cementum (Furseth & Johansen, 1968). Moreover, the anatomical or cross - sectional surfaces have to be ultrapolished, which might result in non-uniform thickness, thus making the microhardness data deceiving. Jones & Boyde (1987) stated that the microhardness values in mineralised tissues relate to the orientation of microstructural elements such as collagen

fibrils and apatite crystals, whereas microradiography assesses the mineral content of the hard tissue. Thus, one could suggest that the two techniques were testing different factors.

Microhardness measurements are not sensitive to minor changes in mineral content whereas mineral changes as low as 5% could be detected by microradiography. In conclusion, the use of microhardness methods for determining mineral loss or gain might be suitable for enamel studies but not for roots because of the large collagen content.

1.12.5 Block face microscopy.

This is a conceptual title to describe a group of methods used to study the structural integrity of the dental hard tissues. The group includes back-scatter electron imaging and Tandem scanning reflected light microscopy.

Back - scattered electron imaging, used on cut and highly polished facets of teeth embedded in PMMA (Poly methyl methacrylate), enabled the fine details of changes in mineralisation levels to be detected (Jones & Boyde, 1987). Using a ring detector configuration with the electron beam at normal incidence to a topography-free surface, the image contrast depends on density variation in the sample surface layer, mainly due to variation in the degree of mineralisation.

When cut surfaces of teeth were air - polished with $NaHCO_3$ powder, and studied with secondary electron

emission mode, or reflected light microscopy, erosion rates were found to be inversely proportional to hardness, which, in turn, was related to both degree of mineralisation and the orientation of structure element (Boyde, 1984).

The Tandem scanning reflected light microscope is the direct view or confocal scanning optical microscope. It permits the study of very thin focussed layers deep to the surface of large samples such as whole or sectioned teeth. Images are formed by reflection or fluorescence.

In enamel, the scattering of light by sound tissue, and by a white spot lesion has been studied using thin slabs (Spitzer & ten Bosch, 1975, 1977; ten Bosch, Borsboom & ten Cate, 1980). These studies with standard equipment yielded quantitative data on the scattering coefficients as a function of wave-length. They showed, as expected, a higher coefficient in lesion material as compared to sound enamel.

The intensity of the back-scattered light is determined by the scattering properties of particles inside the samples and geometric distributions. In case of sound tissues this technique might be valid, but little is known if, in case of remineralisation, mineral will keep the same geometrical arrangement as in sound tissue. In addition, these methods remain only descriptive and might not be suitable for remineralisation studies unless they are made fully quantitative. Jones & Boyde, (1987) claimed that once these are made quantitative they would have more resolution than microradiography.

The block face microscopy methods have the advantages that sections do not need to be produced, and the plane parallel sections needed for the proper interpretation of polarised light microscopy and microradiography can be avoided altogether. The lateral border of a carious dentine lesion could be sharply defined.

1.13 EXPERIMENTAL DESIGN.

In this section, the various *in vitro* and *in situ* experimental designs employed to study remineralisation of the incipient root lesion will be reviewed and the advantages of the *in situ* experiment will be highlighted.

The experimental designs used for enamel studies have been adopted for root surface caries investigations. These designs varied between (1) attacking an enamel specimen with an acid after immersion in a fluoridated solution or slurry for a certain period and (2) the experimental set-up in which specimens are subjected alternately to de- and remineralising solutions to simulate the intraoral pH-changes.

In most studies involving root, the first design was used extensively (Shannon, 1980; Derand & Petersson, 1982; Al-Joburi & Koulouride, 1984). Although, the second technique has the advantage of mimicking the oral environment, surprisingly few experiments have been carried out using this technique.

In vivo and *in situ* experiments have been designed

to study remineralisation of enamel under normal oral environmental conditions, and thus eliminating the *in vitro* disadvantages of using artificial saliva and working at a temperature which is different from that of oral cavity.

Stagnation areas were created by means of orthodontic bands in premolar teeth destined to be extracted for orthodontic reasons (Holmen *et al.*, 1985). Gaps were left between the band and the tooth so that plaque can accumulate there freely and the teeth were subsequently removed after being subjected to a cariogenic challenge, to assess lesion characteristics.

Koulourides *et al.*, (1974) introduced an *in situ* technique which involved using the buccal flanges of a dental prosthesis in which two slabs could be embedded, one acting as a control and the other as experimental. Although this technique contributed to a better understanding of the remineralisation process, the use of slabs, however, suffers from the disadvantage that mineral content varies not only between two different teeth but, also in the same tooth (Groenveld *et al.*,1975). The caries susceptibility, therefore, must vary. In addition, measurement of mineral content can only be made at the end of the experiment.

To obviate the disadvantages of the above experiments, Creanor *et al.*, (1986) developed an *in situ* model in which single sections can be used. The sections are microradiographed at baseline and after treatment with fluoride. Thus, each section acted as its

own control. The flow of saliva was made free in both lingual troughs by making two entrances and two exit holes at the top and bottom of the troughs respectively. (For full detail of appliance construction and features, *vide* Chapter 4).

Teranaka & Koulourides, (1987) extended the *in situ* technique for remineralisation studies in roots. Four slabs (two bovine and two human) were mounted on the recesses of buccal flanges of a partial denture which was worn for a certain period. Although, the *in vitro* disadvantages were eliminated, this technique has not eliminated the disadvantages of using slabs.

The orthodontic band technique is inappropriate for root surface caries studies since experiments can only be done on crowns of teeth destined for extraction.

The appliance of Creanor *et al.*, (1986) was the model of choice for the purposes of this project, for the following reasons: 1. it has obviated the disadvantages of using a slab, 2. it was used successfully for studies related to remineralisation of the incipient lesion in enamel (Creanor, 1987), 3. it has been used to characterise microorganisms associated with enamel demineralisation (Macpherson, 1987); 4. it is well tolerated by the majority of volunteers; 5. it has been used successfully for studies of remineralisation with different fluoride agents (Strang *et al.*, 1987): and finally 6. the technical expertise used to construct these appliances was available for this study.

CHAPTER TWO.
GENERAL MATERIALS AND METHODS.

2.1 INTRODUCTION.

This chapter provides a general account of the experiments described in this thesis. Tooth collection and preparation, lesion production (based on pilot studies as described in Chapter 3), tooth sectioning and grinding techniques are all detailed below. In addition, the microradiographic and microdensitometric procedures used to quantify the mineral content in the lesions are also described.

2.2 TOOTH SELECTION AND PREPARATION.

2.2.1 Introduction.

When the roots of teeth become exposed to the oral fluids as a result of gingival recession, the cementum acquires a densely mineralised surface layer (Forsberg *et al.*, 1960; Selvig & Zander, 1962; Furseth & Johansen, 1968; Westbrook *et al.*, 1974). Therefore, the composition of such exposed cementum varies from unexposed cementum, since the latter is protected from oral fluids by the periodontal tissues.

However, in teeth with long - standing gingival recession the exposed root surfaces are unlikely to have an intact cemental layer. Cementum has a low abrasion resistance, and once exposed is easily worn away by mechanical stimuli such as scaling and tooth - brushing.

In a few cases, abrasion lesions can extend to the pulp (Kitchin, 1941). Phankosol *et al.* (1985a) identified cracks in the surface layer of the *in vitro* produced root lesion. It was thought that the thinness of the cementum was responsible for the cracks (Mellberg & Sanchez, 1986). Therefore, it was decided that remineralisation should be studied in two dental tissues, the dentine and cementum. Thus cementum should be completely removed from some of the tooth surfaces used for this project in an attempt to attain a less chemically and physically, variable surface.

2.2.2 Collection, storage and preparation of the teeth.

The teeth used for experiments in this project were obtained from the clinics of general dental practitioners in the Glasgow area (water fluoride level < 0.03 ppm) and from the Oral Surgery Department, Glasgow Dental Hospital. All experiments were carried out on erupted upper and lower premolar teeth which were extracted for orthodontic purposes and the roots of which had not been exposed to the oral fluids. Immediately after extraction, they were rinsed briefly in tap water to remove blood etc and stored in jars containing distilled water and thymol crystals until required (Feagin, 1984; Al-Joburi & Koulourides, 1984; Teranaka & Koulourides, 1987).

Teeth were scaled gently with a scalpel (No 23 Swan Morton, England B.S) to remove the periodontal membrane remnants from the root surfaces from half of the teeth,

taking extreme care not to scratch the cementum. Six to eight strokes were found to be sufficient for the complete removal of the cementum. Roots were then polished briefly with pumice and a rubber cup. The teeth were thoroughly washed in soapy water and dried. The root surfaces were examined at 10x magnification under the stereomicroscope to confirm there were no scratches or abrasions. Fig. 2.1b shows a polarised light microscope picture of a section from which all cementum has been removed in the manner described above, also, shown in Fig 2.1a an intact non-abraded root surface section on which cementum can been seen. Only those teeth that had similarly clean, smooth surfaces were chosen for this study.

As stated in the introduction to this section, it was decided to prepare samples with and without cementum. Mechanical removal of cementum was used as the method of choice, since demineralising chemicals such as acids might penetrate deep into dentinal tissue (Patterson, Weatherell & Robinson, 1984) and alter its structure. Mellberg & Sanchez (1986) who employed acid, admitted that it affected their underlying dentine and stated they then had to polish thoroughly the etched surface to remove such effects.

2.3 ARTIFICIAL ROOT SURFACE LESION CREATION.

2.3.1 Introduction.

The systems used by different researchers to create subsurface lesions in roots were described in Chapter 1.

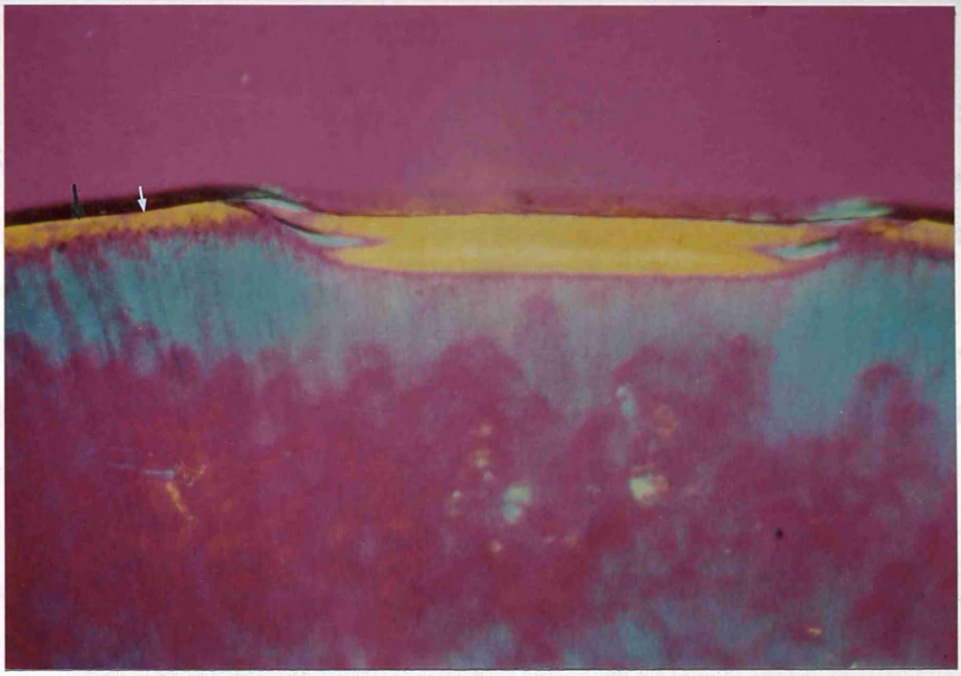


Figure 2.1a Polarised light microscope picture of an intact non - abraded root surface section with a lesion.
Arrow points to cementum surface.

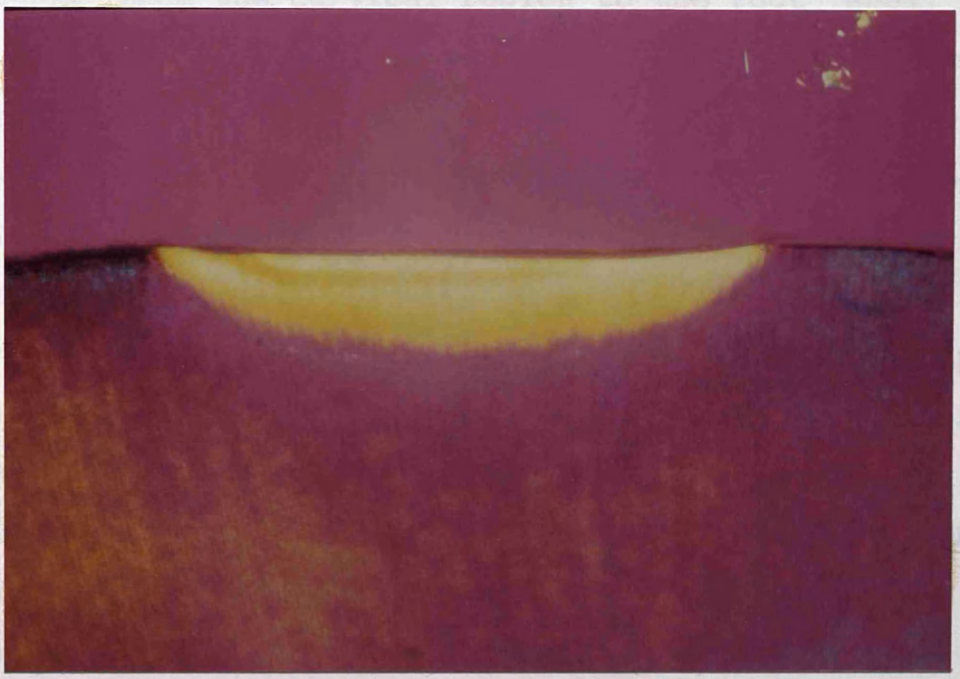


Figure 2.1b Polarised light microscope picture of a lesion in a section from which all cementum has been removed.

The technique chosen for this project was the buffered solution system, because here ingredients are easily controlled, and it has no impurities, such as fluoride, calcium or phosphate as compared to the undialysed gel method (Feagin *et al.*, 1984). Solutions of different composition and pH were prepared in a pilot experiment, (*vide* Section 3.3), before it was established that a pH 5 solution, (*vide infra* for composition), was able to create a lesion of suitable size.

2.3.2 Varnishing and window creation.

Four narrow strips (0.46 mm wide) of adhesive tape were applied longitudinally to the centre of the buccal, mesial, lingual and distal surfaces of the root and extended from the cervical region to the root apex. The whole tooth was then covered with a coat of nail varnish (Max Factor, London, England). The first coat was left in the open air for 1-2 hours before another coat was applied to ensure adequate varnish thickness. The teeth were left overnight to allow the varnish to dry. Under a stereoscopic microscope the adhesive tape was gripped firmly with tweezers at one end and peeled off the root surface, making sure that no adhesive was left on the window site. Figure 2.2 shows two teeth with the adhesive tape and two samples (without the tape) ready for immersion into the artificial caries solution.

2.3.3 Root immersion in the buffered solution.

The varnished tooth, with prepared root windows,

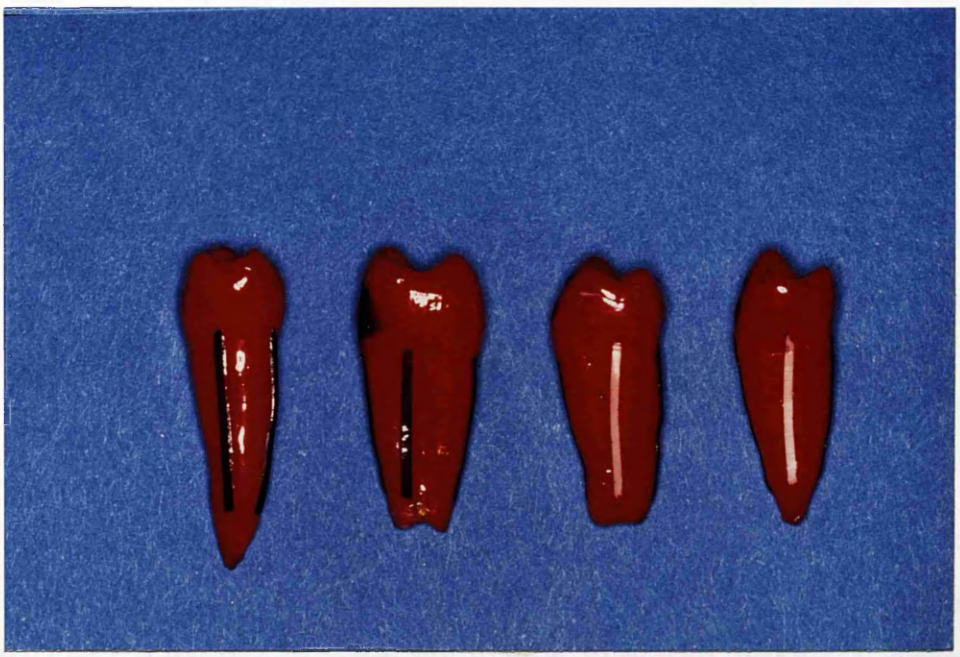


Figure 2.2 Varnished roots, two with the adhesive tape on and two after the tape was removed before immersion into the artificial caries system.

was immersed in 20 ml of pH (5.0) buffered solution in a plastic bottle. The composition of the buffered solution is given in solution No. 13, Tab. 3.1. The surface area per window was = 0.46 (tape width) x 10mm (tape length) i.e 4.6 mm^2 and per tooth was, 0.46 x 10 x 4, i.e 18.4 mm^2 since each root had four windows prepared on it. Therefore, the volume to surface area per window was 5 ml : 4.6 mm^2.

All specimens were immersed in the solutions for five days, following a further five days in a fresh solution. The ten days' immersion period was found to be adequate to produce a lesion of suitable size which still retained an intact surface over the body of the lesion (see Section 3.3.2).

2.4 SECTION PREPARATION.

At the end of the ten day's acid exposure, the tooth was taken out of the solution and washed with water. The apical third of the root was removed using a dental drill (Milbro, Epsom, England) and diamond disc. The tooth was then mounted with the root apex facing uppermost on a specially made square acrylic block, framed in metal, with "Loctite" adhesive (Loctite (U.K) Ltd., Welwyn Garden City, England). The block was fixed securely with screws on the holder of a saw microtome (Fig 2.3). The specimen was left for at least one hour to allow adequate hardening of the adhesive.

Using the saw microtome (E. Leitz (instruments) Ltd., Luton, England), sections were cut perpendicular to

Figure 2.3 The Leitz saw microtome with a specimen fixed on the block which was screwed firmly in position.

the long axis of the tooth to a maximum thickness of 350 µm.

The 350 µm thick sections were placed, one at a time, under a 5 mm thick brass plate covered with a damp gauze. One surface was ground slowly for 10 seconds. The section was then turned over and the other surface ground to remove the concentric rings caused by the saw microtome. Because friction between the section and the damp gauze was greater than that between the section and the carborundum slurry on the glass plate, the section remained immobile under the gauze - covered brass plate. Measurements of section thickness were made at intervals until a final thickness between 130 - 200 µm was achieved. Each section was given a number, and coded with the letter C (for cementum) or D (for dentine). The smaller the number, the more apical was the section.

Section thickness was measured using a digital micrometer (Mitutoyo, Tokyo, Japan; Fig 2.4). Measurements were made on all four aspects of the section i.e mesial, distal, buccal and lingual and a mean of all measurements obtained. Normally, four to six measurements were taken at each site. A section thickness of 130 - 200 µm was found suitable to permit an adequate microradiographic grey level range (see Section 3.4.3).

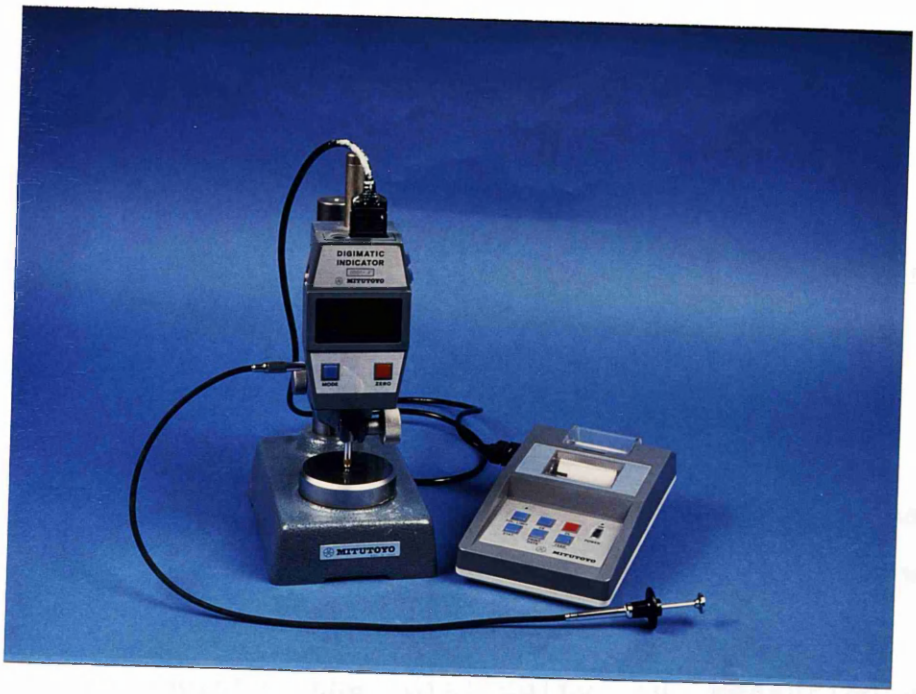


Figure 2.4 The digital microtome used for measurement of root section thicknesses.

2.5 MICRORADIOGRAPHY AND MICRODENSITOMETRY.

2.5.1 Theory.

X-rays are absorbed exponentially in a material and the intensity, I, of X-rays passing through a material (and hence incident on an X-ray plate) is given by:

$$I = I_o \cdot e^{-ut} \qquad -(1)$$

where I_o = the incident X-ray intensity

u = the X-ray linear absorption coefficient for that material

and t = the thickness of the material

Angmar *et al.*, (1963) assumed that enamel consisted of an organic and a mineral component with equivalent thickness t_o and t_m respectively. In addition, to obviate the difficulty in measuring the incident X-ray intensity, they introduced a reference aluminium stepwedge. Thus, for any point in the sample, the X-ray absorption i.e. measured grey level, can be equated against an equivalent aluminium thickness, t_a which gives the same level of greyness.. Thus:-

$$u_a t_a = u_m t_m + u_o t_o \qquad -(2)$$

where u_a, u_m and u_o are the linear absorption coefficients of aluminium, the mineral component and the organic component respectively.

However, the section thickness, t_s is equal to the sum of the thicknesses of the two components, i.e.:

$$t_s = t_m + t_o.$$

Also,

$$V_m/V_s = t_m/t_s$$

where V_m and V_s are the volumes of the mineral component and section respectively.

Thus, substituting into and rearranging equation (2) gives:

$$\text{the percentage mineral content} = (V_m / V_s) \times 100$$
$$= 100.(u_a t_a - u_o t_o) / t_s.(u_m - u_o) \quad - (3)$$

The absorption coefficients depend on the absorbing material and on the radiation source (i.e kilovoltage and target and filtering materials). Angmar *et al.*, (1963) employed a monochromatic CuK source and were able to obtain the absorption coefficients from known data. Thus, substituting these known values into equation (3) gave:

$$\% \text{ volume mineral} = 52.77 \times (t_a/t_s) - 4.54 \quad - (4)$$

Since the section thickness can be measured, the only unknown in this equation is the equivalent aluminium thickness. This is determined for every point in the area of measurement by equating grey levels and

the percentage volume mineral at that point calculated by substituting the appropriate aluminium thickness in equation (4).

Angmar *et al.*, (1963) assumed that hydroxyapatite had an average composition of 37.1 % Ca, 18.1 % P, 43.3 % O, 0.7 % C and 0.3 % H, giving a Ca:P ratio of 2.05. In the application of this equation to root caries studies, it has also been assumed that the hydroxyapatite in dentine has the above composition. In addition, like the enamel studies, it has been further assumed that the Ca:P ratio in remineralised tissues was similar to that in undemineralised dentine.

2.5.2 Microradiography methods.

Root sections were enclosed along with an aluminium step wedge in the centre of two layers of "Cling film" and mounted on Kodak high resolution plate type 1A (Eastman Kodak Company, Rochester, New York, U.S.A.). The plates were then enclosed in a holder, (Fig. 2.5), and exposed to CuK radiation from Marconi X-ray tube (Tx12) in an Enraf Nonius generator for 20 minutes at 20 kv and 30 mA with a target specimen distance of 300 mm. The holders were placed in the X-ray machine during exposure in such a way that the aluminium step wedge was along the Y-axis of the plate. The reason was that the X-ray beam exhibited a variation from "north to south" (vertical) but was homogeneous along the "east to west" (transverse) plane. This permitted microdensitometric measurements of the aluminium wedge to be made at the same vertical level

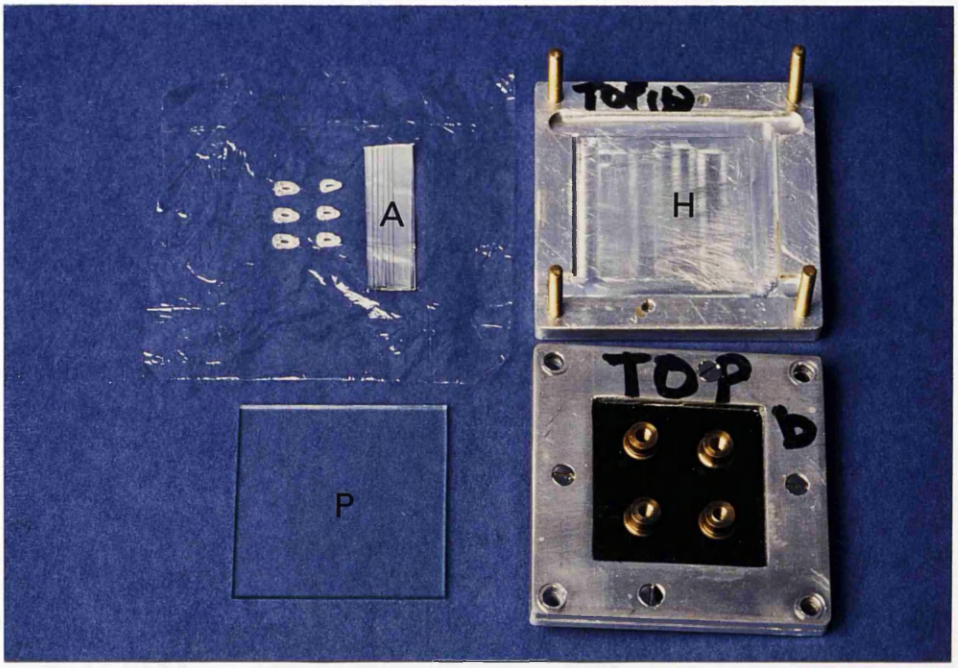


Figure 2.5 Root sections and aluminium step-wedge (A) with radiographic plate (P) and plate holder (H) used for microradiography.

as i.e equal incidence of X-ray intensity, the measurements of the lesion.

2.5.3 Microdensitometry methods.

Analyses were carried out using an ASBA image analyser (Fig. 2.6). It consists of a microscope (Leitz Dialux 22) with a stabilised power supply, and a video camera (ASACA Corporation Type 700BE) mounted on the microscope. The analyser unit, controlled by a Z8002 microprocessor, allows the video signals from the camera to be digitised into 256 grey levels with a resolution of 256 x 256 pixels. Potentiometers on the ASBA front panel, enable the unit to be set up so that the 256 grey levels cover the region of interest. The digitised image is then transferred to a BBC-B computer (Acorn, Cambridge, U.K) for further analysis and storage of results. The software for the ASBA and the BBC-B computers was written by Dr. R. Strang.

Initially the microradiograph was positioned on the microscope stage so that the carious lesion was in the field of view, and the camera rotated until the lesion surface layer was horizontal on the ASBA monitor. The intensity of the microscope light was adjusted to give maximum illumination without saturating the video camera. Thereafter, the camera was blackened and a black level taken. The microscope stage was moved to bring the thickest aluminium wedge into view. An area, 64 X 64 pixels (192 x 192 μm), was sampled in the centre of the screen and average grey level corresponding to that wedge

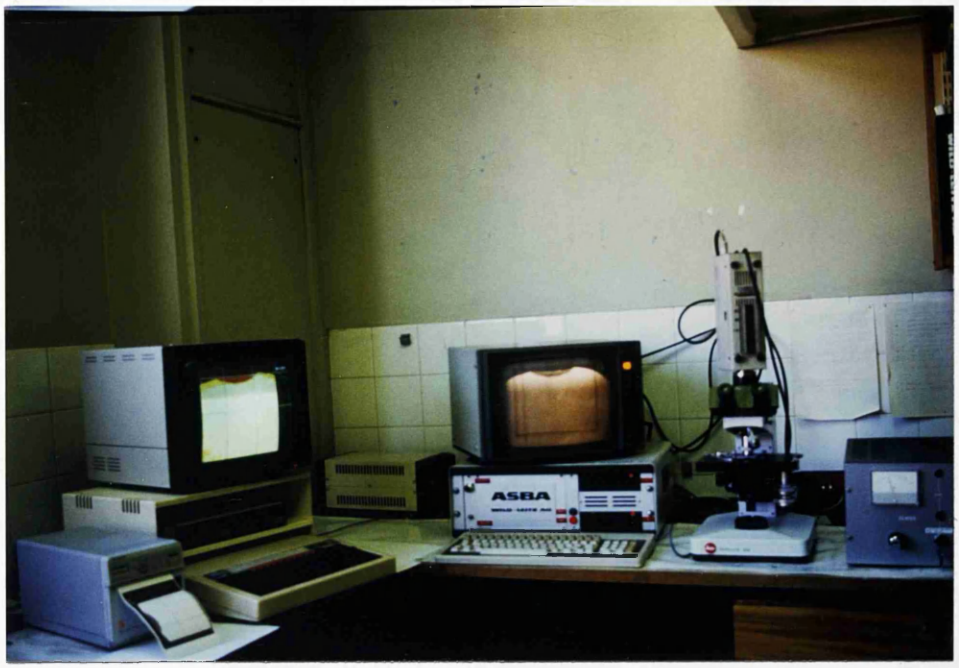


Figure 2.6 The Leitz image analyser and the BBC-B microcomputer for microdensitometric analysis of microradiographs.

calculated and transferred to the BBC computer. The grey levels for the other thicknesses of the stepwedge were calculated and transferred in turn. The radiograph was then positioned so that the lesion was again in the field of view, and the image then transferred to the BBC computer. At the standard magnification used, one pixel corresponded to 4.7 μm. Software colour - coded the grey levels in the image and redisplayed the lesion on the BBC computer (Fig.2.7). At this point, using the keyboard, the width and position of the area of interest on the lesion could be altered for further analysis. This was shown by vertical lines on the monitor (Fig 2.7). The computer then calculated the average microdensitometric profile within these lines and displayed it at the side of the screen. At this stage, the profile was still in terms of grey levels. Horizontal lines were then positioned over the image to limit the profile information stored by the computer. Prints were usually obtained using a Sony Video Graphic Printer (UP-701), which assisted the operator to position the lines in subsequent radiographs of the same lesion.

The grey levels of the profile were converted to per cent volume mineral using the equation derived by Angmar *et al.*, (1963). The profiles and all data were stored on a floppy disc for subsequent analysis.

2.5.4 Measured parameters.

All lesions were normalised to a sound root mineral content of 47% (Nikiforuk, 1986) prior to data handling. As with standard microdensitometric profiles of

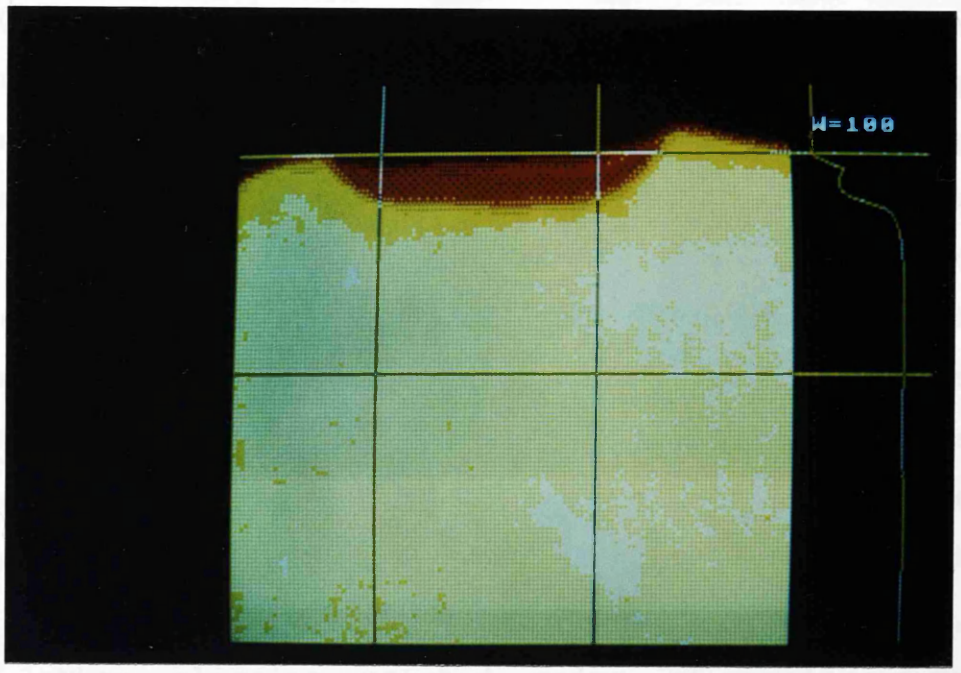


Figure 2.7 Colour-coded image of root subsurface lesion shown on computer monitor with lower horizontal and vertical lines positioned to determine the area of analysis. The upper horizontal line is positioned on a fixed point with respect to the concave nature of that lesion. The average grey level within the area of analysis and the stepwedge values (dotted lines) are shown on the right hand side.

enamel caries, the following measurements were made : (a) lesion depth; (b) the mineral content of the surface zone and lesion body, and (c) the total mineral lost from the lesion. This last parameter is generally termed Δz and has units of % volume mineral x μm.

Quantification of mineral deposition by integration of the areas obtained from mineral density profiles, or by changes in lesion depths, depends on making measurements from a standard reference point on the profile. When such measurements are performed, the comparisons are made between treated and untreated specimens. Usually in enamel studies, it has been common practice to align the mineral density profiles using the outermost mineral - i.e where the curve begins to rise above background (Fig. 2.8a), or else by using the peak mineral content (surface maxima) of surface layer (Fig. 2.8b). When aligning matched pairs of profiles from lesions before and after treatment, one anticipates that an exact reference point on one profile can be identified on the other e.g if the surface layer maximum is used as the reference point, it is assumed that the depth of the maximum mineral density will not shift right or left on the depth axis.

While measurement of surface zone and lesion body mineral contents can be applied directly to root surface caries lesions, the direct application of the Δz parameter will suffer from disadvangtages. It has been demonstrated by Mellberg & Sanchez (1986) that remineralisation of root surface lesions frequently

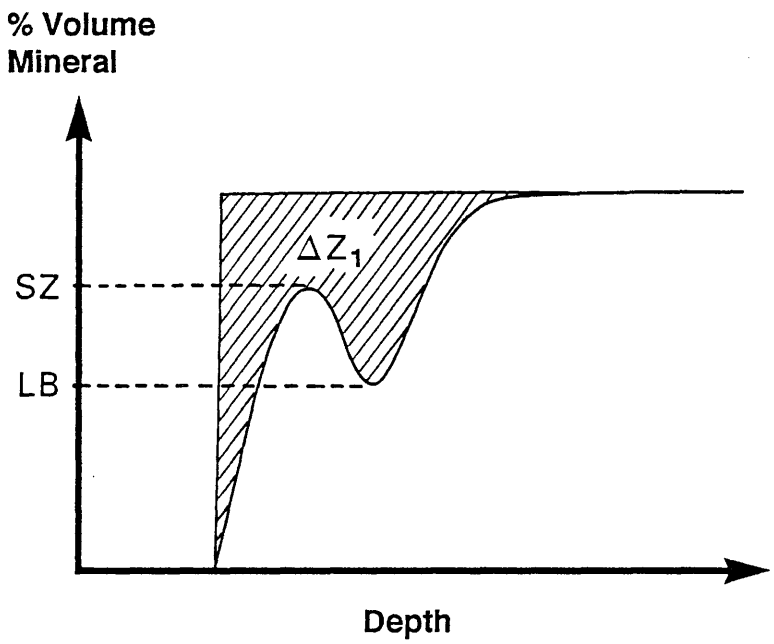


Figure 2.8a Microdensitometric profile of mineral content versus depth in dentine. Definition of (Δz_1) mineral lost (shaded area) from where the curve begins to arise above the background. SZ - % mineral content of the surface maximum. LB - % mineral content of the Lesion Body minimum.

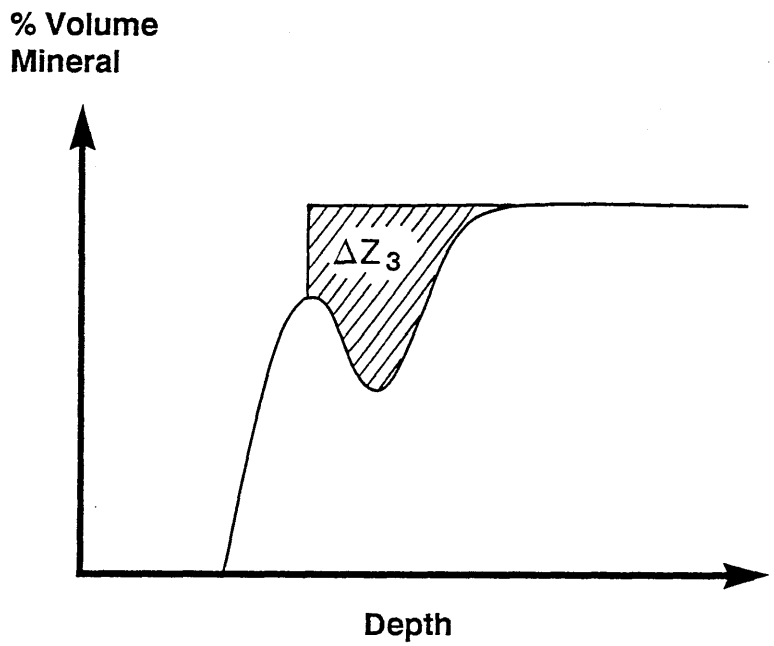


Figure 2.8b Microdensitometric profile of mineral content versus depth in dentine. Definition of (Δz_3) mineral lost (shaded area) from the peak of surface zone.

resulted in mineral being deposited on the surface of the lesion (restoring the surface lost in erosion or shrinkage). Thus, the use of the Δz parameter can result in estimation errors of the amount of mineral deposited. In this situation, the Δz parameter based on an outermost mineral point overestimates the amount of mineral originally lost from the lesion (Fig 2.8a) whereas the z parameter based on the maximum point on the surface zone (Fig. 2.8b) will not allow for any mineral deposited onto the natural surface. Mellberg & Sanchez (1986), suggested using a non-movable landmark which is fixed near the lesion. However, preliminary experiments utilising this suggestion failed to identify a reference point which was constant within a few microns during the repeated coating and removal of varnish during experimental procedures employed with the single section technique in studies reported in this thesis.

It was therefore decided that in addition to both the enamel caries Δz parameters (now termed Δz_1 and Δz_3), a further Δz parameter (Δz_2), would be used which measures the integrated mineral from a fixed anatomical point (Fig 2.9). The baseline for this latter parameter was fixed on the colour -coded representation of the microradiograph (Fig 2.7) and was based on the concave nature of the artificial lesions produced. The main disadvantage of this parameter was that it includes a large area of zero mineral content, therefore, decreasing its sensitivity to small mineral changes. The accuracy and repeatability of this approach will be further discussed

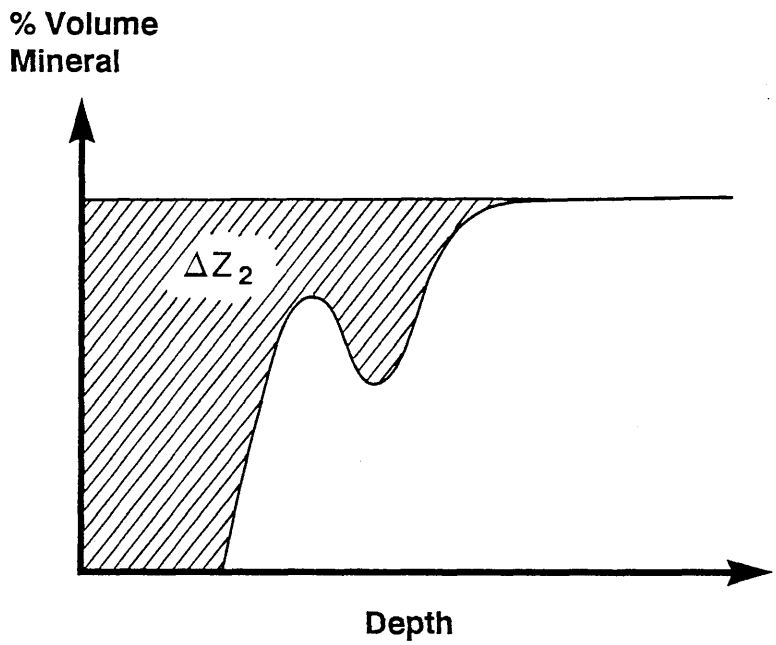


Figure 2.9 Microdensitometric profile of mineral content versus depth in dentine. Definition of (Δz_2) mineral lost (shaded area) with the x-axis origin set on a fixed anatomical point (see also fig. 2.7).

in Section 3.6.

For mineralisation rate measurements, each parameter was plotted against time (0, 2, 4 and 5 weeks), and the least square fit was determined. For most of the lesions studied, the results showed negative slopes for Δz parameters (the integrated mineral loss values decreased with time), whereas they showed positive values for surface zone and lesion body parameter (mineral content increased with time), and in order to maintain consistency in all parameters, the negative values were changed into positive values.

CHAPTER THREE.
PRELIMINARY STUDIES.

3.1 INTRODUCTION.

Since few experimental details are available regarding root subsurface lesion remineralisation and, as natural lesions were not readily obtainable, there was a need for a demineralising system which could produce a standard or reproducible root surface caries lesion.

Moreover, little is known about the application of microradiographic / microdensitometric methods to the study of root caries.

Furthermore, mineral variation might exist between different parts of the roots both at the same horizontal and cervico - apical plane. Such variations were identified for enamel and restricted the use of a control slab or section for *in situ* and *in vitro* experimental designs (Section 1.13).

3.2 AIMS AND OBJECTIVES.

The aims and objectives of this part of the project were therefore:

1. to develop a system capable of producing repeatedly a characteristic subsurface root caries lesion with a microradiographic distinct surface layer overlying a radiolucent area;
2. to investigate the changes required to extend

enamel microradiographic / microdensitometric methods to root caries;

3. to assess inter - lesion, section and tooth mineral variations;

4. to investigate the repeatability of measuring the same lesion on different occasions.

3.3 DEVELOPING AN ARTIFICIAL CARIES SYSTEM.

As mentioned previously natural lesions are of unknown history and difficult to obtain. Therefore, a standard or reproducible artificial caries lesion had to be produced for investigating root remineralisation. The different artificial caries systems have been discussed in detail in Section 1.11.4 and the choice of the buffered solution system was justified. It must be stated that, at the start of this project, there were few published details regarding a buffered solution which could be used for producing a root subsurface lesion.

In this study, the effects of pH, exposure time and solution composition, in particular F^- concentration, were varied in order to obtain a suitable solution which would produce characteristic subsurface lesions in a reasonable time period with the minimum F^- level in the solution.

3.3.1 Materials and methods.

Premolar teeth roots which had not been exposed to the oral environment were used. The roots were prepared and windows created as detailed in Sections 2.2.2.,

2.3.2.

A total of 14 solutions were prepared, the compositions of which are given in Table 3.1. The pH of the solution was adjusted by the addition of 1 M sodium hydroxide.

Three roots were immersed in each solution (1 root : 20 ml) for 1, 2 and 3 week periods. At the end of each period, one root was taken out, sectioned transversely using a saw microtome and ground to a thickness of approximately 150 μm. The sections were microradiographed on a high resolution plates which were then developed by standard methods (*vide* Section 2.5.2).

Microradiographs were inspected under the microscope to assess if subsurface lesions were present. No mineral loss quantification was attempted as the task was only to obtain a subsurface lesion.

3.3.2 Results.

Although demineralisation was seen at each window site exposed to the demineralising solution, none had the characteristic features of a subsurface lesion (pH 5, ppm F^- = 0), (Fig. 3.1). However, when fluoride was added to the same solution (solutions 4, 12), excellent subsurface lesions, with a very distinct surface layer, were produced (Fig. 3.2)

In the non - fluoridated solutions, the depth of the lesion was both time and pH dependent. Three week immersion periods produced relatively deeper lesions compared to one week periods (Fig. 3.3a, 3.3b). When

Table 3.1 Composition of test solutions used to create root lesions.

Solution No	pH	Solution composition $CaCl_2$ mM	NaH_2PO_4 mM	ppm F^-	Result
1	5.0	1.2	1.2	0	N
2	5.3	1.2	1.2	0	N
3	4.5	3.1	3.1	0	N
4	5.0	1.2	1.2	1	P
5	6.0	1.2	2.0	0	N
6	6.0	1.5	2.5	0	N
7	5.5	1.2	2.0	0	N
8	5.5	1.5	2.5	0	N
9	5.5	1.8	3.0	0	N
10	5.5	1.2	1.2	0	N
11	5.5	1.5	1.5	0	N
12	5.0	1.2	1.2	0.5	P
13	5.0	1.2	1.2	0.1	P
14	5.0	1.2	1.2	0.05	N

N = no surface zone present

P = surface zone present

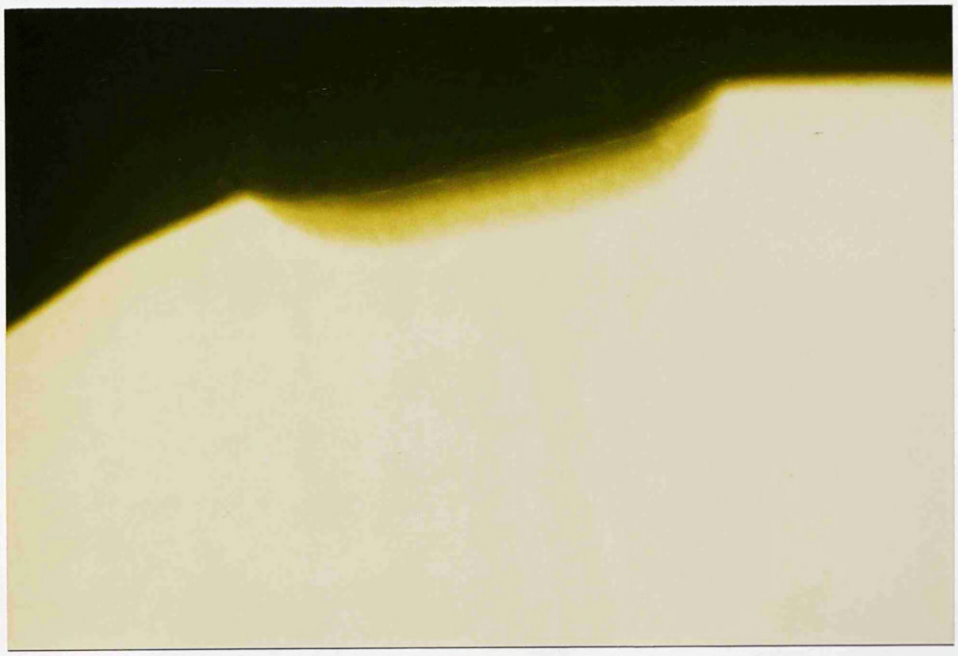


Figure 3.1 A microradiographic picture of a section with a demineralised area (no surface zone formed) after 10 days exposure to a non-fluoridated demineralising solution. pH 5.

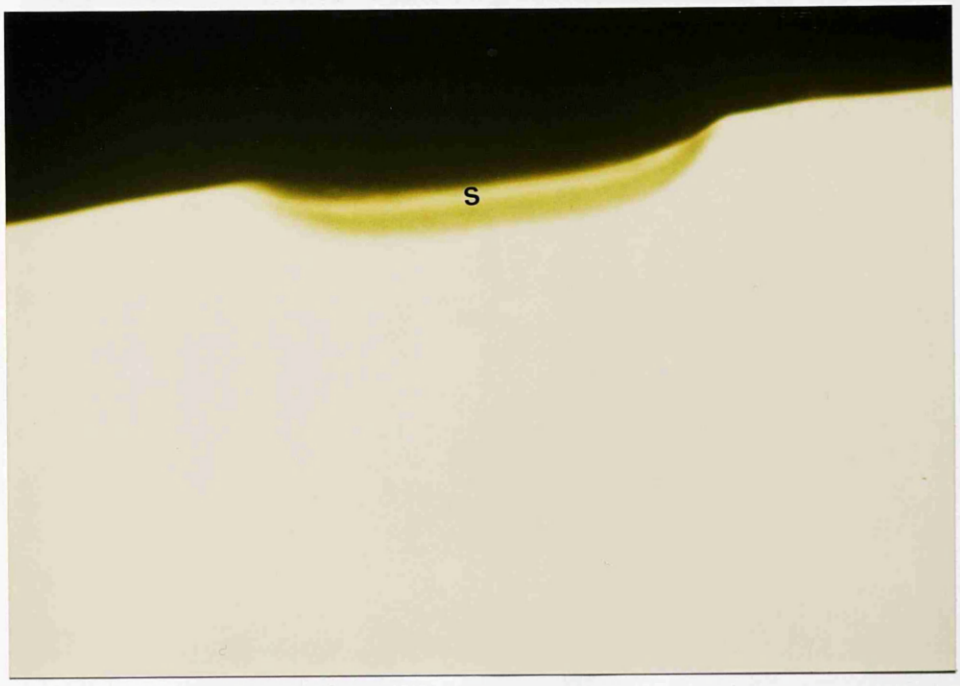


Figure 3.2 A microradiographic picture of a lesion produced after 10 days exposure to a fluoridated (0.1 ppm) demineralising solution. pH 5. S - surface zone

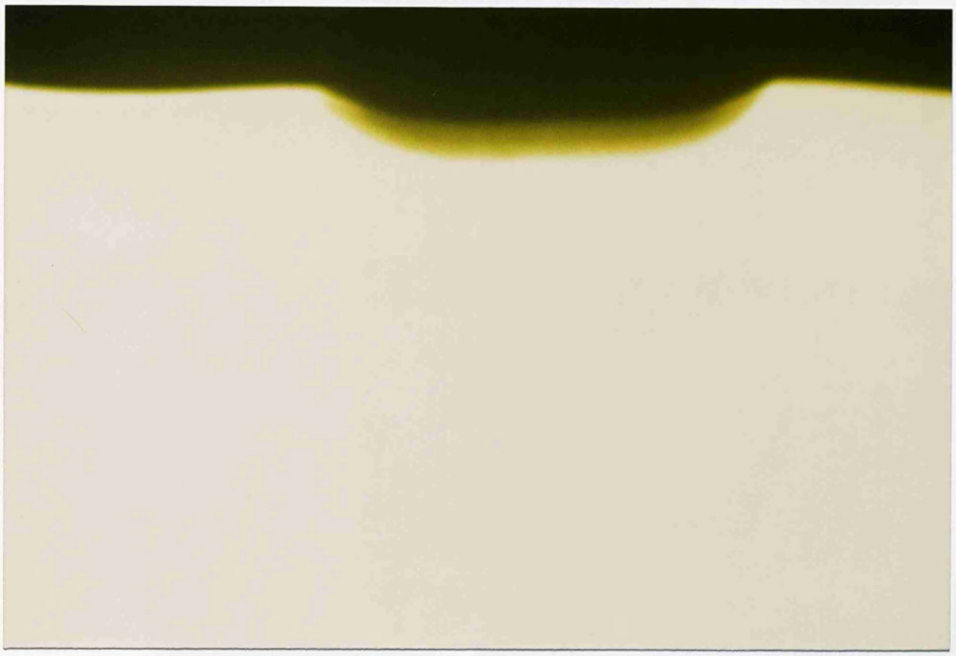


Figure 3.3a A microradiographic picture of a deep lesion produced in a root surface which was immersed in a non-fluoridated demineralising solution for three weeks. pH 5. Note lack of surface zone.

Figure 3.3b A microradiographic picture of a lesion produced in a root surface which was immersed in a non-fluoridated demineralising solution for one week. pH 5. Note lack of surface zone.

immersion periods were similar (3 wks) lesions produced by a pH 5 solution were deeper than those produced by a pH 5.5 solution of the same composition (Fig. 3.4, 3.5).

The surface layer thickness was fluoride concentration and immersion time dependent, both higher concentrations (1 ppm F^-) and longer immersion periods (three weeks) produced thicker surface layers and less deep lesions (Fig. 3.6). However, a concentration as low as 0.05 ppm F^- and an immersion period of 10 days produced a faint surface layer and deeper lesion body (Fig. 3.7). Solutions of 0.1 ppm F^- concentration produced a subsurface lesion with a distinct surface layer and yet had minimum fluoride concentration, and this was adopted as the artificial caries system in this project.

3.3.3 Discussion.

Mellberg (1986), stated that laboratory procedures which easily produce enamel lesions layers might form root lesions with well - defined surface layers. Accordingly, the demineralising solution of Theuns *et al.*, (1983), which was specifically designed to induce enamel, and not root subsurface lesions, was adopted as the basis for this study.

In this series of experiments, the lesions produced by non - fluoridated solutions had no surface layers. However, lesions with distinct, well - mineralised surface layers were produced when as little as 0.1 ppm F^- was added to the initial buffered solution (*vide supra*). The lack of a surface layer in root lesions produced in

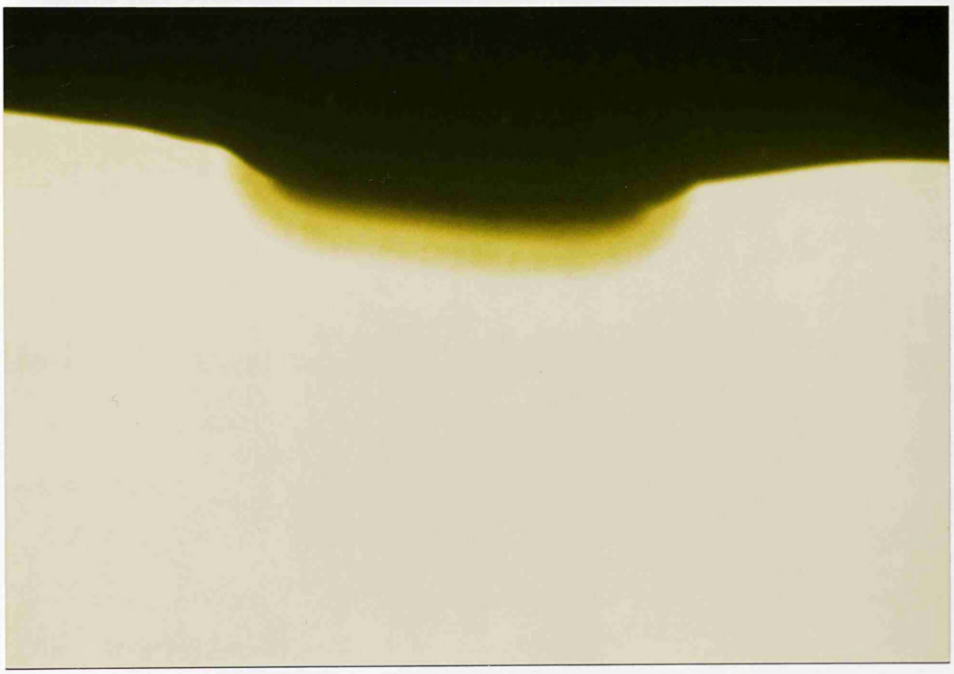


Figure 3.4 A microradiographic picture of a lesion produced after 3 weeks in a pH 5.0 non - fluoridated solution.

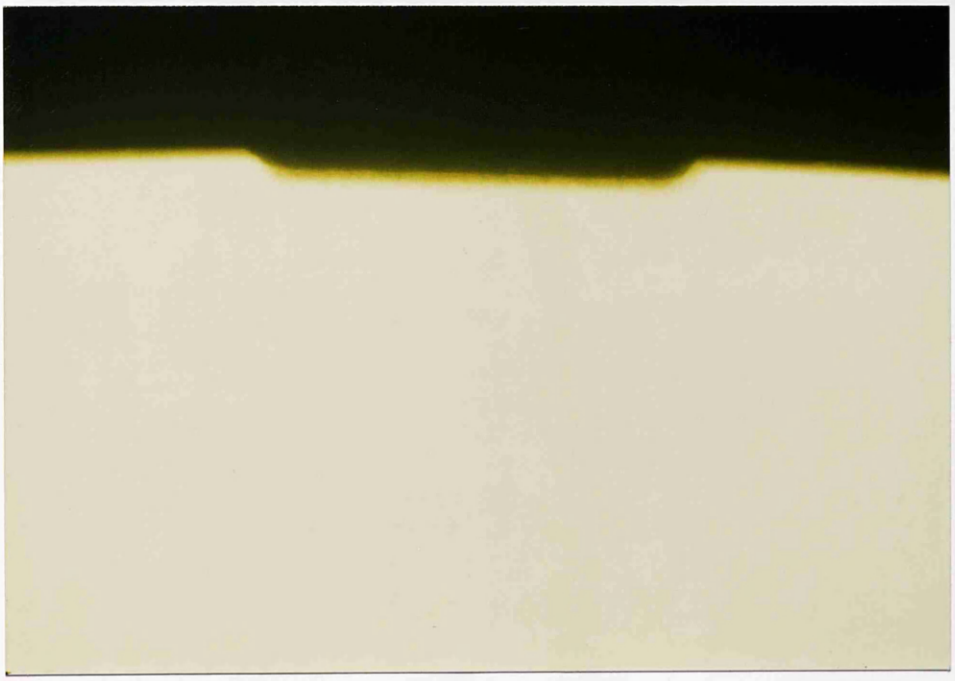


Figure 3.5 A microradiographic picture of a lesion produced after 3 weeks in a pH 5.5 non - fluoridated solution.

Figure 3.6 A microradiographic picture of a lesion produced in a root surface immersed for 3 weeks in a fluoridated (1 ppm) demineralising solution. pH 5. S - the large surface zone.

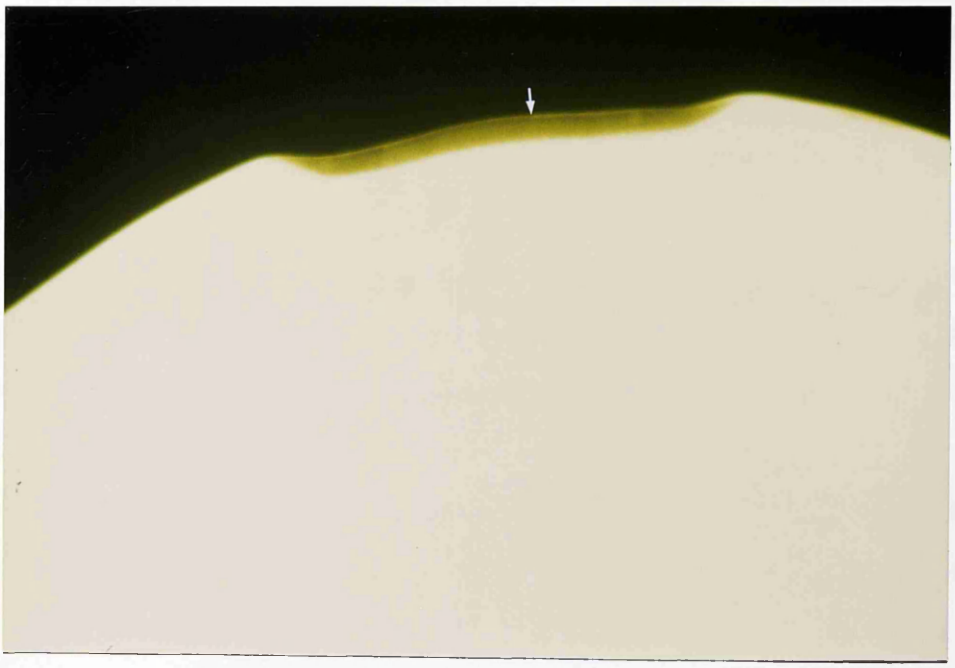


Figure 3.7 A microradiographic picture of a lesion produced after 10 days using a 0.05 ppm F^- demineralising solution. pH 5. Note the ill - defined surface zone (arrow).

non-fluoridated solutions might be explained by the fact that root tissues are less mineralised and, therefore, more soluble in acids than enamel (Feagin, 1984; & Mellberg, 1986). The absence of a surface layer from a lesion in a microradiograph does not mean that there was no surface layer present, as such a layer would have only been seen if it has more mineral than the adjacent tissue. Therefore, fluoride could have contributed to depositing enough mineral into the surface layer for it to be distinguishable in a microradiograph.

Higher fluoride concentration and long immersion periods had a positive effect on the thickness of the surface layer. This emphasises the remineralisation effect of fluoride.

It could be argued that fluoride uptake by the lesion from the artificial system might influence the subsequent uptake from dentifrice treatment *in situ* or fluoride solution treatment *in vitro*. However, such an argument was refuted by the results of two *in situ* and *in vitro* pilot studies in which substantial remineralisation of lesions, which were produced by the same system, was achieved.

Calcium and phosphate were added to the artificial caries systems since Phankosol *et al.*, (1985a) emphasised the importance of its availability in such a system.

Because the critical pH of root hard tissues ranges from about 6.8 - 6.0 (Hoppenbrouwers *et al.*, 1986), a slight decrease in the pH of the plaque fluid *in vivo* should lead to root demineralisation. Therefore, one

would expect an artificial caries system of a lower pH to induce a deeper lesion than a system of relatively higher pH. However, although lesions were produced by two different systems, the results reported here regarding the correlation of lesion depth penetration and pH are in agreement with those of Phankosol *et al.*, (1985b).

In the case of non-fluoridated solutions, longer immersion periods increased the lesion size, as lesions produced after the three week immersion period were relatively larger than those in the one week group. This situation might have resulted from the extended action of the acid on the root surface. In contrast, increased immersion periods of fluoridated solutions had an inhibitory effect on lesion progression.

3.4 OPTIMISATION OF MICRORADIOGRAPHIC PARAMETERS FOR DETERMINATION OF DENTINE MINERAL CONTENT.

3.4.1 Introduction.

Microradiographic / microdensitometric methods have been used frequently to study enamel remineralisation (Section 1.12.2). However, there are mineral and structural differences between enamel and cementum / dentine, and, since few experimental details are available regarding microdesitometric / microradiographic procedures in roots; the aim of this study was to determine the range of dentine section thickness, and microradiographic exposure times suitable for optimal microdensitometric analysis.

3.4.2 Material and methods.

An aluminium stepwedge (0, 27, 54, 81, 108, 135, 162 μm) was loaded on a high resolution plate type I A and exposed to radiation (20 Kv Cu Kα, 30 mA) for exposure times of 10, 20, 30 and 40 minutes. Target specimen distance = 300 mm. Grey levels for each step were determined using a Leitz image analyser. In addition, equivalent aluminium thicknesses were calculated using Angmar's equation (see Section 2.5) and a value for sound dentine, of 47% vol. mineral, calculated for dentine section thicknesses of (a) 50, (b) 75, (c) 100, (d) 125, (e) 150, (f) 175 and (g) 200 μm.

3.4.3 Results.

Plots of the measured grey levels are shown in Figs. 3.8 - 3.11 for the different 10, 20, 30 and 40 minutes exposure times respectively. The vertical lines on these plots indicate the calculated equivalent aluminium thickness values for different dentine section thicknesses listed above.

The graphs for the 30 and 40 minute exposures exhibit a flat response at low aluminium thickness values (Figs. 3.10, 3.11) and were thus deemed to be overexposed. The responses for both 10 and 20 minute exposures (Figs. 3.8, 3.9) were deemed suitable, with an arbitrary choice of 20 minute exposure being used in subsequent studies as this was the exposure time used in enamel caries investigations employing the same equipment. Inspection of

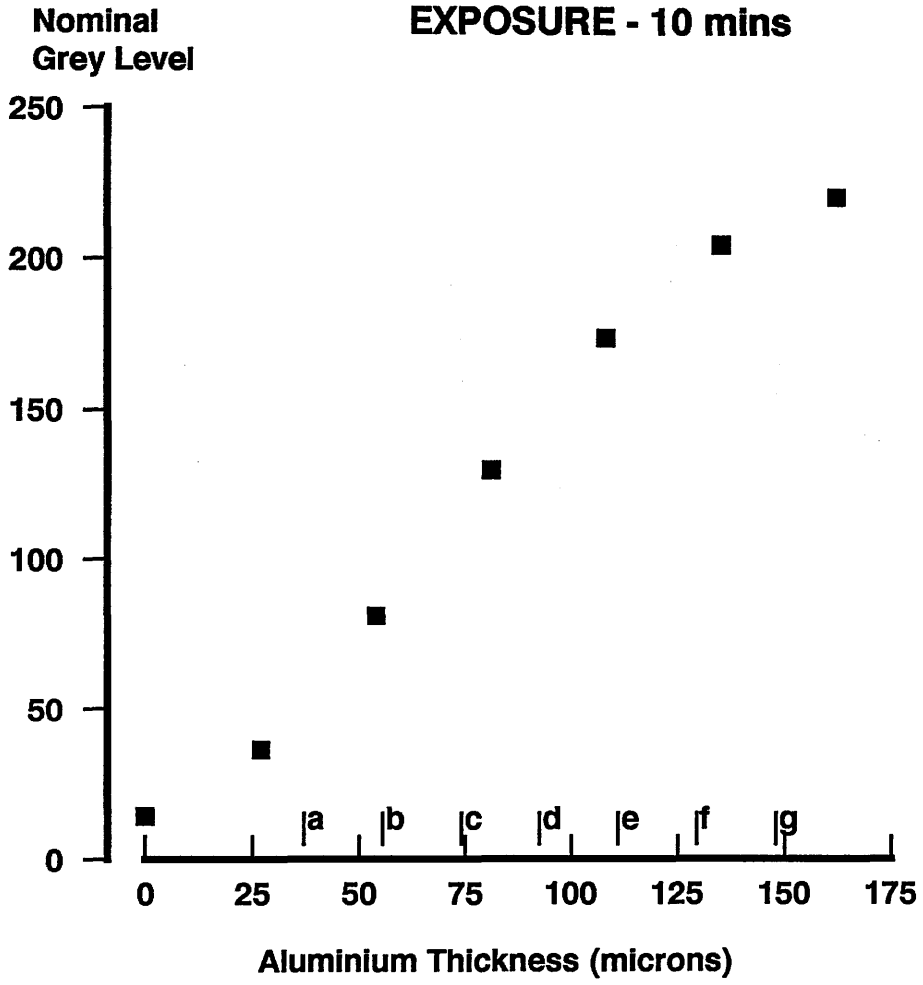


Figure 3.8 Graph of measured Grey Levels for different thicknesses of aluminium (solid squares). Vertical lines correspond to a calculated (using Angmar's equation) equivalent aluminium thickness for dentine thicknesses of (a) 50, (b) 75, (c) 100, (d) 125, (e) 150, (f) 175 and (g) 200 μm.
X-ray exposure: 20 kV, 30 mA, 10 mins.

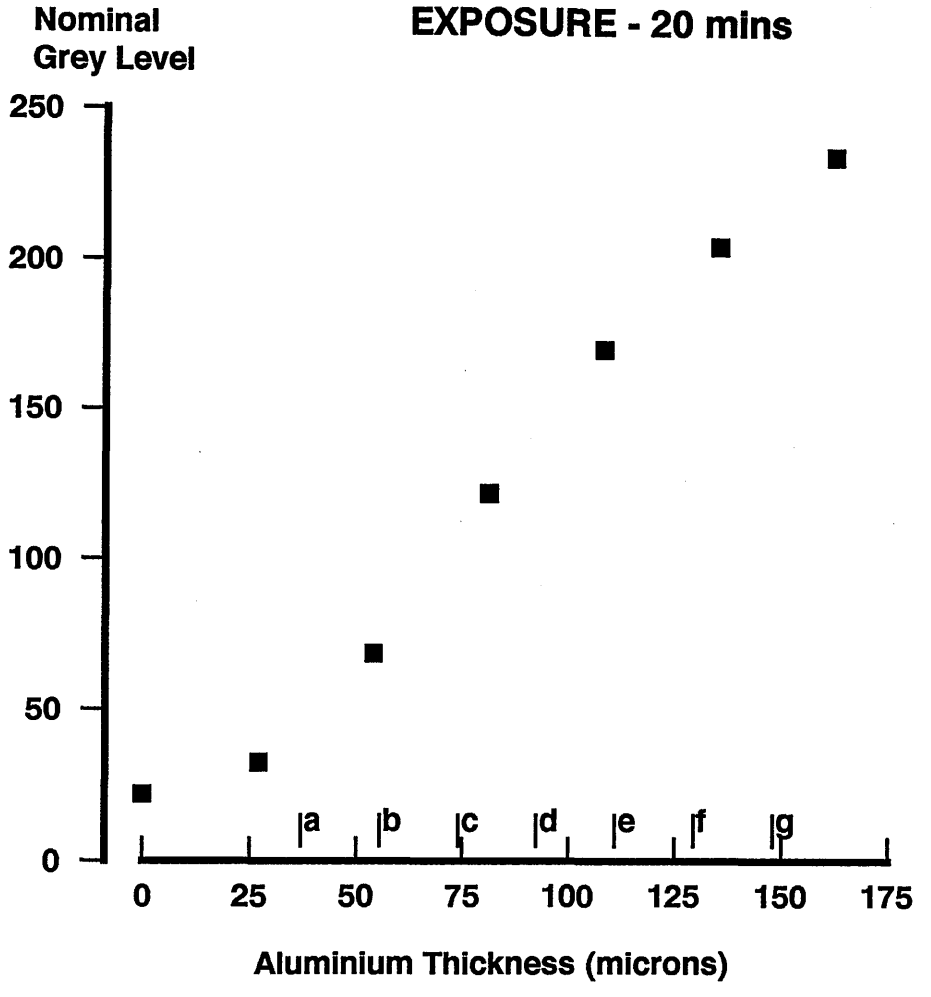


Figure 3.9 Graph of measured Grey Levels for different thicknesses of aluminium (solid squares). Vertical lines correspond to a calculated (using Angmar's equation) equivalent aluminium thickness for dentine thicknesses of (a) 50, (b) 75, (c) 100, (d) 125, (e) 150, (f) 175 and (g) 200 μm.
X-ray exposure: 20 kV, 30 mA, 20 mins.

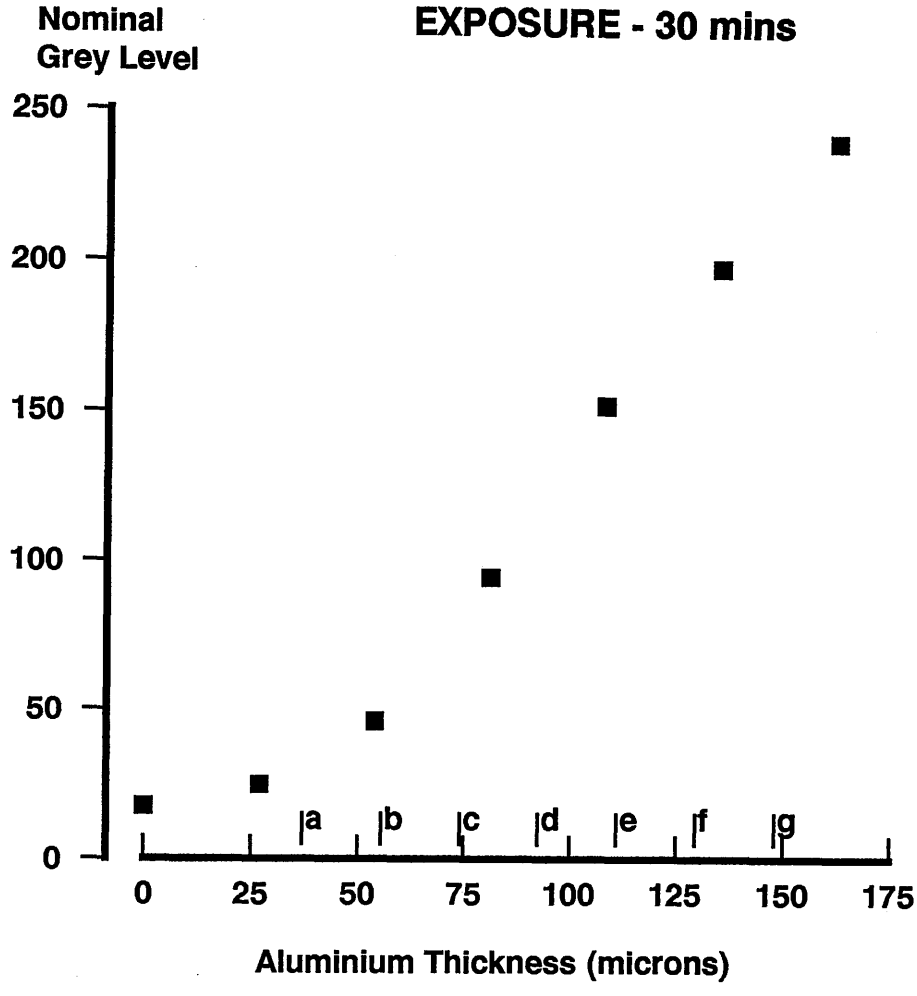


Figure 3.10 Graph of measured Grey Levels for different thicknesses of aluminium (solid squares). Vertical lines correspond to a calculated (using Angmar's equation) equivalent aluminium thickness for dentine thicknesses of (a) 50, (b) 75, (c) 100, (d) 125, (e) 150, (f) 175 and (g) 200 μm.
X-ray exposure: 20 kV, 30 mA, 30 mins.

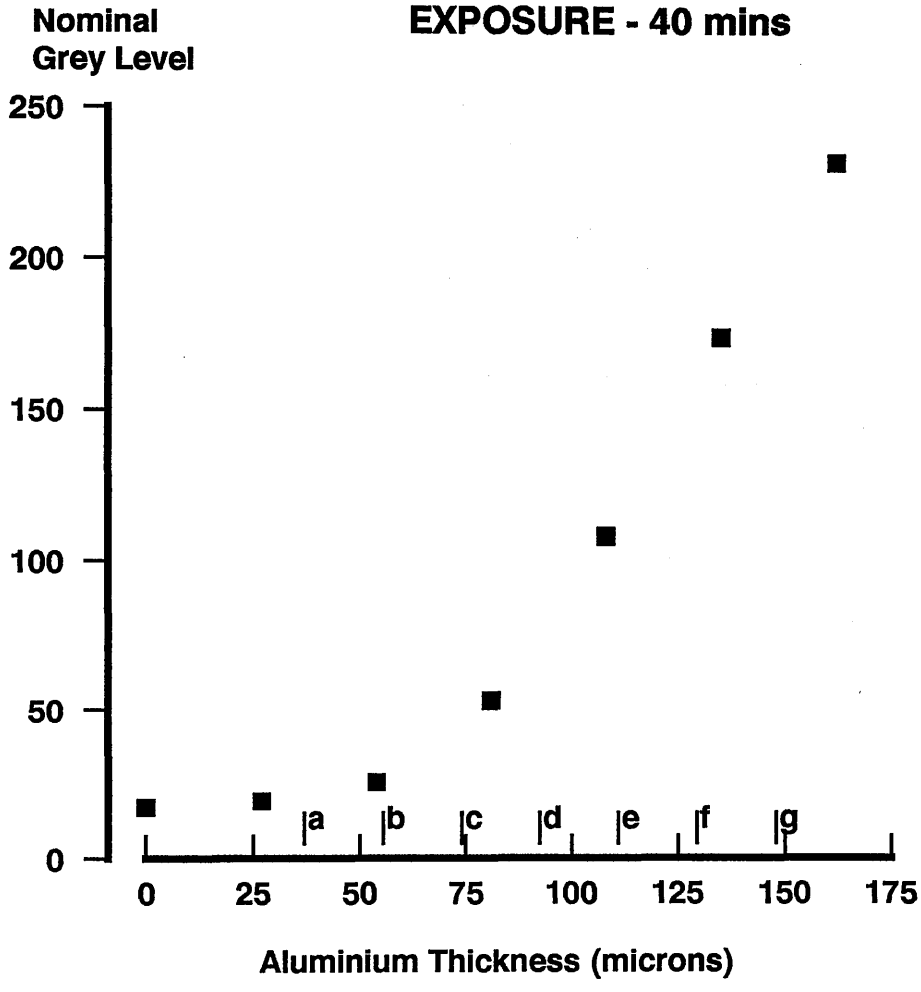


Figure 3.11 Graph of measured Grey Levels for different thicknesses of aluminium (solid squares). Vertical lines correspond to a calculated (using Angmar's equation) equivalent aluminium thickness for dentine thicknesses of (a) 50, (b) 75, (c) 100, (d) 125, (e) 150, (f) 175 and (g) 200 μm.
X-ray exposure: 20 kV, 30 mA, 40 mins.

the vertical lines representing the calculated equivalent aluminium thicknesses reveals that sound dentine section thicknesses between 130 and 200 μm would have grey levels near the top of the scale and thus would give adequate accuracy in determining mineral content in carious lesions with lower mineral content. It was decided to use a nominal thickness of 150 μm in subsequent experiments as this would allow accurate determination of mineral content in hypermineralised areas.

3.5 LESION VARIATIONS IN THE SAME SECTION, BETWEEN SECTIONS AND BETWEEN DIFFERENT TEETH.

3.5.1 Introduction.

Although roots which had not been exposed to the oral fluids were employed in the experiments of this project and the outermost layer was removed by cleaning and polishing, mineral variations could be expected in different sites at the same horizontal level and also, along the cervical - apical plane. These variations, if any, could have taken place during the root development and mineralisation stages. Murakami *et al.*, (1987) reported that cementum contained varying levels of fluoride and these were higher in the surface, than in the deep layers. Also, cementum thickness increases towards the root apex. Therefore, it might well be that the cervical third mineral differs from middle and apical third mineral. Moreover, there are some anatomical situations in which enamel does not meet cementum,

resulting in part of the root being covered with dentine, and the remaining part with cementum. Thus the aims in this section were to investgate different variations namely:

1. Between lesions of the same root section.
2. Between lesions in different sections of the same tooth.
3. Between lesions in sections taken from different teeth, but created by the same buffered solution.

3.5.2 Materials and methods.

Six premolar teeth were employed in this study. Roots were cleaned and pumiced to remove the periodontal ligament remnants and the surfaces of three roots were abraded to expose dentine. Lesions were created as detailed in Chapter 2. Sections were cut and ground to a thickness of approximately 150 μm and were loaded on a high resolution X - ray plate type I A, and microradiographed as described in Section 2.5.2. The microradiographic plates were developed using standard techniques.

Five sections per tooth, each with four lesions, distal mesial, buccal and lingual, representing different parts of the root along the cervico-apical plane, were included in this study.

Microdensitometric profiles were obtained and the mineralisation of the surface zone and lesion body were calculated. In addition, Δz_1 was determined as detailed in

3.5.3 Results.

Inter - lesion variations.

The mean values for the three parameters, for distal, mesial, buccal and lingual lesions for both dentine and cementum lesions are shown in Fig 3.12 - 3.17.

In cementum lesions, the mean (± S.E) per cent volume mineral of the surface zone (Fig. 3.12) for the four lesions, distal, mesial, buccal and lingual were: 28.1 (1.4), 27.1 (1.1), 27.9 (1.2) and 27.7 (1.4) % volume mineral respectively. The corresponding values for the lesion body (Fig. 3.13) parameter were: 22.3 (1.1), 21.0 (1.4), 22.4 (1.3) and 20.5 (1.6) and the integrated mineral loss (Δz_1) values were: 2322 (87), 2273 (127), 2418 (113) and 2536 (110) % volume mineral x μm for distal, mesial, buccal and lingual lesions respectively (Fig. 3.14).

No significant statistical differences were found between all measured parameter values of all lesions when tested by unpaired t test.

In dentine lesions, the mean (± S.E) per cent volume mineral of the surface zone (Fig. 3.15) for the four lesions, distal, mesial, buccal and lingual, were: 26.4 (1.6), 28.1 (1.4), 23.1 (0.9) and 27.8 (1.4) respectively. The corresponding values of the lesion body (Fig. 3.16) were 21.1 (1.3), 20.3 (0.7), 22.6 (0.9) and 21.1 (1.1) and those of integrated mineral loss (Δz_1)

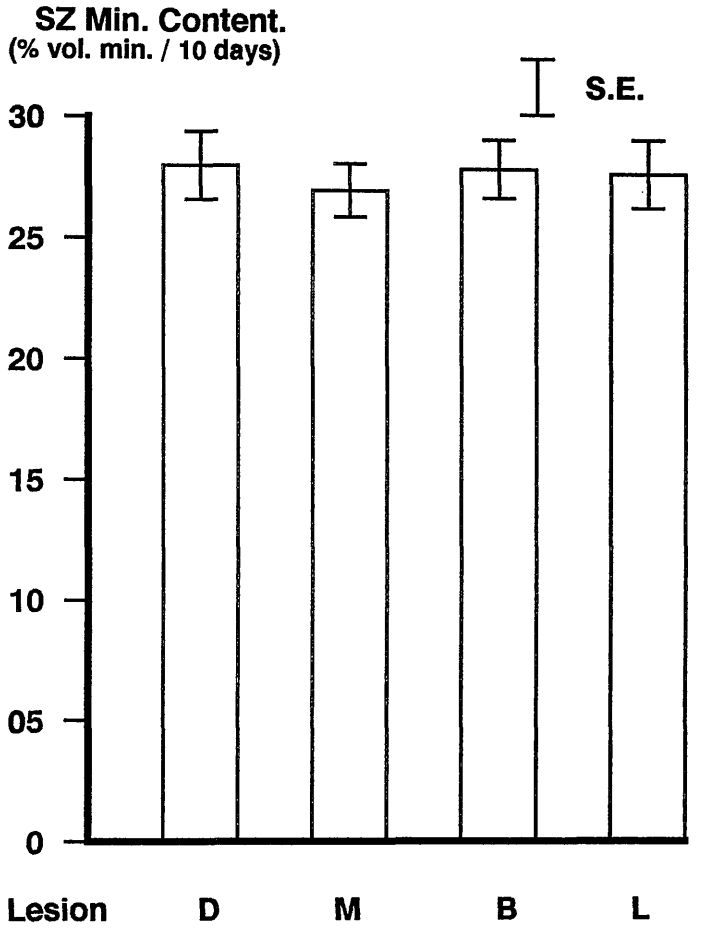


Figure 3.12 Inter - lesion variations: Mean Surface Zone (SZ) mineral content for distal (D), mesial (M), buccal (B) and lingual (L) non - abraded root (cementum) lesions.

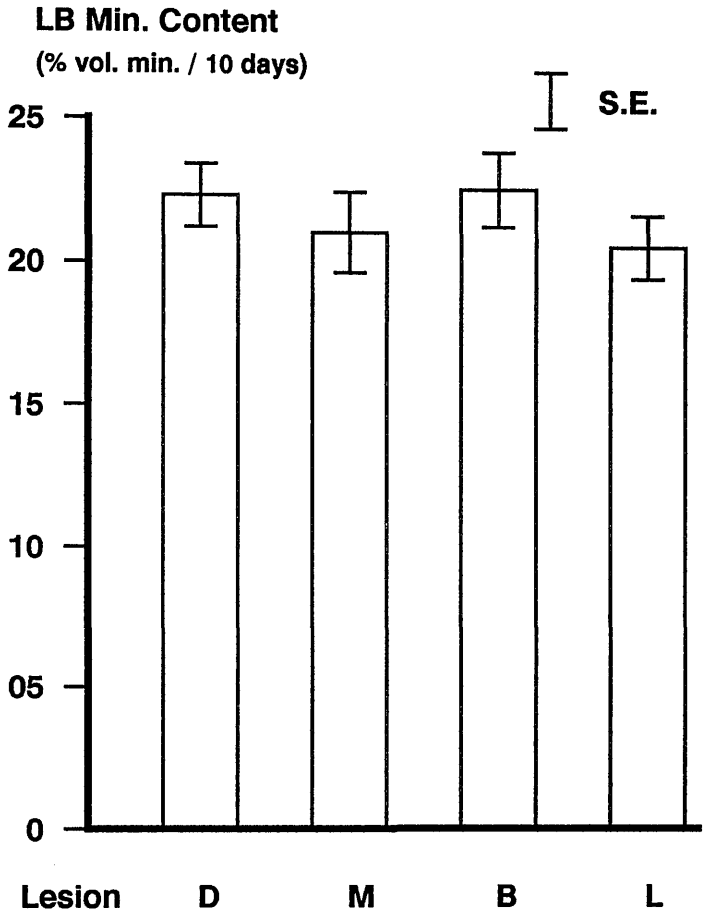


Figure 3.13 Inter - lesion variations: Mean Lesion Body (LB) mineral content for distal (D), mesial (M), buccal (B) and lingual (L) non - abraded root (cementum) lesions.

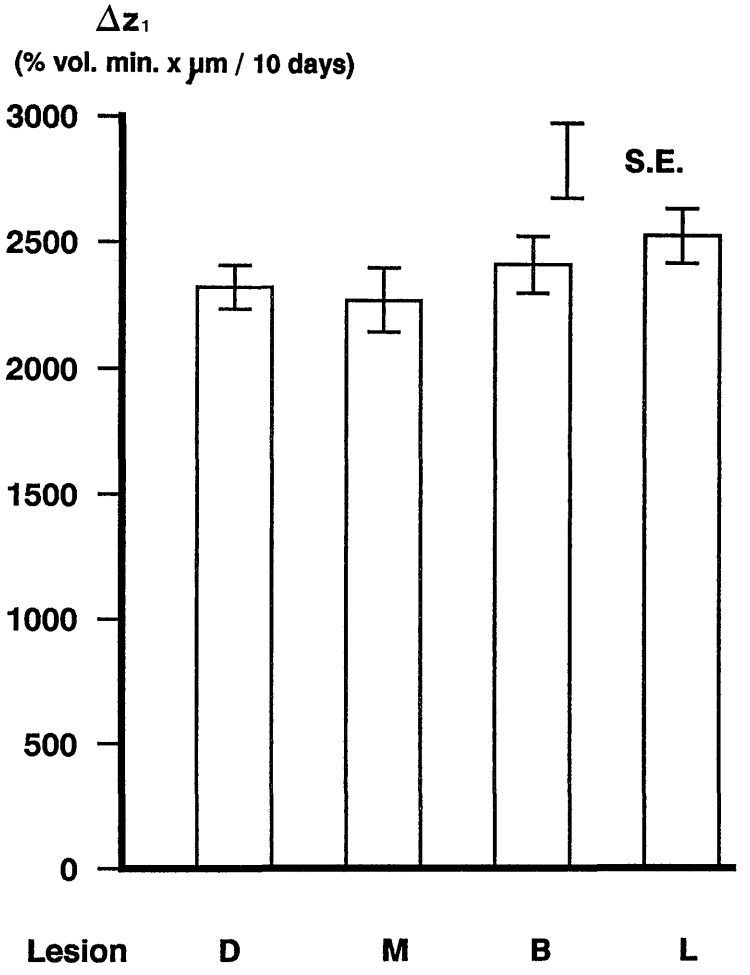


Figure 3.14 Inter - lesion variations: Mean mineral lost (Δz_1) for distal (D), mesial (M), buccal (B), and lingual (L) non - abraded root (cementum) lesions.

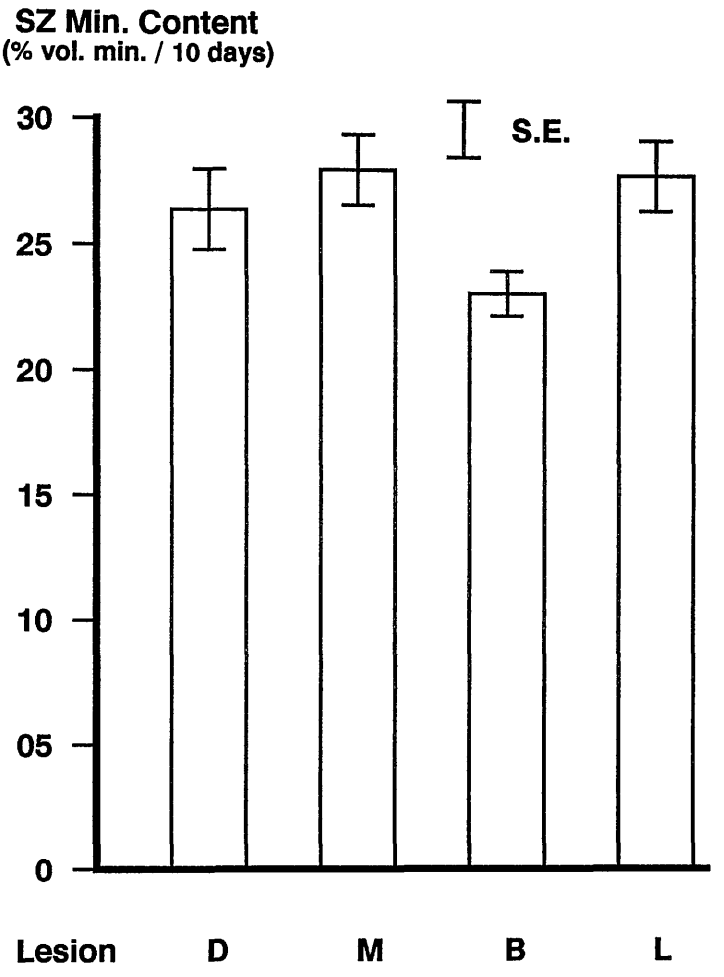


Figure 3.15 Inter - lesion variations: Mean Surface Zone (SZ) mineral content for distal (D), mesial (M), buccal (B), and lingual (L) abraded (dentine) root lesions.

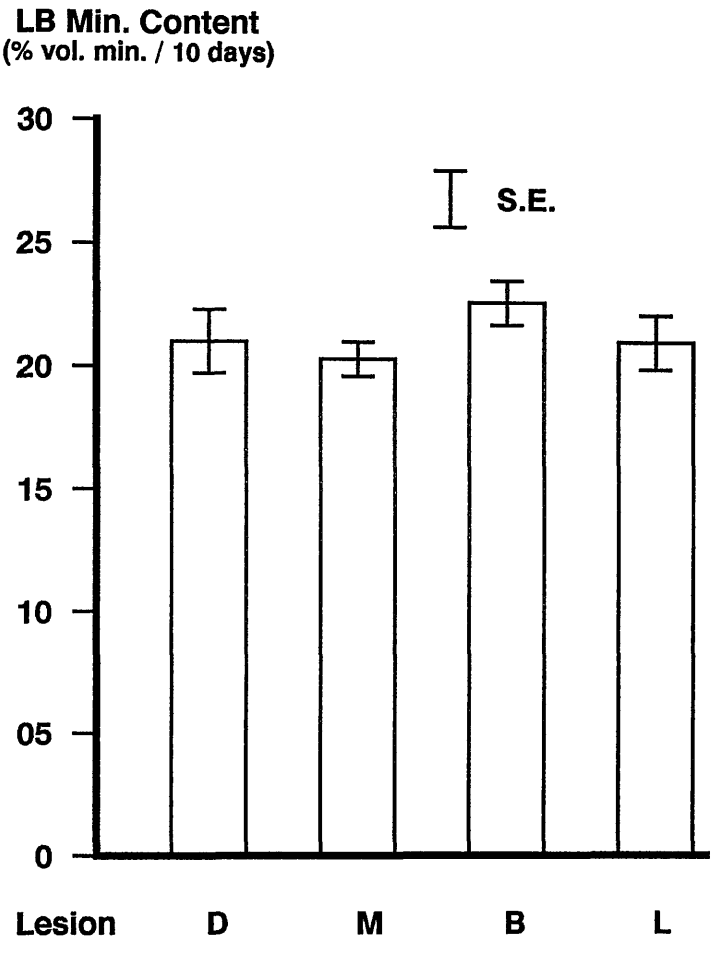


Figure 3.16 Inter - lesion variations: Mean Lesion Body (LB) mineral content for distal (D), mesial (M), buccal (B), and lingual (L) abraded root (dentine) lesions.

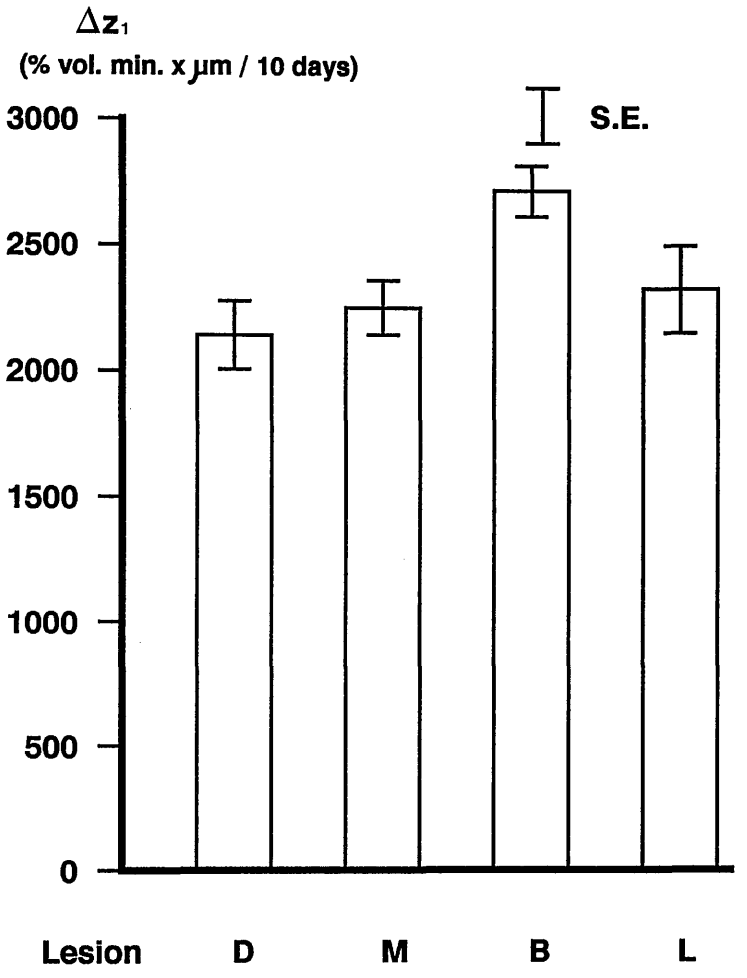


Figure 3.17 Inter - lesion variations: Mean mineral lost (Δz_1) for distal (D), mesial (M), buccal (B), and lingual (L) abraded (dentine) lesions.

values (Fig. 3.17) were 2139 (135), 2248 (108), 2714 (101) and 2328 (175) for distal, mesial, buccal and lingual lesions respectively. The mean per cent volume mineral of the buccal lesion's surface zone is significantly lower ($0.01 < p < 0.05$), and therefore the integrated mineral loss values were higher ($p < 0.05$), than for any of the other lesions. Lesions body values were not significantly different for any of the lesions.

Inter - section variations.

Five cementum and five dentine sections were included in this investigation. These sections were chosen to represent different parts of the root along the cervico - apical plane and their numbers represent serial cutting, the smaller the number the more apical the section.

The mean per cent volume mineral of the surface zone and lesion body and Δz_1 values of the four distal, mesial, buccal and lingual lesions were calculated for each section. These values are represented in the histograms shown in Figs. (3.18 - 3.23).

In sections with cementum, the mean ($\pm$ S.E) per cent volume mineral of the surface zones (Fig. 3.18) of the five sections were: 23.3 (2.1), 26.3 (1.4), 25.3 (1.4), 31.8 (1.6) and 26.5 (1.2) % vol. min. for section 1, 2, 4, 6, 7. The corresponding lesion body values (Fig. 3.19) were 18.0 (0.7), 18.5 (0.8), 18.4 (0.6), 22.8 (1.0) and 21.7 (0.6) % vol. min. and integrated mineral loss values, Δz_1 (Fig. 3.20), were 2437 (113), 2412 (297), 2236 (274), 1956 (93) and 2230 (154) % vol. min. x μm.

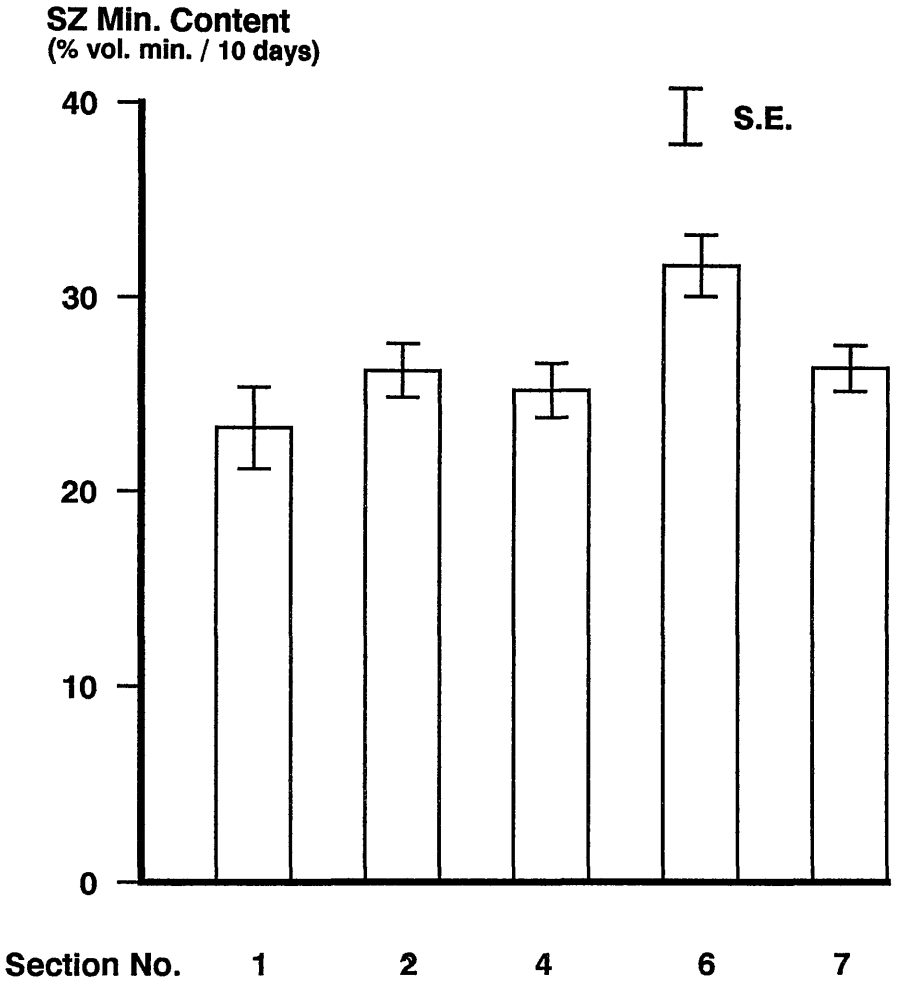


Figure 3.18 Inter - section variations: Mean Surface Zone (SZ) mineral content of the four lesions of each section obtained from the same non - abraded root (cementum).

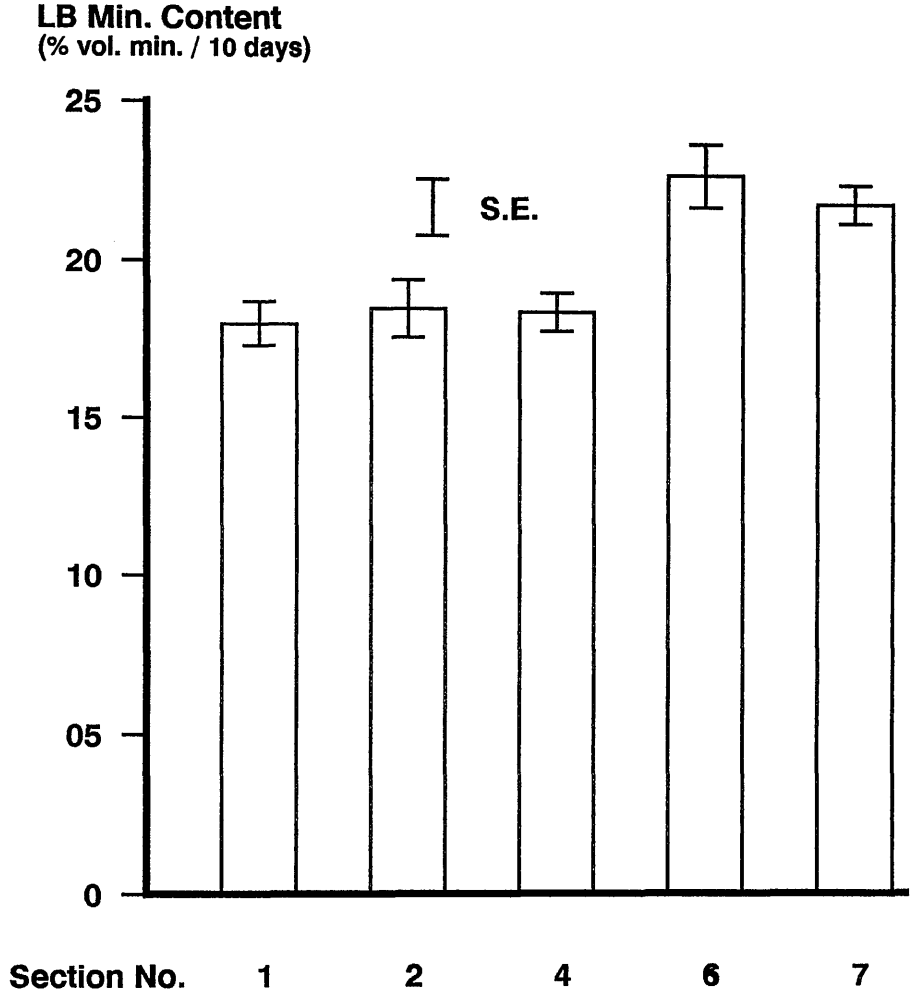
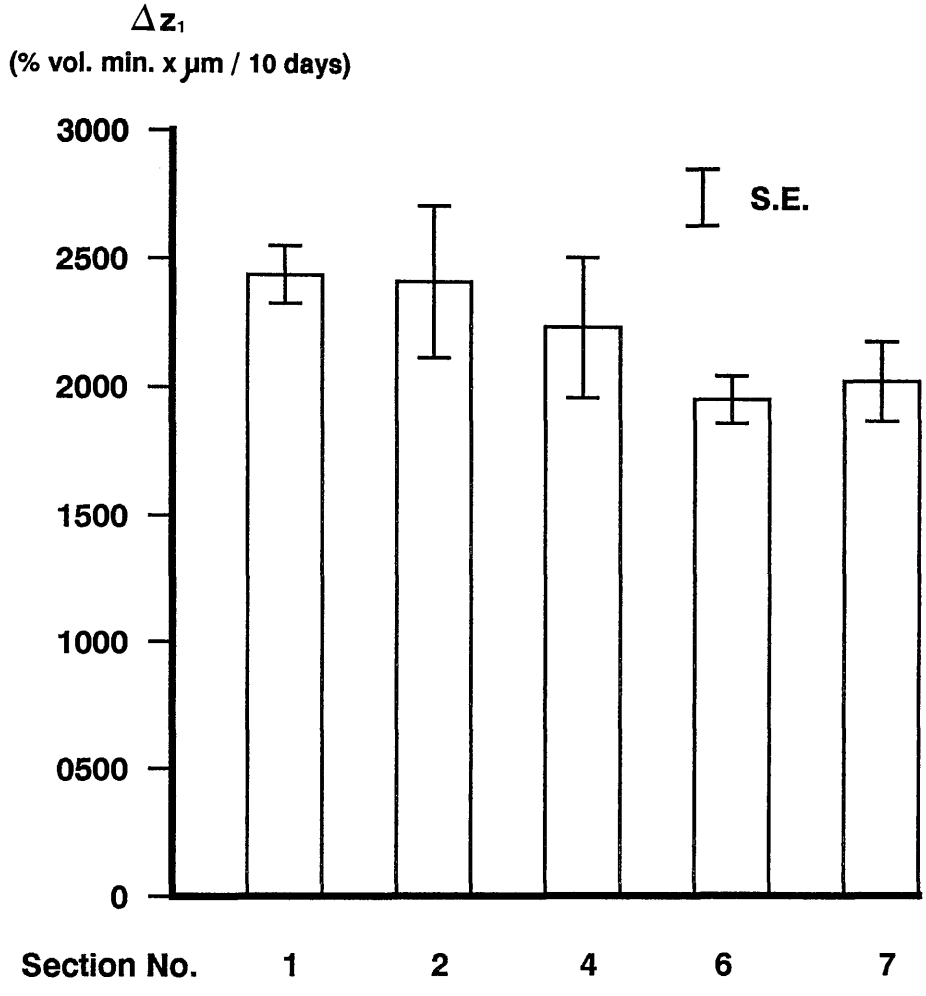


Figure 3.19 Inter - section variations: Mean Lesion Body (LB) mineral content of the four lesions of each section obtained from the same non - abraded root (cementum).



Figue 3.20 Inter - section variations: Mean mineral lost (Δz_1) of the four lesions of each section obtained from the same non - abraded root (cementum).

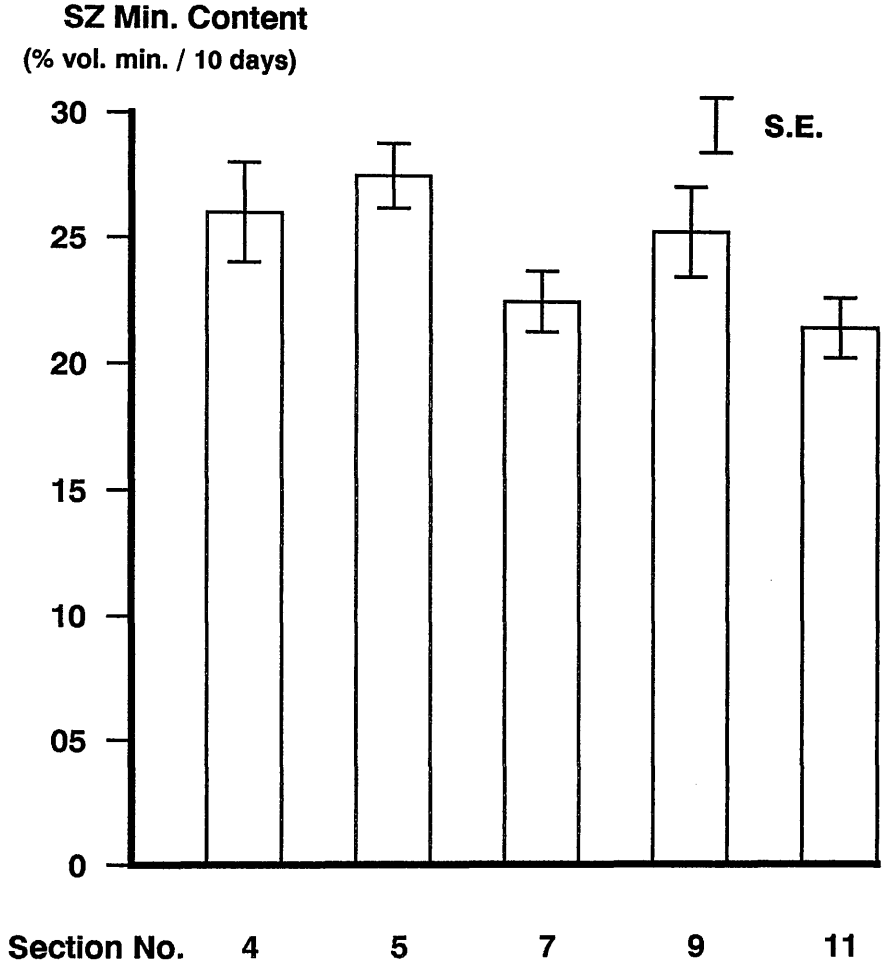
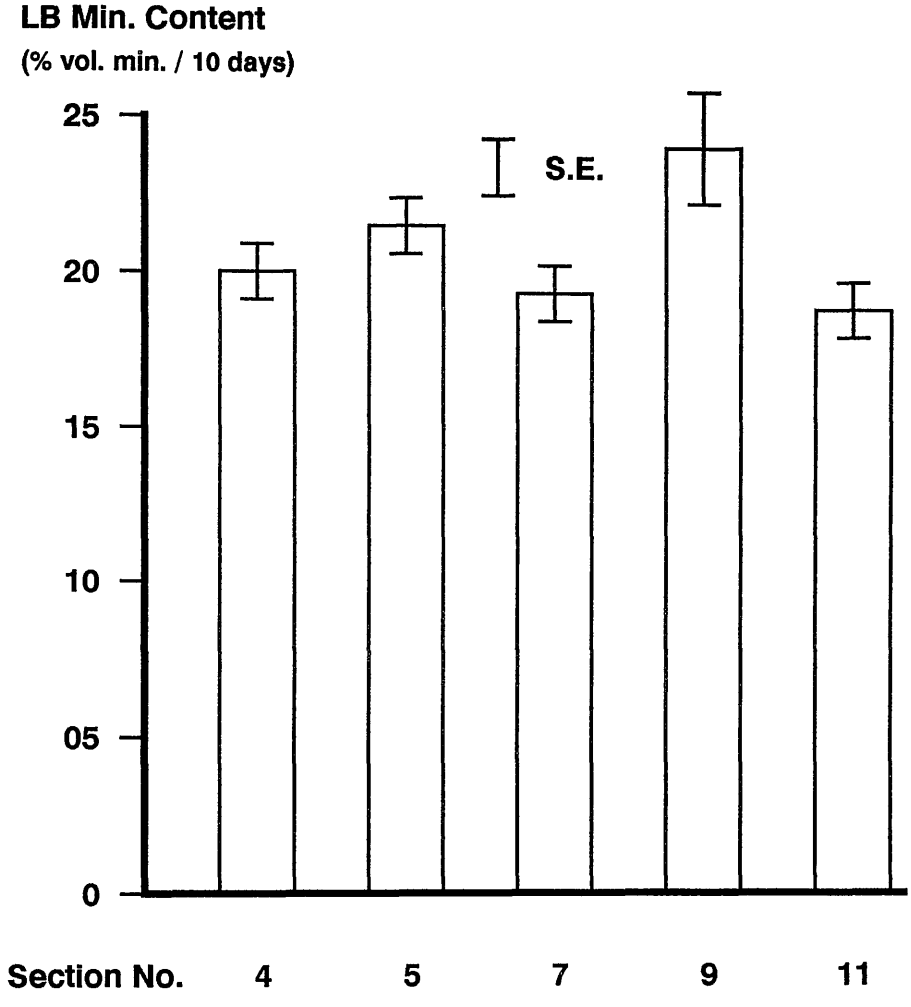


Figure 3.21 Inter - section variations: Mean Surface Zone (SZ) mineral content of the four lesions of each section obtained from the same abraded root (dentine).



Figue 3.22 Inter - section variations: Mean Lesion Body (LB) mineral content of the four lesions of each dentine sections obtained from the same abraded root (dentine).

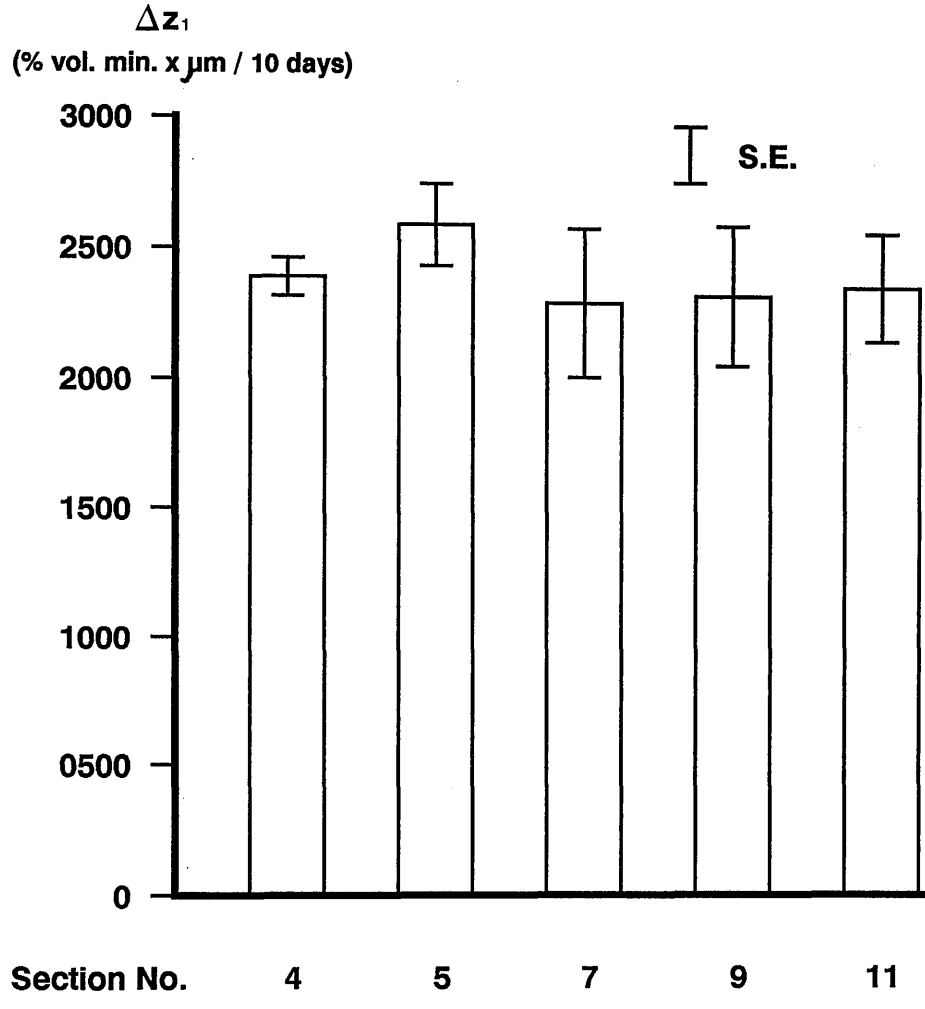


Figure 3.23 Inter - section variations: Mean mineral lost (Δz_1) of the four lesions of each section obtained from the same abraded root (dentine).

Significant differences were found ($p < 0.001$) to exist between the section representing a cervical area (Section no. 6) and that representing an apical area (Section no. 2). The significance levels between apical and cervical sections decreased, going towards the cervical third.

In abraded sections (dentine), the mean ($\pm$ S.E) per cent volume mineral of the surface zones (Fig. 3.21) of the five sections (4, 5, 7, 9 and 11) which were chosen to represent different parts of the roots were: 21.5 (1.2), 26.0 (2.1), 27.5 (1.3), 22.5 (1.2) and 25.3 (1.2) % vol. min. The corresponding lesion body (Fig. 3.22) values were: 20.0 (0.9), 21.5 (0.9), 19.3 (0.9), 24.0 (1.8) and 18.8 (0.9) % vol. min. and Δz_1 (Fig. 3.23) values were 2390 (72), 2589 (158), 2290 (284), 2317 (268) and 2349 (206) % vol. min. x μm.

Significant differences were found ($0.001 < p < 0.01$) to exist between cervical section 5 and the apical section 11.

Inter - tooth variation.

For each root, the means of all lesions in all sections of each measured parameter were calculated and are shown in histograms (Fig. 3.24 - 3.26).

In roots with cementum, the mean ($\pm$ S.E) per cent volume mineral content of the surface zones (Fig. 3.24) were, 32.4 (0.8), 24.2 (0.7), 26.6 (0.9) for the three teeth. The corresponding values of both lesion body (Fig. 3.25) for the same teeth were: 27.1 (0.6), 17.7 (0.6) and

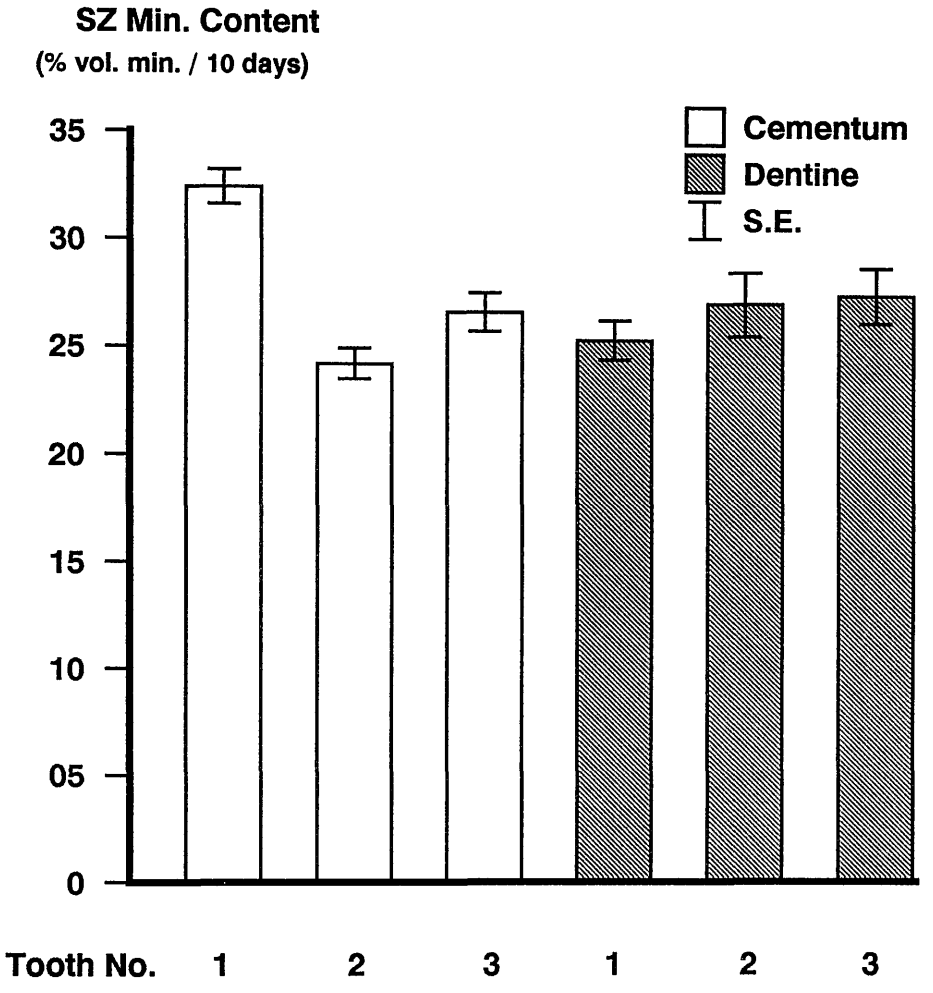


Figure 3.24 Inter - tooth variations: Mean Surface Zone (SZ) mineral content for all lesions on all sections for three non - abraded (cementum) and three abraded (dentine) roots.

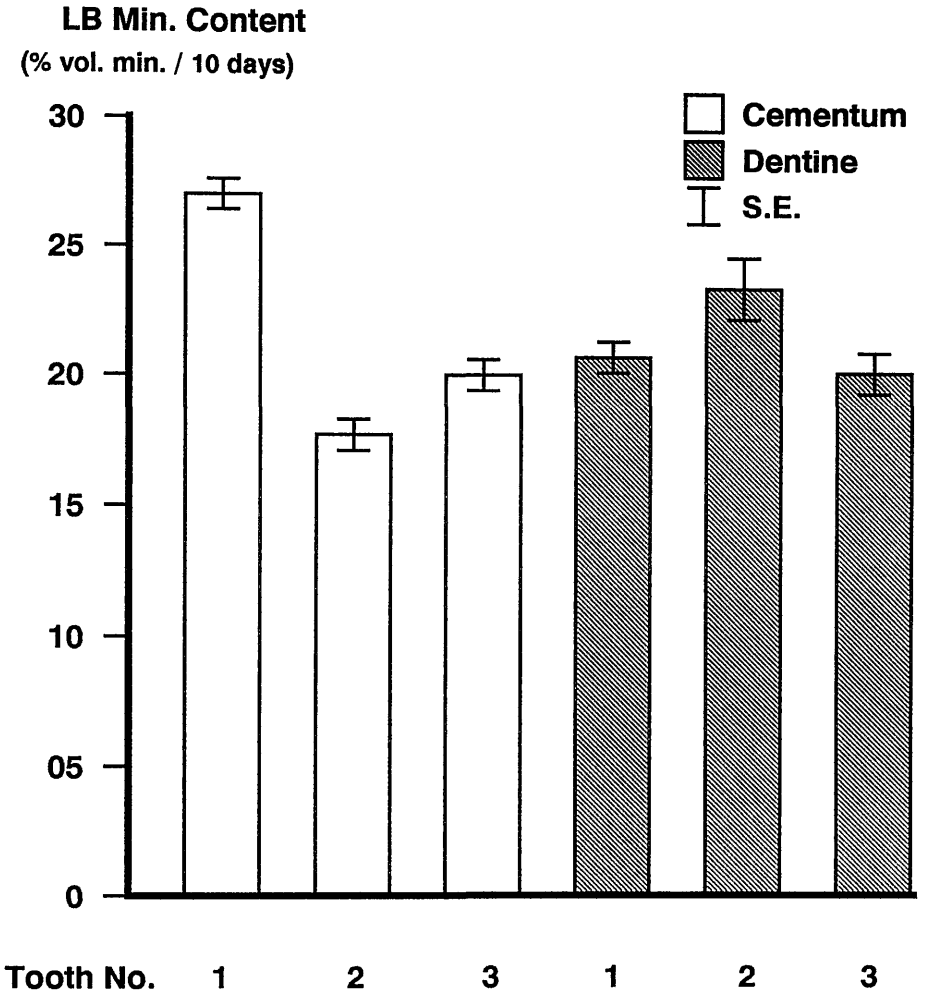


Figure 3.25 Inter - tooth variations: Mean Lesion Body (LB) mineral content for all lesions on all sections for three non - abraded (cementum) and three abraded (dentine) roots.

19.9 (0.6) % vol. min.; and the Δz_1 (Fig. 3.26) values were: 2250 (98), 2659 (71) and 2254 (90) % vol. min. x μm.

Significant variations were found to exist between both tooth 1 and 2 and also between teeth 3 and 2 in both surface zone and integrated mineral loss parameters. The lesion body mean value of tooth 1 was significantly higher ($p < 0.001$) than that of tooth 2 and 3.

In roots with abraded surfaces (dentine), the mean ($\pm$ S.E) per cent volume mineral content of the surface zones (Fig. 3.24) were: 25.3 (0.9), 26.9 (1.5) and 27.4 (1.2) % vol. min. and for the lesion bodies (Fig. 3.25) 20.6 (0.6), 23.4 (1.2) and 20.0 (0.8) % vol. min. for teeth nos. 1, 2 and 3 respectively. The mean ($\pm$ S.E) percent of the integrated mineral loss values (3.26) were: 2388 (88), 2141 (114) and 2628 (191) vol. min. x μm. for the same teeth respectivley.

For the surface zone, no significant variations were found but integrated mineral loss values were significantly higher in tooth 3 than in tooth 2 ($0.01 < p < 0.05$). In addition, lesion body values were significantly higher for tooth 2 in comparison to tooth 3 ($0.001 < p < 0.01$).

Moreover, when comparisons were made between abraded and non - abraded roots, for the surface zone parameter, significant differences were found between tooth (cementum) 1 and each of the three dentine teeth (1, 2, 3). Also, the integrated mineral loss values for tooth 2 (cementum) and 2 (dentine) were significantly different ($p < 0.001$). The high lesion body value of tooth 1 and the

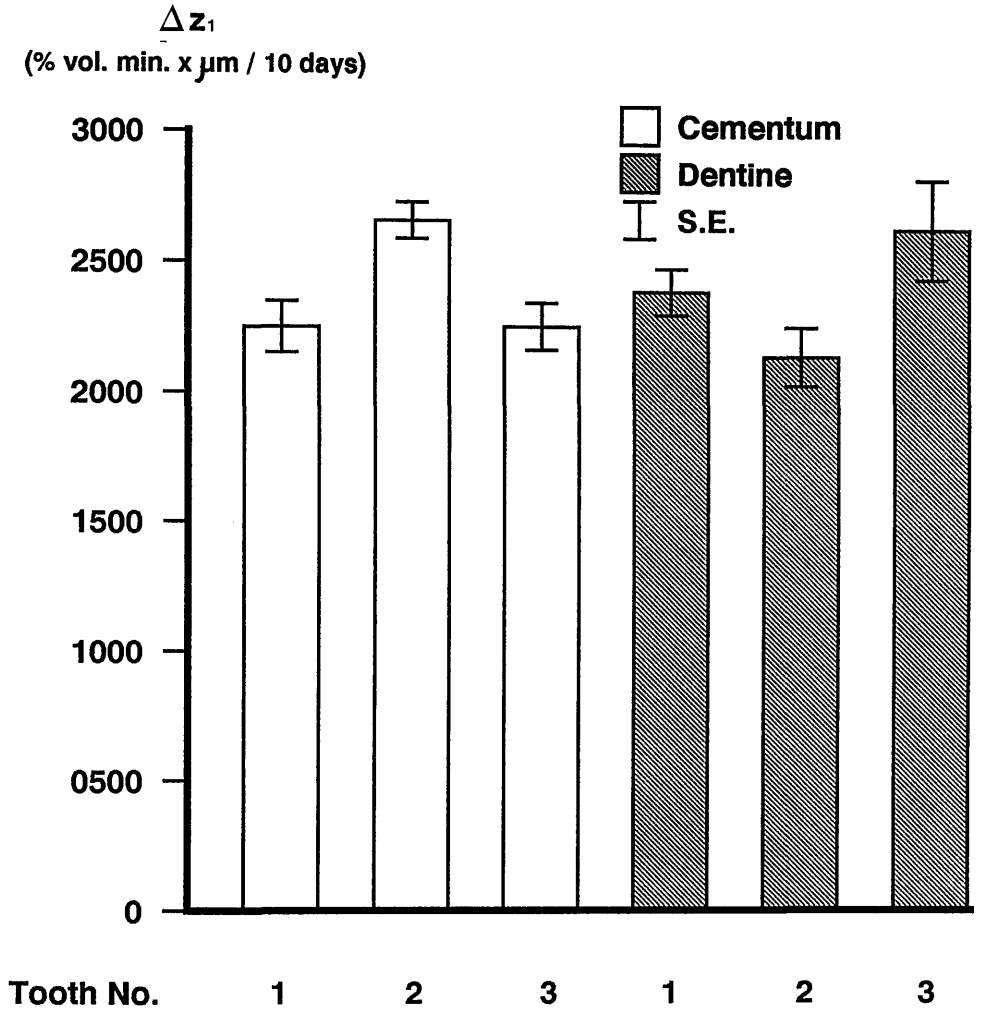


Figure 3.26 Inter - tooth variations: Mean mineral lost (Δz_1) for all lesions on all sections for three non - abraded (cementum) and three abraded (dentine) roots.

low value of tooth 2, caused significant differences to exist between those teeth and all dentine teeth ($p < 0.001$).

3.5.4 Discussion.

The results reported here showed no significant variation between distal, mesial, buccal and lingual lesions, therefore, no critical errors would result if the data for all lesions were combined in the results shown in the following chapters, 4 and 5.

However, due to inter - tooth variation, the sections obtained from the same tooth were distributed amongst all groups in the *in vitro* experiments, and between the subjects in the *in situ* experiments.

3.6 REPEAT MEASUREMENTS.

To test the repeatability of repositioning the horizontal line on subsequent microradiographs, three lesions were used and a microdensitometric profile of each lesion obtained on four different occasions. Using the microradiographic prints, the horizontal line was repositioned in the 2nd, 3rd and 4th measurements at the level determined in the first measurement.

Fig. 3.27 shows the % volume mineral x μm obtained, for the same lesion, in each of the four measurements. The measurements for lesion A were: 6939, 6889, 6934 and 7079 % volume mineral x μm. and the mean of these was 6960 % volume mineral x μm. Those for lesion B, were: 3401, 3455,

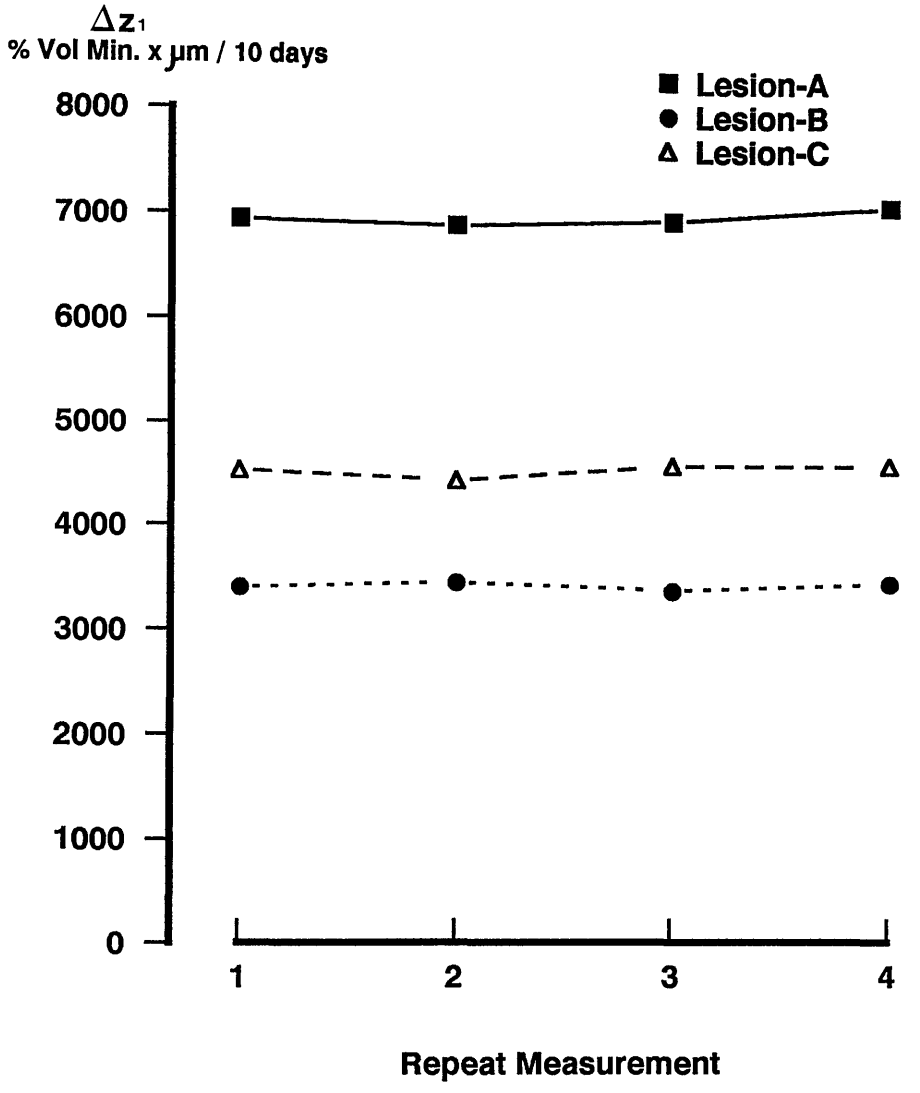


Figure 3.27 A graph showing the repeatability of Δz_1 measurements on four different occasions for three lesions (A, B and C).

3375 and 3445 % volume mineral x μm, and the mean was: 3418, % volume mineral x μm. The measured mineral loss for lesion C were: 4528, 4437, 4583 and 4587 % vol. min. x μm and the mean was of these was: 4533 % volume mineral x μm.

These figures suggested that the difficulty of repositioning the horizontal lines on the same anatomical landmark in subsequent microradiographs could be minimised by taking microradiographic prints at base - lines and referring to these while handling a subsequent microradiograph of the same lesion.

CHAPTER FOUR.
IN SITU STUDIES.

4.1 INTRODUCTION.

Although the precise cariostatic mechanism of fluoride is not fully understood (Section 1.9), there is general agreement that fluoride acts on the incipient lesion by reducing the mineral lost during an acid attack and by enhancing remineralisation of any subsurface lesion formed. This implies frequent fluoride delivery to the oral environment. Fluoride is commonly delivered to the teeth either via dentifrices or solutions. The latter will be used for *in vitro* experiments reported in Chapter 5.

Although root caries might behave differently, clinical trials studying coronal caries prevention have shown dentifrices to be efficient anti - caries agents and a 20% caries reduction using NaF at 1000 ppm concentration has been reported (Reed, 1973). Stephen *et al.*, (1988) demonstrated a 10 % caries reduction by using 1500 ppm MFP and this was increased to 17% when the dose was raised to 2500 ppm. An impressive caries reduction range between 40 - 59% was recorded when sodium monofluorophosphate with the abrasive calcium carbonate was used (Von der Fehr & Moller, 1978).

However, in spite of the public's daily use of commercial dentifrices, little decrease in root caries prevalence has been noticed. Since the fluoride concentration in these dentifrices is about 1000 ppm,

therefore, it is hypothesised that higher concentrations might be more efficient in affecting the disease.

Although, *in situ* experiments have been proved valuable for studying fluoride's remineralisation efficacy on enamel subsurface lesion, few similar experiments have been reported for the roots. Outlined in Section 1.12 were the advantages of an intra-oral appliance designed to study remineralisation of the incipient lesion under the normal oral environment. As few experiments have investigated remineralisation of the incipient root lesion, the intra - oral appliance and the single section technique used for enamel caries studies were adopted for the root caries studies reported in this Chapter. With each lesion microradiographed at baseline and after different fluoride exposure periods, the single section technique allows remineralisation to be assessed in each lesion without the need for a control lesion.

4.2 AIMS AND OBJECTIVES.

The aims and objectives of this investigation were:

1. to study remineralisation of incipient caries lesions in abraded and non - abraded root surfaces,
2. to investigate the effect on remineralisation of root subsurface lesions using different sodium monofluorophosphate dentifrice concentrations namely: placebo, 1000, 2500, 5000 ppm.

4.3 MATERIALS AND METHODS.

4.3.1 The intra - oral appliance design and construction.

The lower removable appliance, as described by Creanor *et al.* (1986), was employed in this study. However, a cobalt-chromium framework, with lingual plate and clasps, was used instead of the previously described all acrylic design as the former improved retention and was more comfortable for the volunteers (Fig. 4.1).

The stages involved in the construction of an appliance were as follows:- upper and lower impressions were taken in alginate (Xantalgin, Bayer, Newbury, England) and from these, models were poured in dental stone. Undercut areas present on the lower cast were blocked out with plaster and the cobalt-chromium framework constructed with clasps in the lower left and right second premolar and first molar regions. This was then inserted into the volunteer's mouth and any necessary adjustments made. Pieces of wax (16 x 9 x 1 mm) were added to the lingual lower first and second molar regions on either side of the cast. The model was then duplicated and acrylic lingual flanges added to the appliance framework. In this way recessed troughs were incorporated into the fitting surfaces of the flanges, the dimensions corresponding to that of the wax templates. Channels were cut in the centre of the upper and lower edges of the acrylic trough areas to allow free flow of saliva through the sites. Root sections were mounted on the

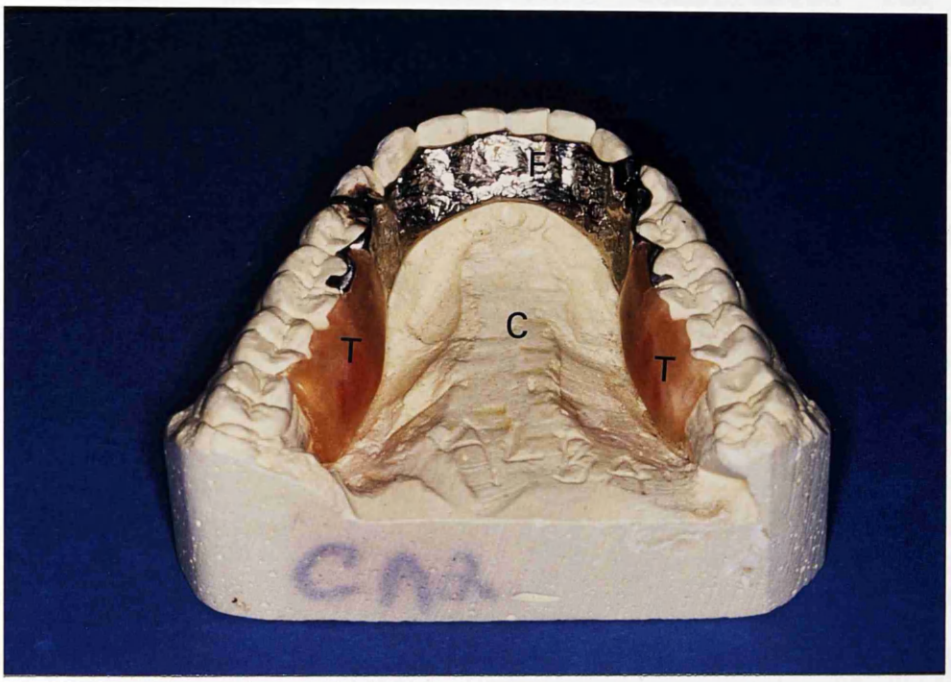


Figure 4.1 The intra - oral appliance.
F - metal framework, T - trough areas, and
C - cast.

recessed sites without contacting the natural tooth surface or lingual gingivae.

4.3.2 General methods.

Intra - oral appliances were constructed for six volunteers all of whom worked in the Glasgow Dental Hospital and School. Sections were prepared of abraded and non-abraded human premolar roots in which artificial carious lesions were created previously as detailed in Chapter 2.

For each experimental run of five weeks' duration, four specimens (2 non-abraded, cementum and 2 abraded, dentine) were mounted on the troughs of each appliance (Fig. 4.2). Subjects were provided with a copy of the "Experiment protocol" (Appendix I) and were asked not to rinse after dentifrice use for maximum benefit (Featherstone *et al.*, 1982) On each occasion, subjects were provided with the dentifrice to be tested at least one week prior to appliance insertion. Each was asked to wear the appliance for two weeks (i.e 28 dentifrice exposures). Microradiography of the sections was carried out, as detailed in Chapter 2. At each assessment period, the sections were removed from the appliance using amyl acetate and then taken through two concentrations of alcohol and eventually into deionised water. Subsequently, the sections were remounted on the appliances and returned to the volunteers. The subjects were asked to continue using the test dentifrices when not participating in the test, during the 2 - 3 days necessary to remove the

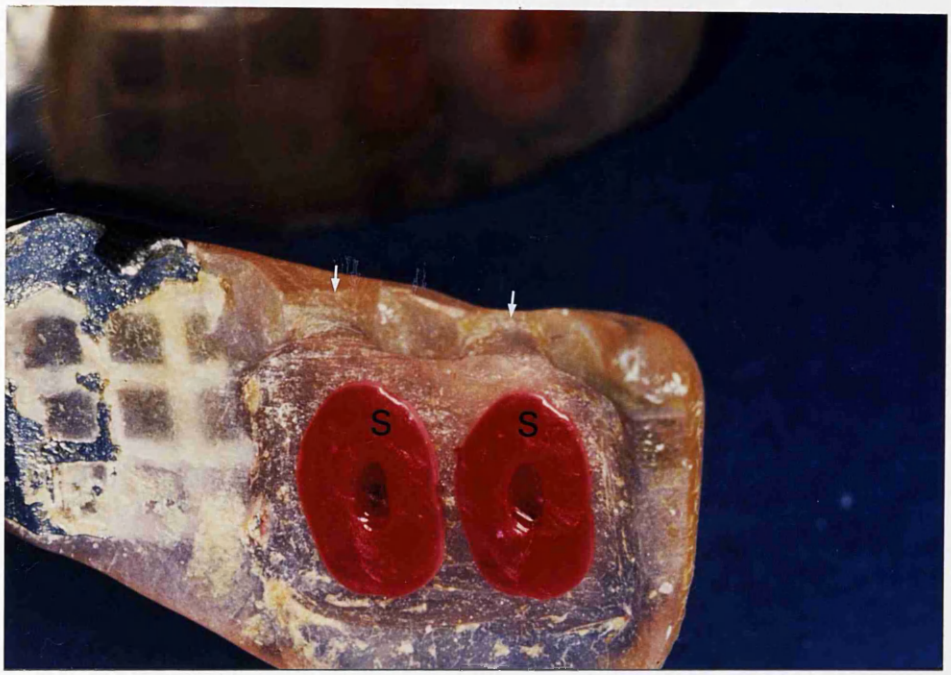


Figure 4.2 A close view of the trough area onto which two varnished root sections(S) are mounted. Salivary flow was facilitated by two holes (arrows) at the upper side of the trough.

specimens, carry out microradiography and replace them on the appliances. This ensured that baseline salivary fluoride levels would not be altered by using a different dentrifrice.

The dentifrices employed were provided by Unilever Research (Gibbs Dental Division, Port Sunlight, England). The fluoride concentrations tested were: placebo, 1000, 2500 and 5000 ppm (Formulation detailed in Appendix II).

4.3.3 Experimental protocol.

Since each paste was identically packaged and marked only with a code, the volunteers were unaware of the dentifrice being tested. The general plan of the experimental protocol is detailed in Table 4.1, illustrating the cross-over arrangement employed. Before commencing the placebo run, the non-fluoridated dentifrice was distributed to the subjects at least 2 weeks prior to appliance wearing. To ensure that all fluoride dentifrices were tested before sudden departure of any of the volunteers, the placebo dentifrice run for all volunteers was always carried out at the end of each experiment.

4.3.4 Method of analysis.

Only those lesions which lasted longer than 4 weeks without breakage were included in the analysis. The lesion parameters (zs, SZ and LB) were plotted against time and a "least squares fit" carried out on the data. The resultant gradient was taken as a measure of the mineralisation rate for that lesion (see Section 2.8).

Table 4.1 Cross-over design used in *in situ* study.

	Dentifrice fluoride concentration (ppm)			
Volunteer	Run 1	Run 2	Run 3	Run 4
A	1000	2500	5000	0
B	1000	2500	5000	0
C	2500	5000	1000	0
D	2500	5000	1000	N.A.
E	5000	1000	2500	0
F	5000	1000	2500	0

N.A. - Volunteer (D) not available for this run.

The mean slope and standard error of a group of lesions, for each tissue and per each volunteer, under the same fluoride treatment were calculated.

Comparisons between mineralisation rates of each fluoride treatment and between those for cementum and dentine lesions were carried out using unpaired t test.

4.4 RESULTS.

The mean mineralisation rates and standard errors for each measured parameter for cementum and dentine lesions for each volunteer are given in Tables (4.2 - 4.7) and are shown in figures (4.3 - 4.26).

4.4.1 Fluoride concentration effect on non - abraded root surface lesion for subject A, (Figs. 4.3, 4.4, Tab. 4.2).

For the Δz_1 parameter (Fig. 4.3), although the mean remineralisation rate increased with increasing fluoride concentration, no significant differences were found to exist between these means.

The concentration effect was also evident for the Δz_2 parameter (Fig. 4.3) of the same lesions, but none of the fluoride concentrations tested were significantly superior to another.

The only signifcant result noted for Δz_3 parameter (Fig. 4.3) was found between the 5000 ppm F^- and the placebo ($0.001 < p < 0.01$).

However, the remineralisation rate of the surface zone (Fig. 4.4) for both the 1000 and 5000 ppm F^- pastes

Table 4.2 Mean Mineralisation rates for the Δz, Surface Zone (SZ) and Lesion Body (LB) parameters. Volunteer A.

ppm F⁻	n	Δz_1	Δz_2	Δz_3	SZ	LB
		(% Vol. min x μm/wk)			(% Vol. min. / wk)	
Non-abraded (cementum) lesions.						
0	7	70.9 (36.5)	358.4 (58.9)	65.8 (30.5)	2.5 (0.4)	1.7 (0.4)
1000	6	87.7 (44.6)	378.4 (84.1)	127.1 (45.7)	4.2 (0.6)	2.6 (0.6)
2500	5	97.1 (74.6)	460.4 (156.6)	167.7 (95.3)	3.8 (1.0)	2.9 (0.8)
5000	7	205.3 (102.4)	520.6 (58.5)	258.6 (66.3)	5.8 (0.7)	3.4 (0.6)
Abraded (dentine) lesions.						
0	8	118.6 (34.2)	405.5 (76.2)	123.7 (27.7)	2.7 (0.3)	2.2 (0.3)
1000	8	149.4 (43.9)	843.8 (43.0)	220.2 (32.2)	5.9 (0.3)	3.2 (0.4)
2500	8	145.8 (28.1)	581.9 (71.0)	195.7 (23.9)	4.7 (0.4)	2.9 (0.3)
5000	7	84.9 (323.9)	725.7 (62.3)	384.1 (56.6)	4.6 (0.5)	3.8 (0.4)

n = number of lesions available for analysis.
Values in parenthesis are Standard Errors

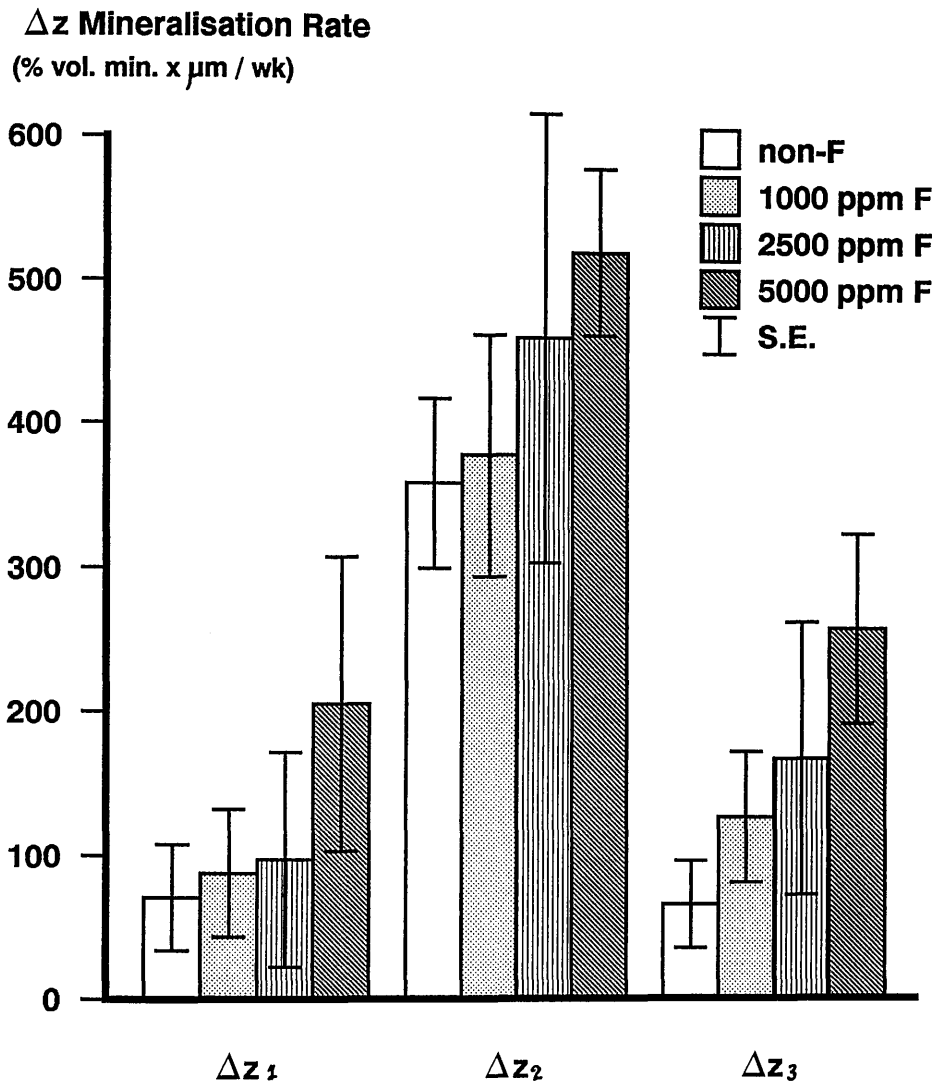


Figure 4.3 Histogram of the mean mineralisation rates for the Δz parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer A, non - abraded (cementum) root lesions.

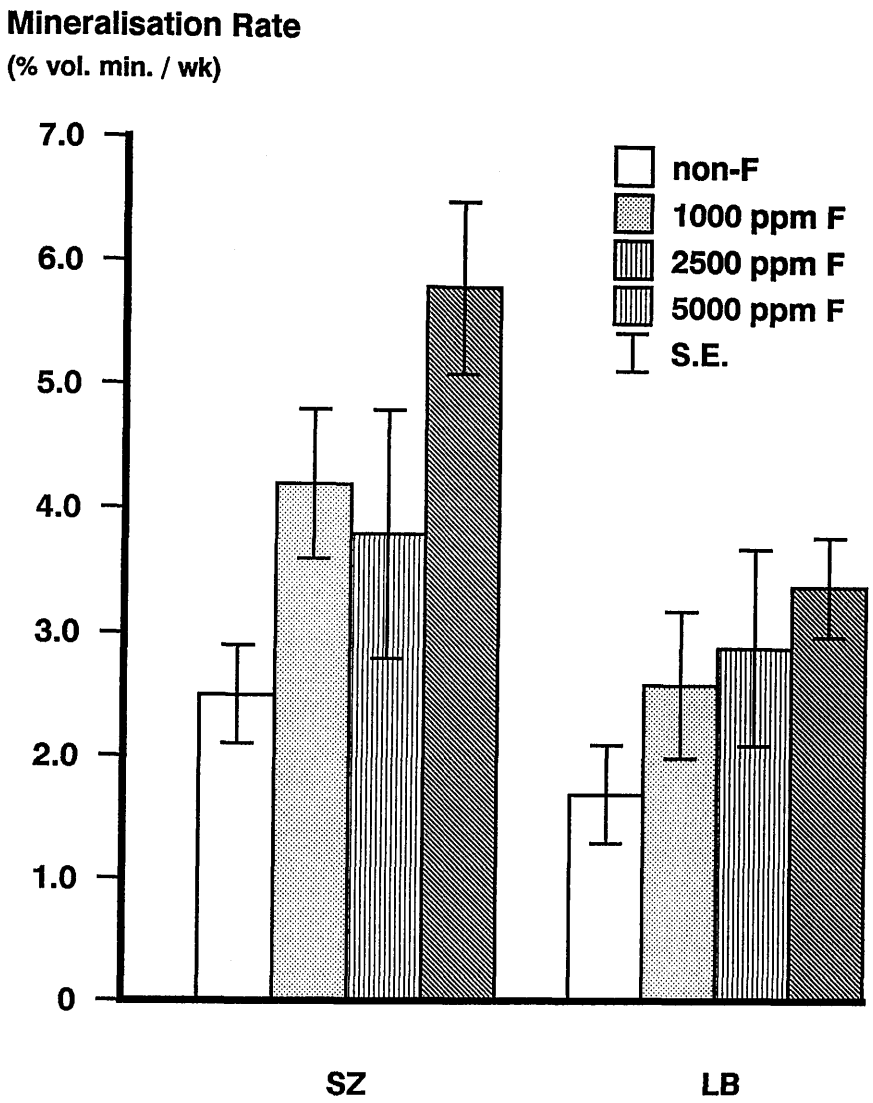


Figure 4.4 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer A, non - abraded (cementum) root lesions.

increased significantly ($0.01 < p < 0.05$, $0.001 < p < 0.01$) compared to placebo.

For the lesion body parameter (Fig. 4.4), the remineralisation rate increased with increasing fluoride dose. However, only the value for the 5000 ppm F^- paste was significantly different from the placebo ($p < 0.001$).

4.4.2 Fluoride concentration effect on abraded root surface lesion for subject A, (Figs. 4.5, 4.6, Tab. 4.2).

For the Δz_1 parameter (Fig. 4.5), no particular trend was evident. None of the fluoride concentrations was significantly different from the others or the placebo.

For the Δz_2 parameter (Fig. 4.5), the remineralisation rates for both 1000 ppm F^- and 5000 ppm F^- pastes were significantly higher than the placebo ($p < 0.001$, $0.001 < p < 0.01$ respectively). In addition, the value for the 1000 ppm F^- was significantly ($p < 0.001$) higher than that of the 2500 ppm F^-. It was also higher, but not significantly, than that of the 5000 ppm F^- paste.

For the Δz_3 parameter (Fig. 4.5), the remineralisation rate achieved by treating the lesions with 5000 ppm F^- was superior to the other three treatments ($p < 0.001$) and the 1000 ppm F^- dose was also significantly ($0.001 < p < 0.01$) superior to that of the placebo.

Compared to the placebo, the surface zone (Fig. 4.6) remineralisation rate of these (dentine) lesions increased significantly ($p < 0.001$) for all three fluoride

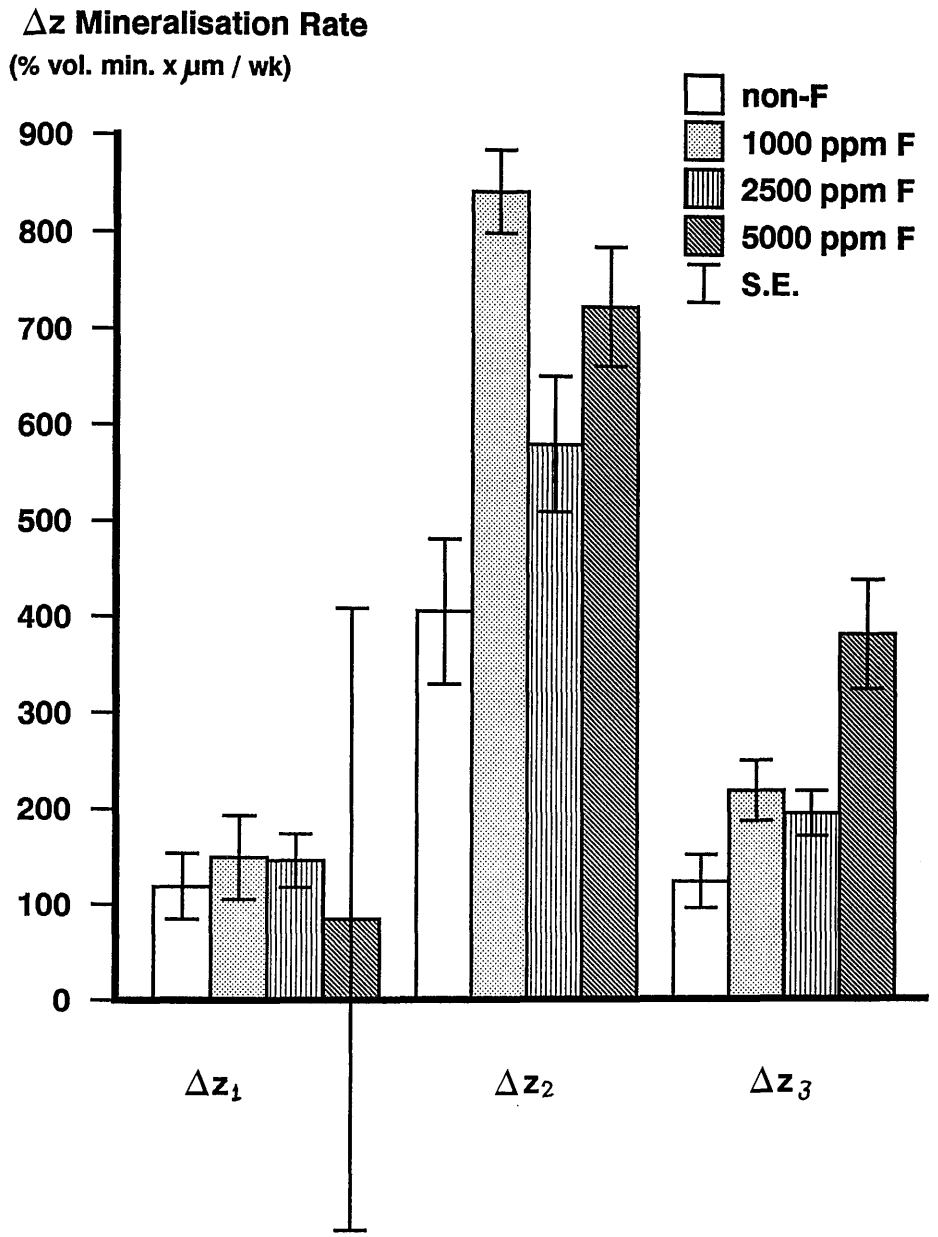


Figure 4.5 Histogram of the mean mineralisation rates for the Δz parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer A, abraded (dentine) root lesions.

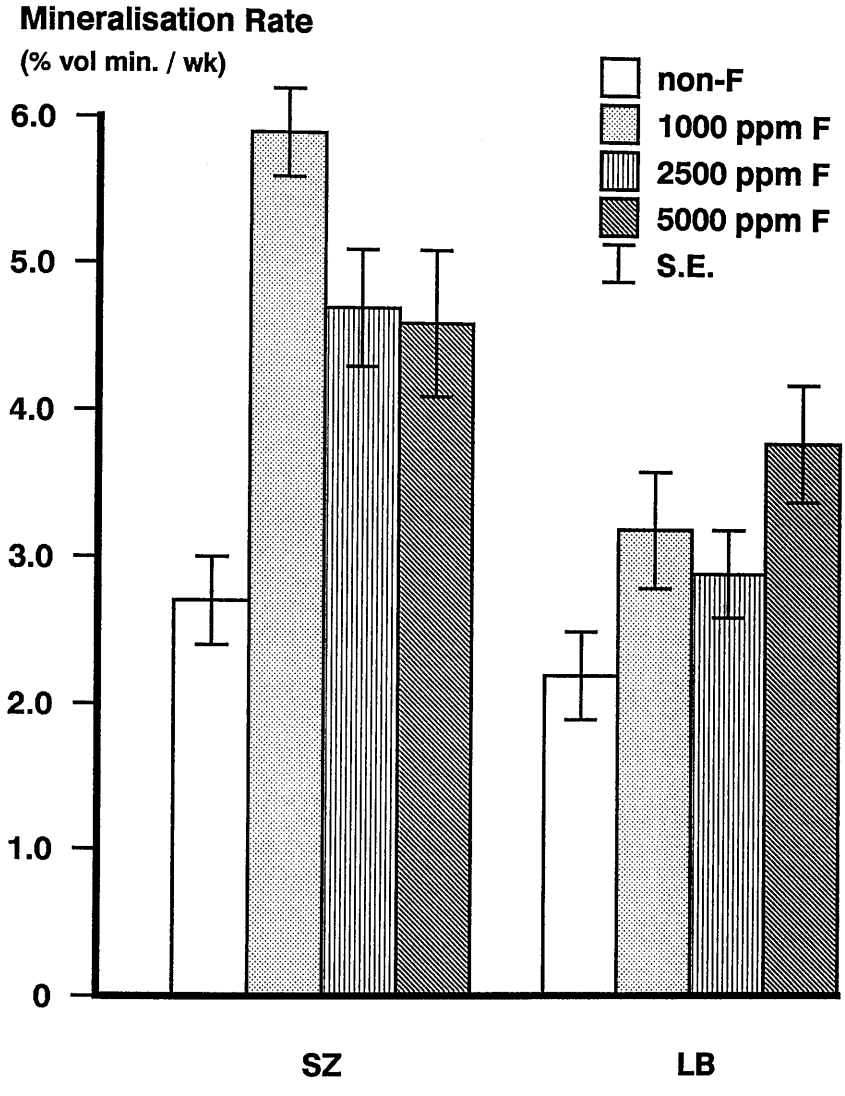


Figure 4.6 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer A, abraded (dentine) root lesions.

treatments with the 1000 ppm F^- paste being significantly superior to 2500 and 5000 ppm F^- treatments ($0.001 < p < 0.01$).

The lesion body (Fig. 4.6) mineralised significantly when lesions were exposed to the 1000 and 5000 ppm F^- as compared to the placebo treatment. However, there was no significant difference between 2500 ppm F^- and the placebo. The lesion body remineralisation rate for the 5000 ppm F^- paste was found to be significantly superior to that of 2500 ppm F^- paste.

Comparisons between remineralisation of cementum and dentine lesions.

For the 1000 ppm F^- paste, the Δz_2 remineralisation rates for dentine lesions were significantly higher ($p < 0.001$) than that for cementum lesions. However, for the same parameter, the rates for 5000 ppm F^- paste cementum lesions were significantly higher than for dentine lesions ($0.01 < p < 0.05$).

None of the other concentrations for all parameters showed significant differences.

4.4.3 Fluoride concentration effect on non - abraded root surface lesiom for subject B, (Fig. 4.7, 4.8, Tab.4.3).

For this subject, for all parameters, remineralisation rates were higher for the 2500 ppm F^- treatment than any of the other fluoride concentrations.

For the Δz_1 parameter (Fig. 4.7), lesions treated with the placebo dentifrice demineralised significantly (p

Table 4.3 Mean Mineralisation rates for the Δz, Surface Zone (SZ) and Lesion Body (LB) parameters. Volunteer B.

ppm F⁻	n	Δz_1	Δz_2	Δz_3	SZ	LB
		(% Vol. min x μm/wk)			(% Vol. min. / wk)	
Non-abraded (cementum) lesions.						
0	8	-642.6 (125.0)	-572.1 (147.4)	-688.8 (133.9)	0.2 (0.3)	-1.3 (0.5)
1000	8	68.1 (61.0)	288.1 (36.2)	62.6 (38.1)	3.6 (0.6)	1.8 (0.4)
2500	8	422.5 (80.2)	715.5 (103.4)	345.3 (44.1)	5.2 (0.8)	4.0 (0.3)
5000	8	43.9 (75.4)	518.4 (45.7)	164.5 (30.1)	4.2 (0.3)	2.6 (0.1)
Abraded (dentine) lesions.						
0	4	-1187.1 (169.5)	-1141.3 (187.7)	-1263.1 (163.2)	0.1 (0.2)	-3.4 (0.4)
1000	8	35.6 (52.3)	516.6 (51.0)	114.3 (37.2)	5.3 (0.6)	2.2 (0.4)
2500	7	28.6 (147.2)	334.1 (107.0)	111.7 (73.0)	2.8 (0.8)	1.9 (0.6)
5000	8	301.0 (53.0)	660.2 (108.8)	262.3 (36.1)	5.6 (0.8)	3.1 (0.4)

n = number of lesions available for analysis.
Values in parenthesis are Standard Errors

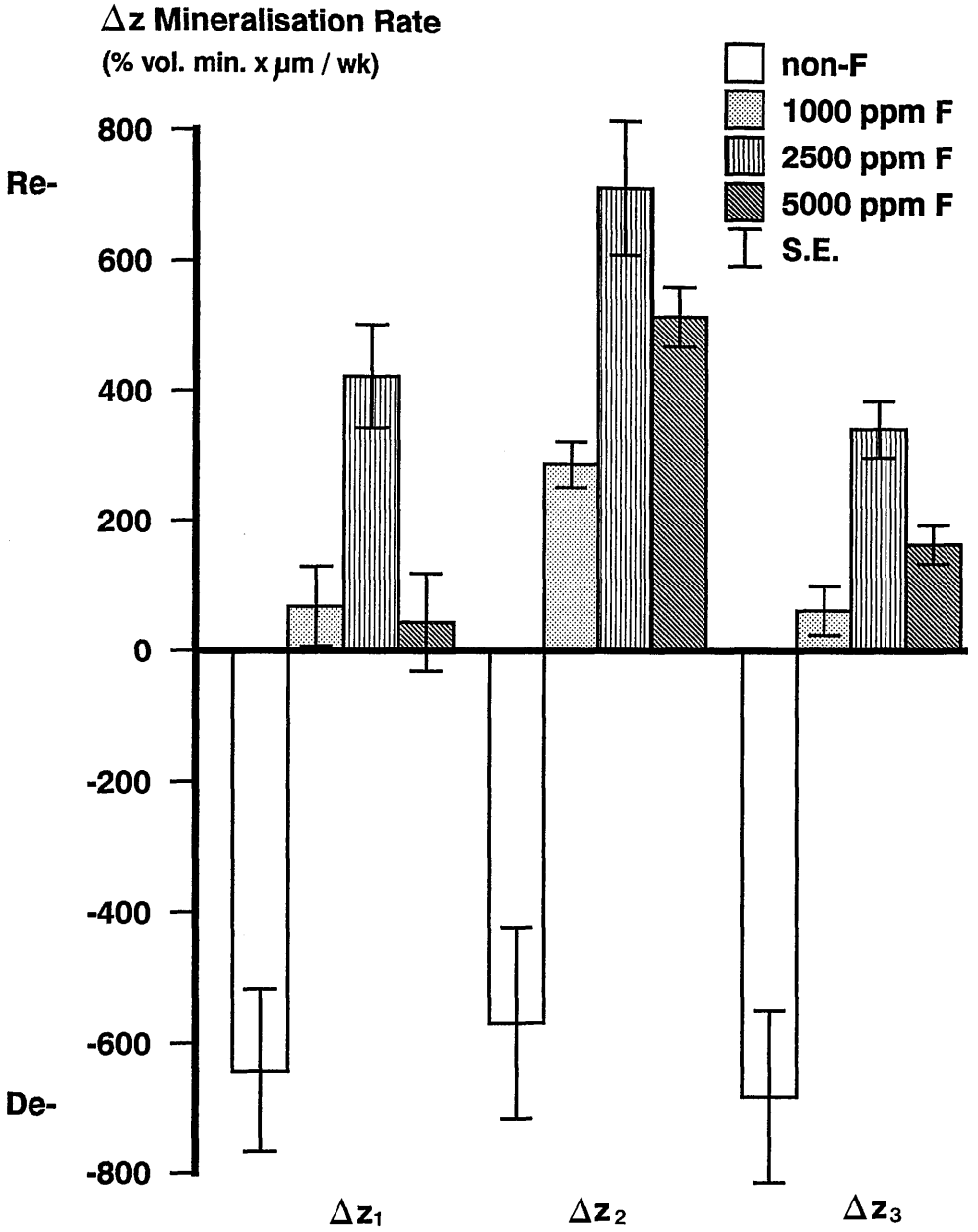


Figure 4.7 Histogram of the mean mineralisation rates for the Δz parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer B, non - abraded (cementum) root lesions.

< 0.001) further compared to those treated with 1000, 2500 and 5000 ppm F^- which remineralised. The 2500 ppm F^- paste was superior to the other two 1000 and 5000 ppm F^-, ($p < 0.001$). However, the 5000 and 1000 ppm F^- pastes were not significantly different from each other ($p > 0.05$).

For the Δz_2 parameter (Fig. 4.7, there was a significant demineralisation when lesions were subjected to the placebo compared to the other three fluoride pastes. Also, the 2500 ppm F^- paste was significantly ($p < 0.001$) superior to both 1000 and 5000 ppm F^-, and the 5000 ppm F^- was significantly ($p < 0.001$) superior to the 1000 ppm F^- paste.

The situation for Δz_3 parameter (Fig. 4.7) was similar to the above one, here also demineralisation resulted from lesions' exposure to the placebo while mineral was gained significantly ($p < 0.001$) after exposing similar lesions to the three fluoride treatments. Moreover, remineralisation rates which resulted from treating lesions with 2500 ppm F^- pastes were significantly higher ($p < 0.001$) than those which resulted from either 1000 or 5000 ppm F^- pastes. The 5000 ppm F^- paste was significantly ($0.01 < p < 0.05$) superior to 1000 ppm F^- paste.

The mineral content of both surface zone and lesion body (Fig. 4.8) increased significantly ($p < 0.001$) when lesions were exposed to the three fluoride pastes compared to placebo which resulted in further demineralisation of the lesion body and a non significant remineralisation of the surface zone. Although mean

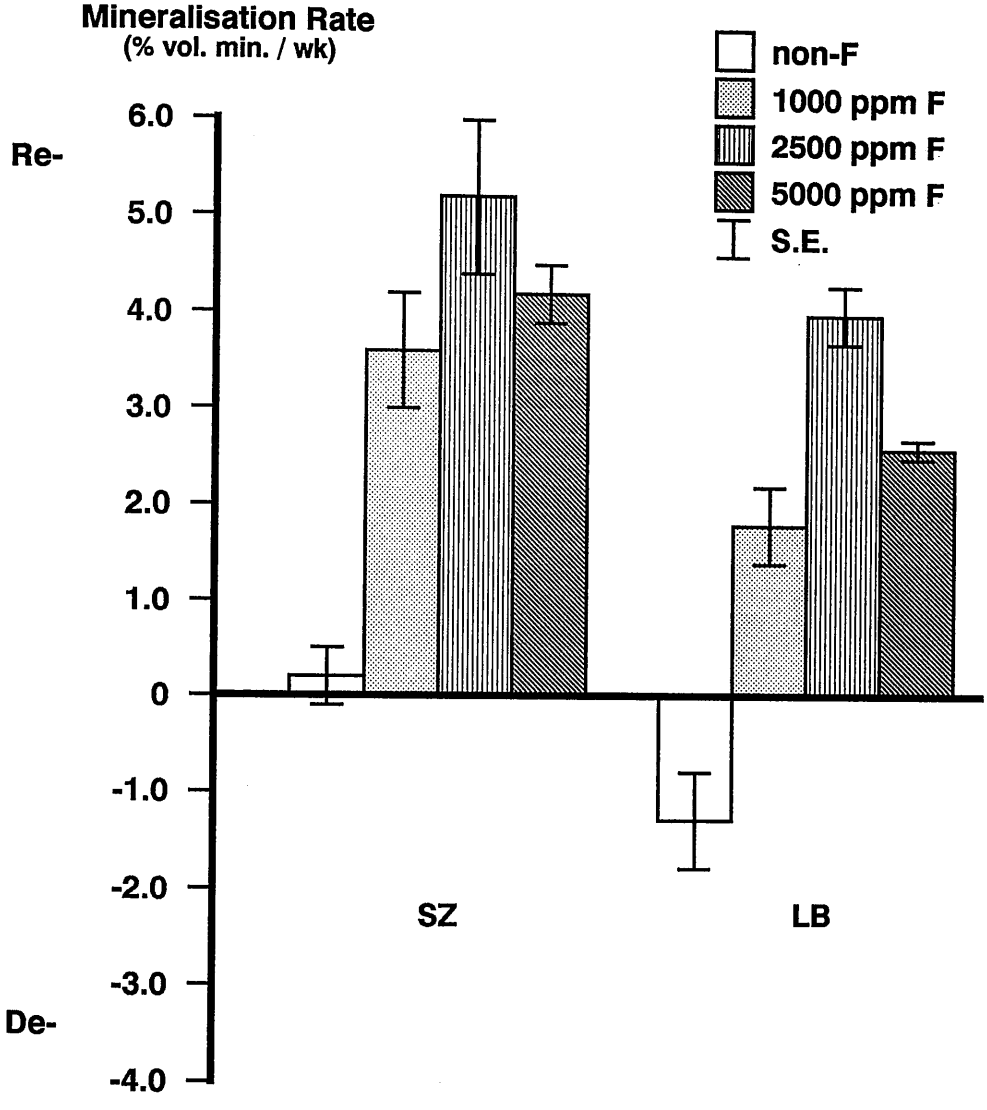


Figure 4.8 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer B, non - abraded (cementum) root lesions.

(±S.E) remineralisation rates for the surface zone parameter were higher (5.2 (0.8) % volume mineral / week) for 2500 ppm F^- lesions than for those of 1000, (3.6 (0.6) vol. min. / week) and 5000 ppm F^-, (4.2 (0.3) vol. min. / week), these were not significantly different from each other. However, for the lesion body parameter, significant differences were noted between 2500 ppm F^- and both 1000 and 5000 ppm F^- pastes ($p < 0.001$), also 5000 ppm F^- was found superior to 1000 ppm F^- paste, ($0.01 < p < 0.05$).

4.4.4 Fluoride concentration effect on abraded root surface lesion for subject B, (Figs. 4.9, 4.10, Tab. 4.3).

Dentine lesions demineralised further after exposure to the placebo dentifrice compared to the significant ($p < 0.001$) remineralisation after exposure to the three fluoride dentifrices. Here, 5000 ppm F^- resulted in higher remineralisation rates of varying significance than the other fluoride concentraions for all measured parameters.

For the Δz_1 parameter (Fig. 4.9) remineralisation rates for the 1000, 2500 and 5000 ppm F^- pastes were significantly different from the demineralisation which resulted from lesion exposure to the placebo. However, among the three fluoride concentrations, the 5000 ppm F^- was found to be significantly superior to the other two 1000 ($p < 0.001$) and 2500 ppm F^- ($0.01 < p < 0.05$).

For the Δz_2 parameter (Fig. 4.9), integrated mineral loss increased further as a result of lesions'

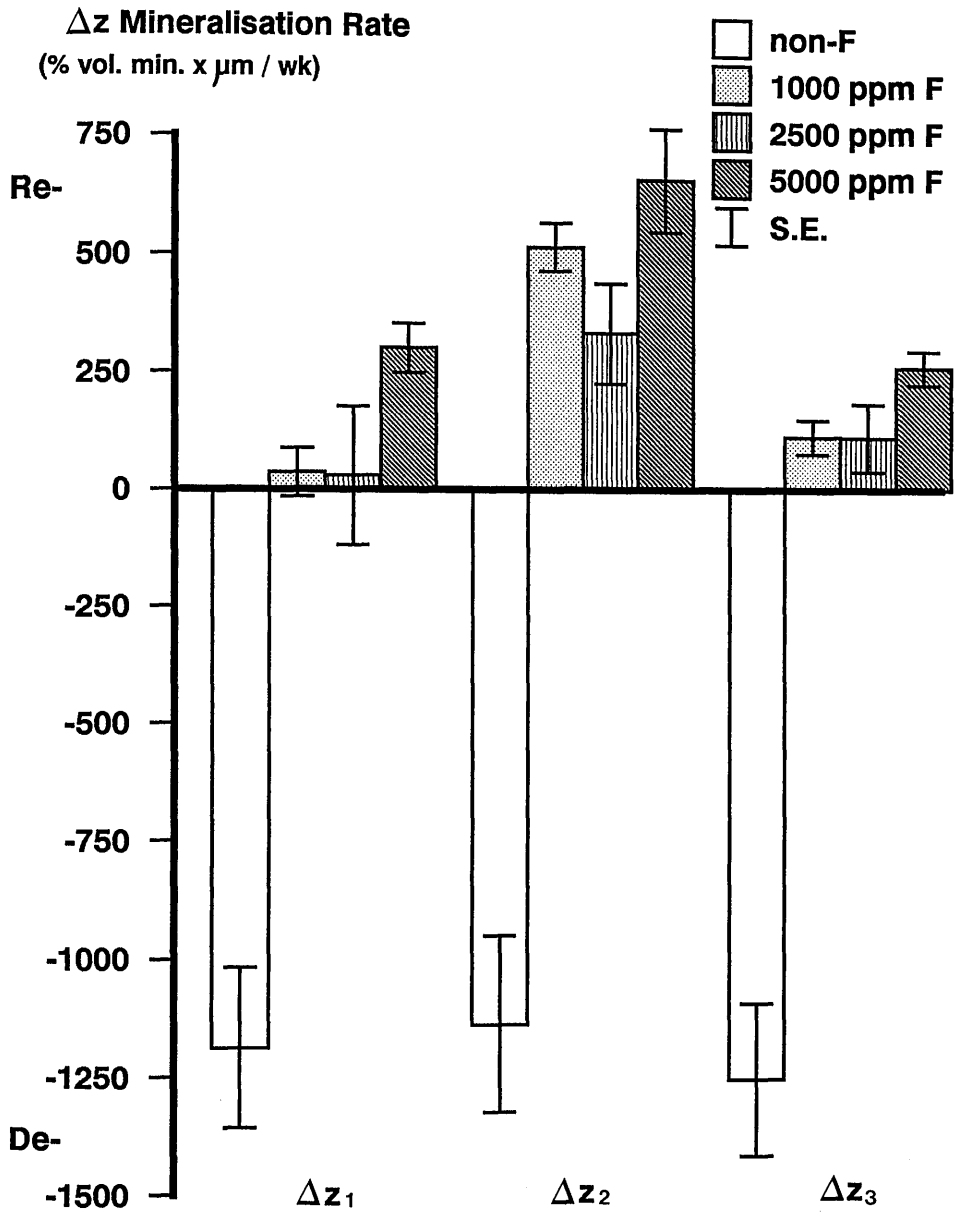


Figure 4.9 Histogram of the mean mineralisation rates for the Δz parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer B, abraded (dentine) root lesions.

exposure to placebo dentifrice. However, the mineral loss was significantly reduced ($p < 0.001$) when lesions were treated with each of the three fluoride concentrations. In addition, a significant differece was found between 5000 ppm F^- (superior) and 2500 ppm F^- pastes ($0.001 < p < 0.01$).

For the Δz_3 parameter (Fig. 4.9), exposure of the lesions to the placebo resulted in demineralisation which was significantly different from the remineralisation found on exposing similar lesions to the 1000, 2500 and 5000 ppm F^- dentifrices, ($p < 0.001$). The remineralisation rates for 5000 ppm F^- dentifrice were significantly higher ($p < 0.001$) than both 1000 and 2500 ppm F^- dentifrices.

The mineral content of the surface zone and lesion body (Fig. 4.10) of all lesions increased with 1000, 2500 and 5000 ppm F^- pastes and this was significantly different from the demineralisation with the placebo ($p < 0.001$). Both 1000 and 5000 ppm F^- pastes were significantly superior ($0.001 < p < 0.01$) to 2500 ppm F^- paste for the surface zone parameter. However, for the lesion body the 5000 ppm F^- paste was found superior to the 2500 ppm F^- paste only ($0.01 < p < 0.05$).

Comparisons between remineralisation of cementum and dentine lesions.

For all Δz parameters, the demineralisation which resulted from exposing lesions to the placebo dentifrice was significantly higher for dentine lesions than cementum lesions ($0.001 < p < 0.01$). In addition, the lesion body

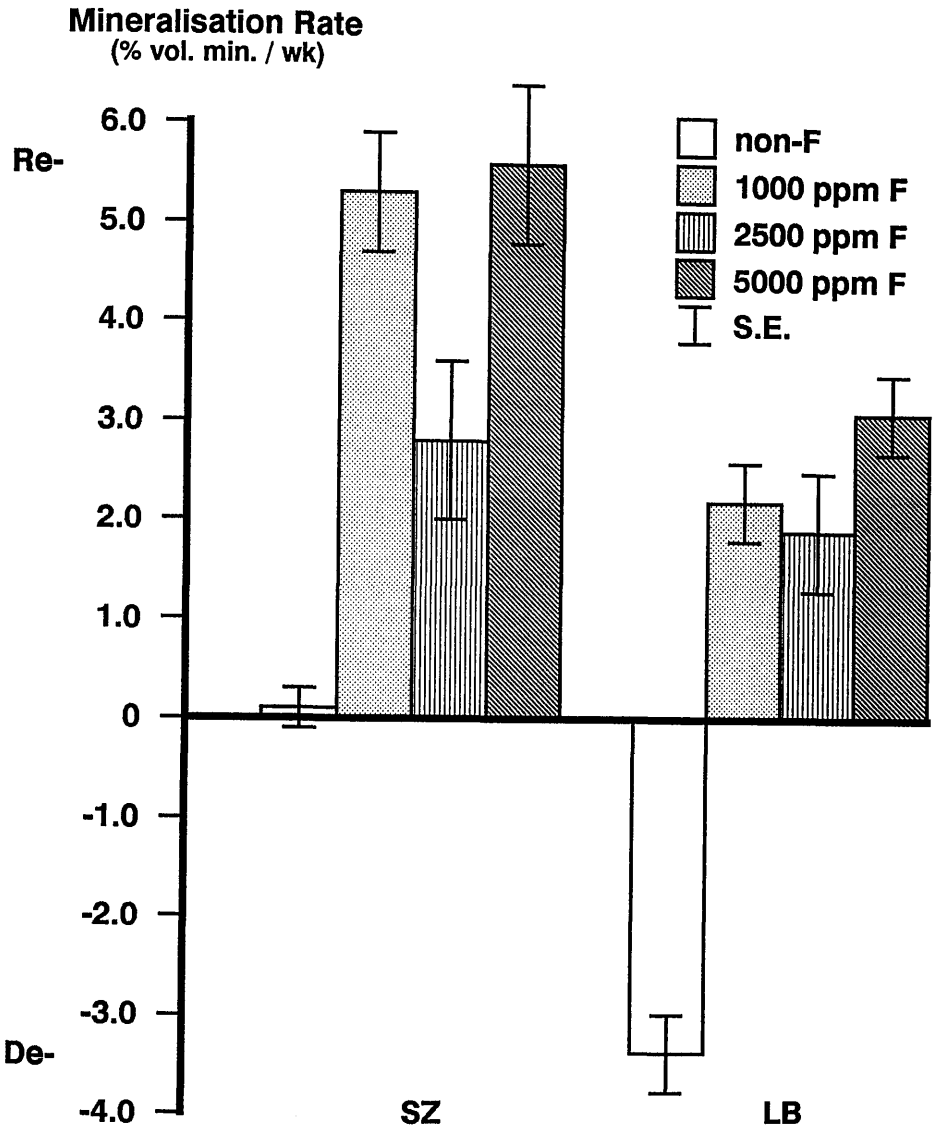


Figure 4.10 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer B, abraded (dentine) root lesions.

of dentine demineralised significantly further more than cementum lesions ($p < 0.001$). However, no significant difference was found to exist between the remineralisation of both tissue lesions' surface zones.

For Δz_2 parameter, the remineralisation rates for 1000 ppm F^- dentine lesions were significantly higher than those for cementum lesions for the same concentration ($p < 0.001$). Also, for the surface zone parameter, the dentine lesions values were significantly higher than those for cementum lesions of the same concentration ($0.01 < p < 0.05$).

For the 2500 ppm F^- lesions, remineralisation for cementum lesions was significantly better than for dentine lesions for Δz_1, Δz_2 ($0.001 < p < 0.01$), Δz_3, lesion body ($p < 0.001$) and surface zone ($0.01 < p < 0.05$).

For the 5000 ppm F^- treatment, the remineralisation rates for dentine lesions were significantly better than cementum lesions ($p < 0.001$) in Δz_1 only, while the remineralisation rates for all other parameters were not significantly different.

4.4.5 Fluoride concentration effect on non - abraded root surface lesion for subject C (Figs. 4.11, 4.12, Tab 4.4).

In this subject, no particular trend was noted and in some occasions the remineralisation rates of the placebo treatment were higher than those of the three fluoride treatments. The detailed results were as follows:

For the Δz_1 parameter, no significant differences between any of the four dentifrices tested was evident.

For the Δz_2 parameter, exposing the lesions to the placebo dentifrice resulted in remineralisation rates which were significantly higher than those for the 1000 ppm F^- paste ($p < 0.001$). Among the three fluoride concentrations tested, the remineralisation rates for the 5000 ppm F^- were significantly higher than for 2500 ($0.01 < p < 0.05$) and 1000 ppm F^- pastes ($p < 0.001$).

For the Δz_3 parameter (Fig. 4.11), the only significant difference was between the 5000 ppm F^- and the 2500 ppm F^- ($0.01 < p < 0.05$) pastes.

The mineral content of the surface zone and lesion body (Fig. 4.12) increased with all fluoride pastes and the placebo. The surface zone remineralisation rates achieved by treating the lesions with the 5000 ppm F^- paste were significantly higher than those with the 1000 ppm F^- and placebo pastes, ($0.01 < p < 0.001$). In addition, those of the 2500 ppm F^- paste were significantly higher than for the 1000 ppm F^- or placebo pastes, ($0.01 < p < 0.05$). However, none of the dentifrices tested was found to be significantly different from each other for the lesion body parameter.

Table 4.4 Mean Mineralisation rates for the Δz, Surface Zone (SZ) and Lesion Body (LB) parameters. Volunteer C.

ppm F^-	n	Δz_1	Δz_2	Δz_3	SZ	LB
		(% Vol. min x μm/wk)			(% Vol. min. / wk)	
Non-abraded (cementum) lesions.						
0	7	269.5 (31.8)	680.1 (42.0)	264.0 (23.5)	5.0 (0.5)	4.1 (0.3)
1000	5	232.0 (83.5)	449.6 (75.5)	326.4 (51.8)	5.0 (0.6)	3.8 (0.4)
2500	7	212.9 (50.5)	681.0 (83.9)	226.1 (38.3)	6.5 (0.5)	4.4 (0.3)
5000	8	256.7 (79.8)	907.7 (101.9)	337.8 (47.2)	6.9 (0.5)	4.1 (0.7)
Abraded (dentine) lesions.						
0	7	157.7 (32.1)	581.1 (32.8)	174.5 (34.5)	3.1 (0.6)	3.2 (0.2)
1000	8	238.2 (59.3)	746.1 (69.1)	242.0 (26.9)	5.3 (0.3)	3.2 (0.2)
2500	12	158.1 (46.4)	732.1 (90.3)	182.7 (32.2)	6.0 (0.5)	2.8 (0.3)
5000	7	226.8 (74.7)	604.3 (47.3)	334.3 (26.3)	4.5 (0.4)	3.8 (0.3)

n = number of lesions available for analysis.
Values in parenthesis are Standard Errors

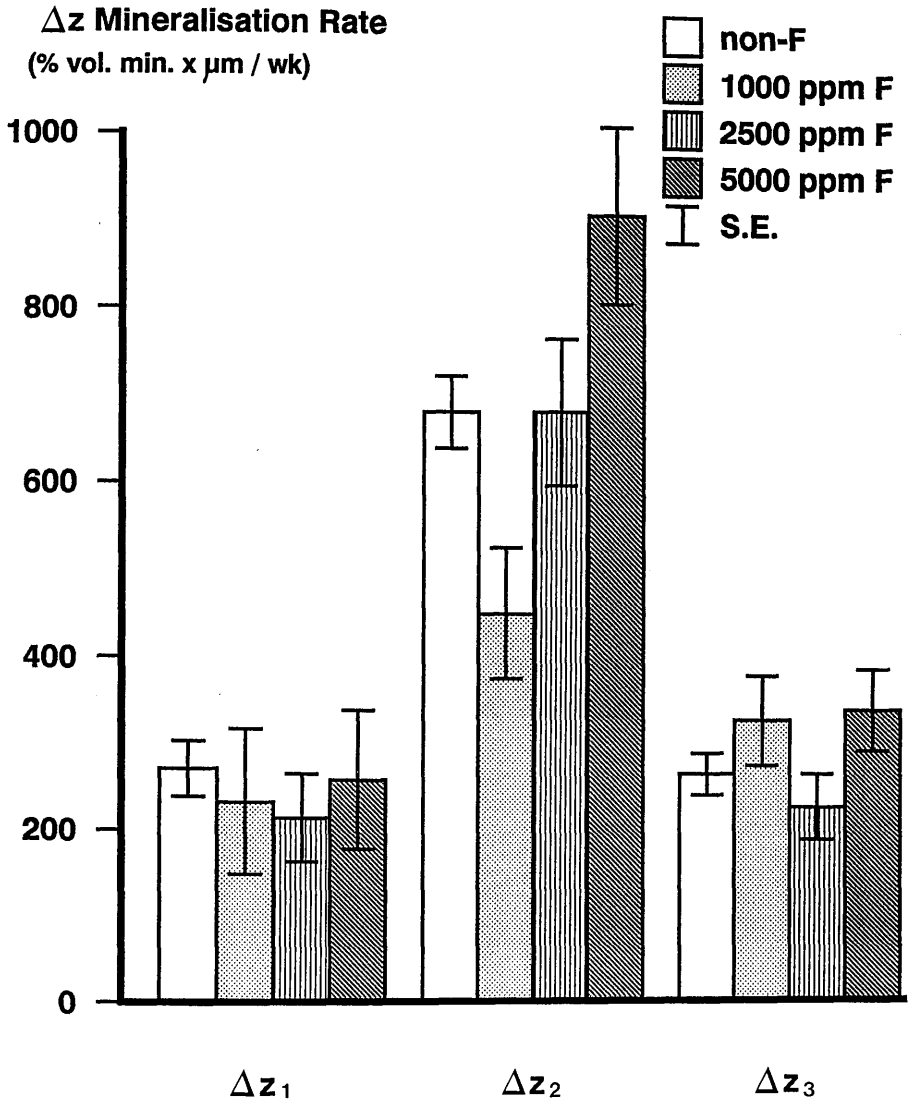


Figure 4.11 Histogram of the mean mineralisation rates for the Δz parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer C, non - abraded (cementum) root lesions.

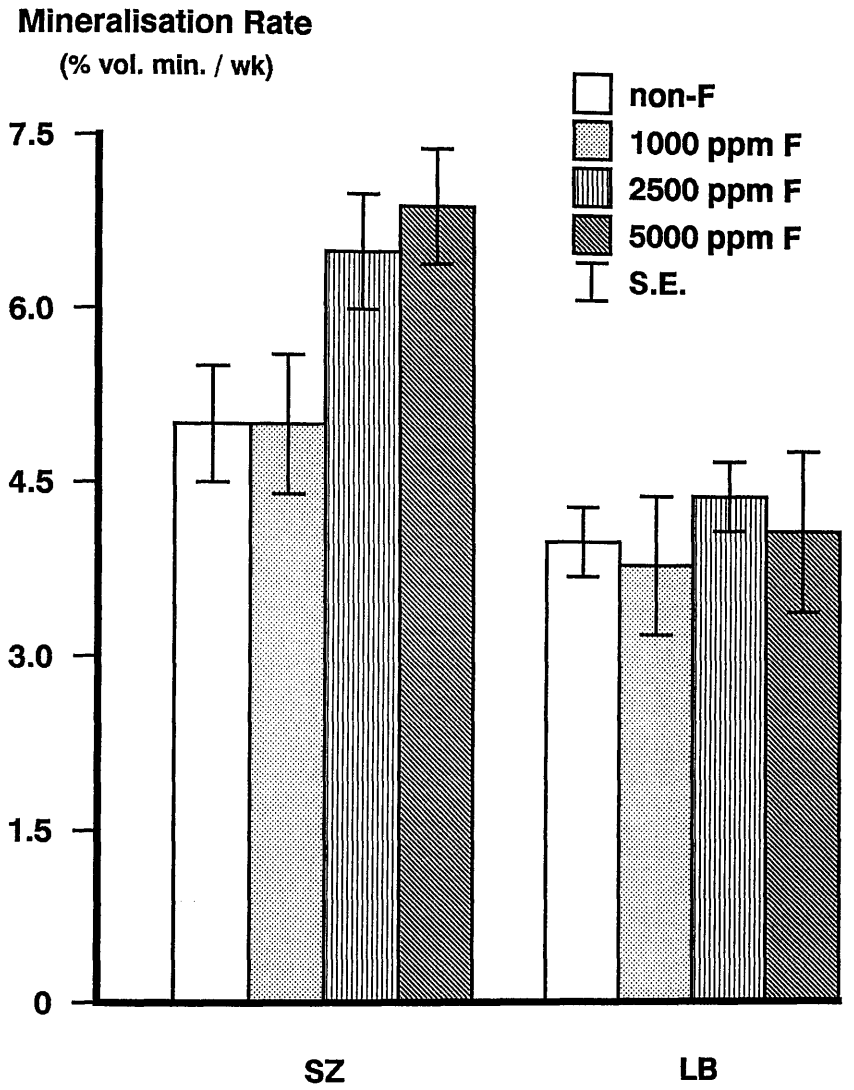


Figure 4.12 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer C, non - abraded (cementum) root lesions.

4.4.6 Fluoride concentration effect on abraded root surface lesion for subject C (Figs. 4.13, 4.14, Tab 4.4).

All lesions remineralised when exposed to the fluoride and placebo dentifrices. There was no particular trend evident except that both the 1000 and 2500 ppm F^- pastes showed higher mean remineralisation rates than the placebo and 5000 ppm F^- pastes, for the z_2 and surface zone parameter

For the Δz_1 parameter (Fig. 4.13), there were no significant differences between the remineralisation rates for the four treatments. For the z_2 parameter, a significant difference was noted between 1000 ppm F^- and the placebo pastes.

However, for the Δz_3 parameter (Fig. 4.13), the remineralisation rates for the 5000 ppm F^- paste were found to be significantly higher than those of the placebo and 2500 ppm F^- paste, ($p < 0.001$) and the 1000 ppm F^- paste ($0.01 < p < 0.05$).

The mineral content of the surface zone and lesion body (Fig. 4.14) increased with all four pastes, and for the surface zone, this was significantly higher with the 1000, 2500 ($p < 0.001$) and 5000 ppm F^- ($0.01 < p < 0.05$) than the placebo pastes. However, no significant differences between the fluoridated pastes were found for the surface zone remineralisation rates. Apart from a significant difference between the remineralisation rates for the 5000 and 2500 ppm F^- pastes no other significant differences were evident for the lesion body parameter.

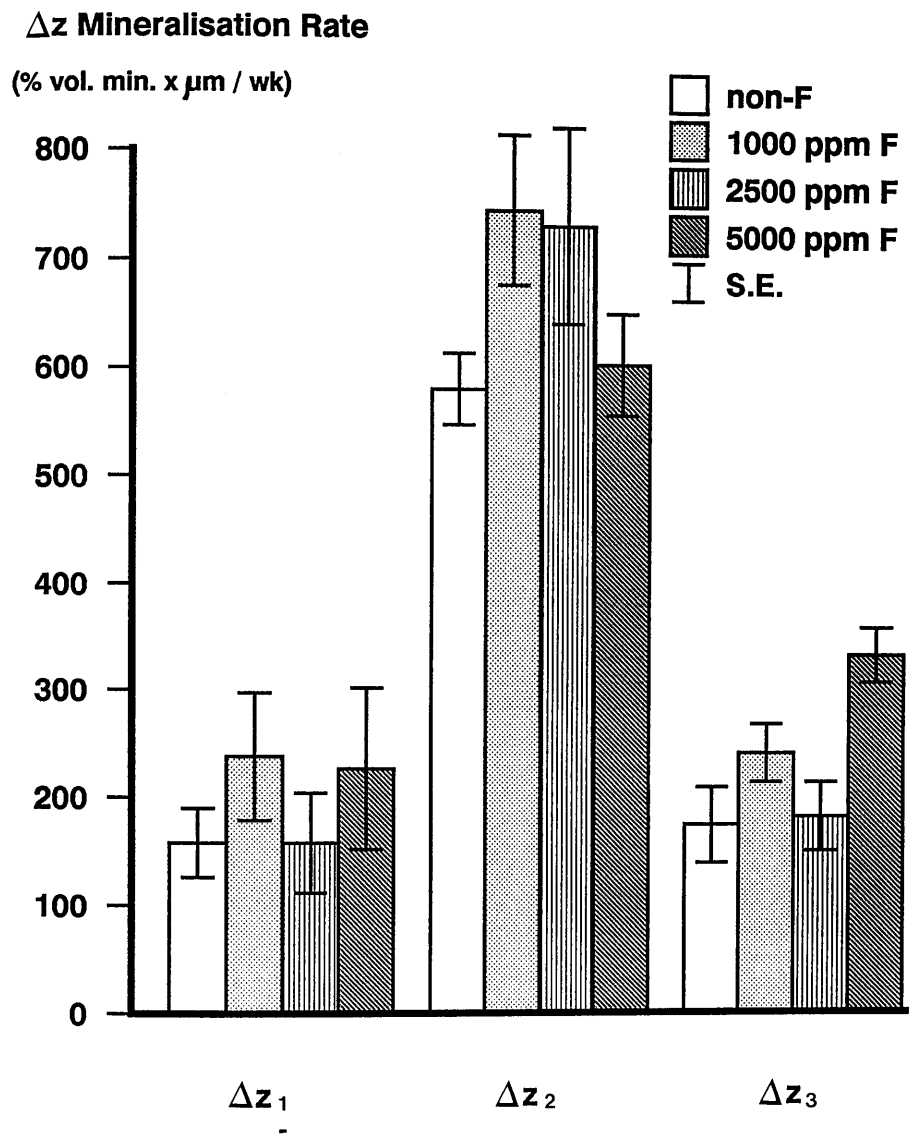


Figure 4.13 Histogram of the mean mineralisation rates for the Δz parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer C, abraded (dentine) root lesions.

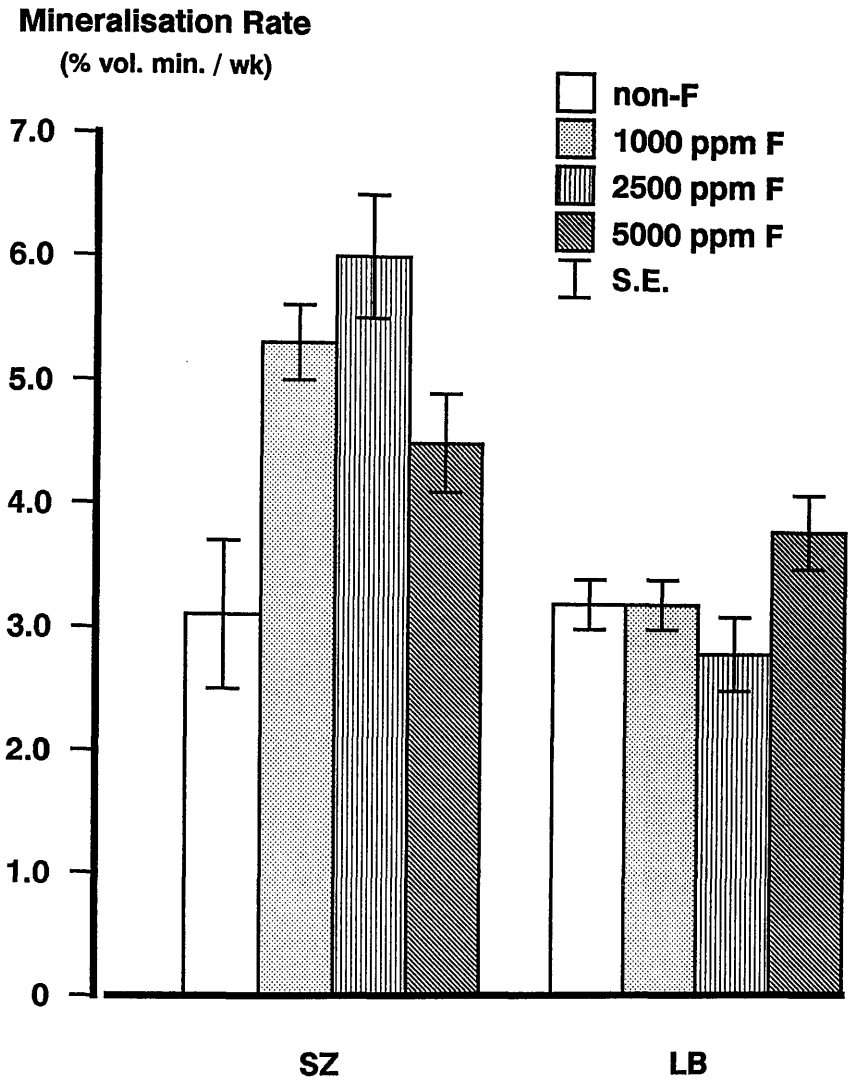


Figure 4.14 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer C, abraded (dentine) root lesions.

Comparisons between remineralisation of cementum and dentine lesions.

For the placebo treatment, remineralisation occurred in cementum and dentine lesions for all parameters and this was significantly higher in cementum lesions than dentine lesions for Δz_1, surface zone, ($0.001 < p < 0.01$), Δz_2, Δz_3 ($0.01 < p < 0.05$) and lesion body ($p < 0.001$).

For the 1000 ppm F^- treatment, remineralisation rates were significantly higher for dentine lesions than for the cementum lesions ($p < 0.001$) for z_2 only.

For the 2500 ppm F^- paste, the remineralisation for dentine was significantly higher than for cementum lesions ($p < 0.001$), for all parameters.

For the 5000 ppm F^- treatment, the cementum lesion remineralisation rates were greater than those of dentine lesions ($0.01 < p < 0.05$) for Δz_2. However, the surface zone of the dentine lesions remineralised significantly better ($p < 0.001$) than cementum lesions.

4.4.7 Fluoride concentration effect on non - abraded root surface lesion for subject D (Figs. 4.15, 4.16, Tab. 4.5).

Unfortunately, this subject left before the non - fluoride dentifrice could be tested.

For all Δz parameters (Fig. 4.15), the 5000 ppm F^- dentifrice's remineralisation rates were higher than the 1000, 2500 ppm F^- dentifrices. The detailed differences were as follows:

For the Δz_1 parameter (Fig. 4.15), the

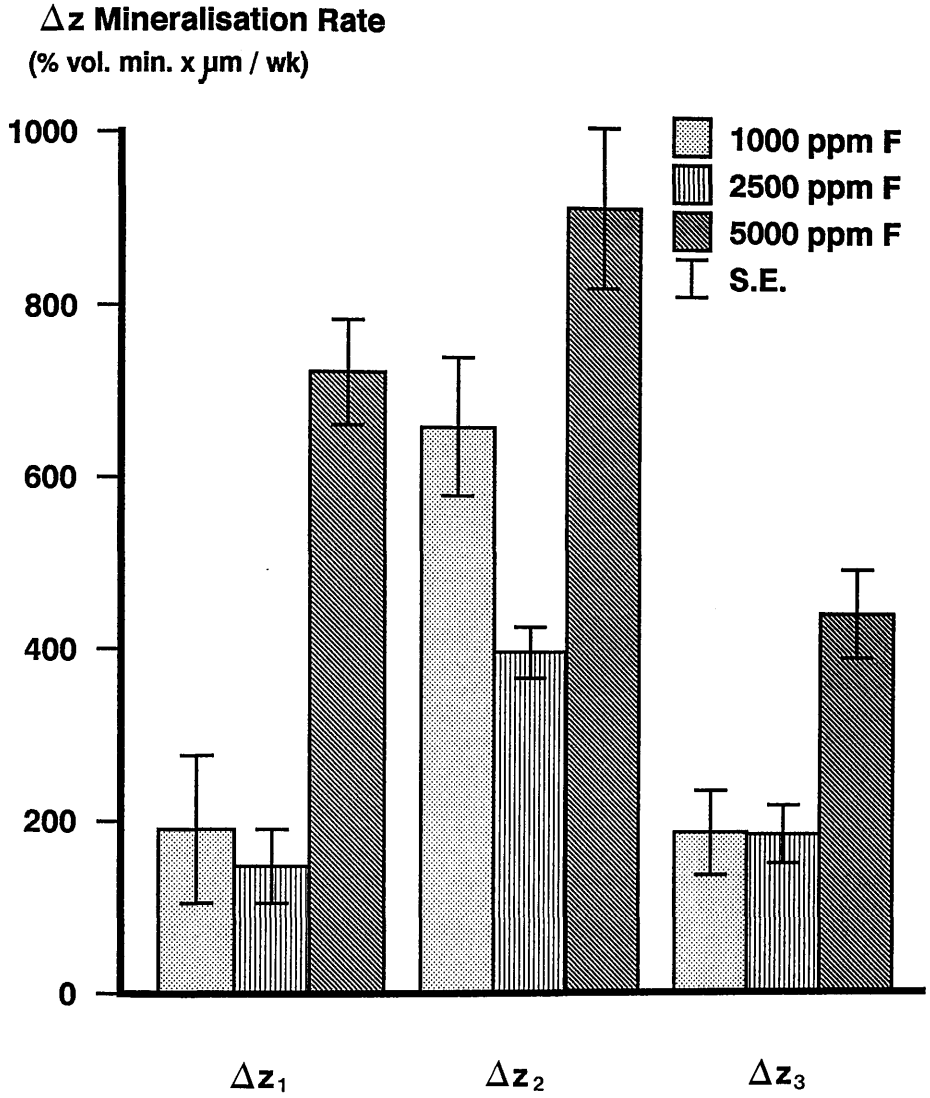


Figure 4.15 Histogram of the mean mineralisation rates for the Δz parameters for the three test dentifrices. (+ve rates indicate remineralisation).
Volunteer D, non - abraded (cementum) root lesions.

remineralisation rates for 5000 ppm F^- paste were significantly higher than those for the two other fluoride pastes tested ($p < 0.001$).

For the Δz_2 parameter (Fig. 4.15), again the 5000 ppm F^- dentifrice achieved a significantly better remineralisation than the 1000 ppm F^- ($0.01 < p < 0.001$) and the 2500 ppm F^- dentifrice lesions ($p < 0.001$). Also, the 1000 ppm F^- dentifrice resulted in significantly ($p < 0.001$) higher remineralisation rates than the 2500 ppm F^- dentifrice.

The picture for Δz_3 parameter (Fig. 4.15) was similar to that of Δz_1, here again exposing lesions to 5000 ppm F^- paste resulted in remineralisation rates which were significantly higher than those of 1000 and 2500 ppm F^- pastes ($p < 0.001$).

For the surface zone parameter (Fig. 4.16), the 1000 ppm F^- was significantly superior to 2500 ppm F^- treatment which was the only significant difference elucidated between the three fluoride treatments.

However, for the lesion body parameter (Fig. 4.16), a trend was evident; the mean ($\pm$S.E) remineralisation rate for 5000 ppm F^- lesions was 3.3 (0.5), against 3.0 (0.3) and 2.5 (0.4) % volume mineral / week for 2500 and 1000 ppm F^- pastes respectively.

4.4.8 Fluoride concentration effect on abraded root surface lesion for subject D (Figs. 4.17, 4.18, Tab. 4.5).

Apart from the Δz_1 parameter (Fig. 4.17) where the 5000 ppm F^- paste was superior to the other two fluoride

Table 4.5 Mean Mineralisation rates for the Δz, Surface Zone (SZ) and Lesion Body (LB) parameters. Volunteer D.

ppm F^-	n	Δz_1	Δz_2	Δz_3	SZ	LB
		(% Vol. min x μm/wk)			(% Vol. min. / wk)	
Non-abraded (cementum) lesions.						
1000	8	190.9	659.8	187.3	6.5	2.5
		(85.9)	(81.4)	(48.8)	(0.7)	(0.4)
2500	8	147.5	396.6	185.3	4.8	3.0
		(43.3)	(30.3)	(33.9)	(0.4)	(0.3)
5000	8	722.5	914.7	443.1	5.6	3.3
		(60.9)	(93.2)	(52.2)	(0.3)	(0.5)
Abraded (dentine) lesions.						
1000	5	284.8	809.3	396.9	6.6	4.2
		78.1)	(107.0)	(33.3)	(0.1)	(0.9)
2500	7	284.2	802.2	343.8	5.6	3.9
		(135.0)	(72.8)	(30.1)	(0.5)	(0.5)
5000	6	466.6	765.9	362.4	4.7	3.8
		(80.9)	(109.3)	(32.1)	(0.7)	(0.3)

n = number of lesions available for analysis.
Values in parenthesis are Standard Errors

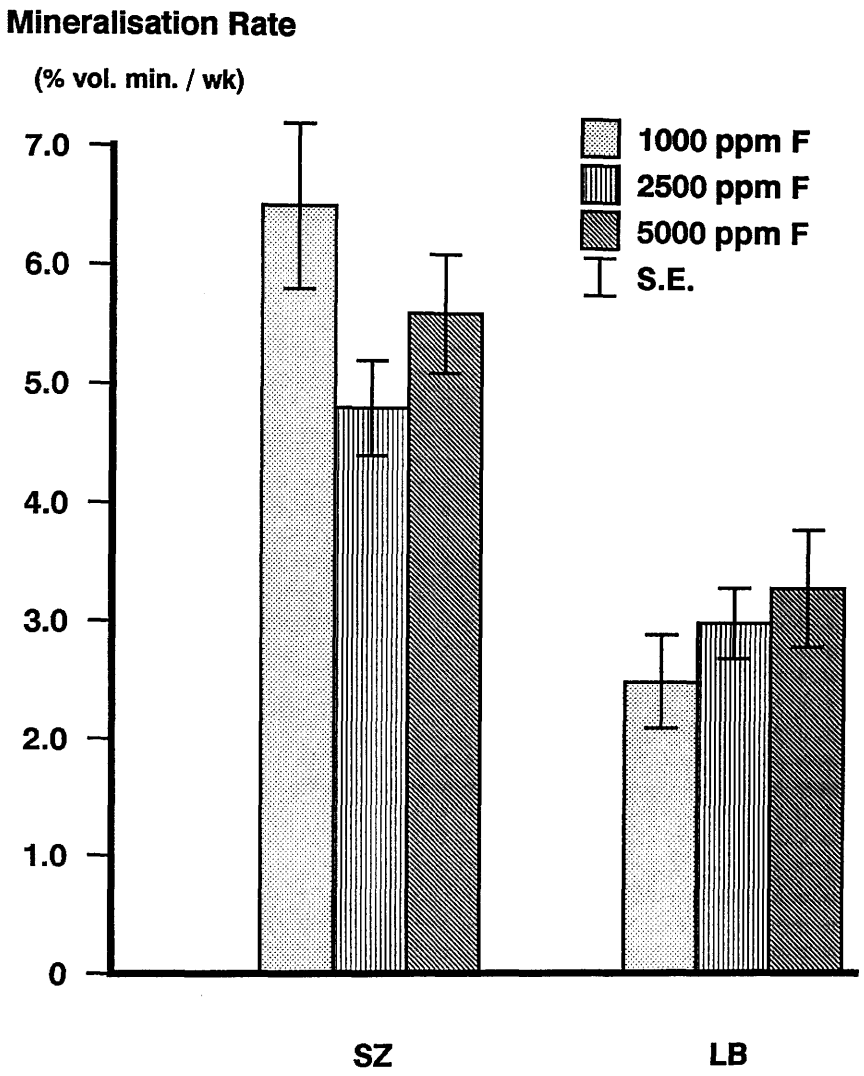


Figure 4.16 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the three test dentifrices. (+ve rates indicate remineralisation).
Volunteer D, non - abraded (cementum) root lesions.

dentifrices, there was no particular trend evident here.

For both the Δz_2 and the Δz_3 parameters (Fig. 4.17), the remineralisation rates for the tested fluoride pastes were approximately the same and no significant differences were noted.

However, the remineralisation rates of the surface zone (Fig. 4.18) decreased with increasing dentifrice fluoride concentration. For the 5000 ppm F^- paste, remineralisation rates were significantly lower than those of 1000 ppm F^- paste ($0.001 < p < 0.01$) which was the only significant difference found for this parameter.

The picture for the lesion body parameter (Fig. 4.18) was similar to the surface zone, although significant differences in remineralisation rates were evident.

Comparisons between the remineralisation of cementum and dentine lesions.

For the 1000 ppm F^- lesions, the remineralisation rates for cementum lesions were significantly lower than dentine lesions ($p < 0.001$) for all parameters except surface zone where there was no significant difference.

For the 2500 ppm F^- lesions, remineralisation rates for the dentine lesions were significantly greater than that for cementum lesions ($p < 0.001$) for the Δz_2 and the Δz_3 parameters. For the 5000 ppm F^-, remineralisation rates for the cementum lesions were significantly greater than that for dentine lesions ($0.001 < p < 0.01$).

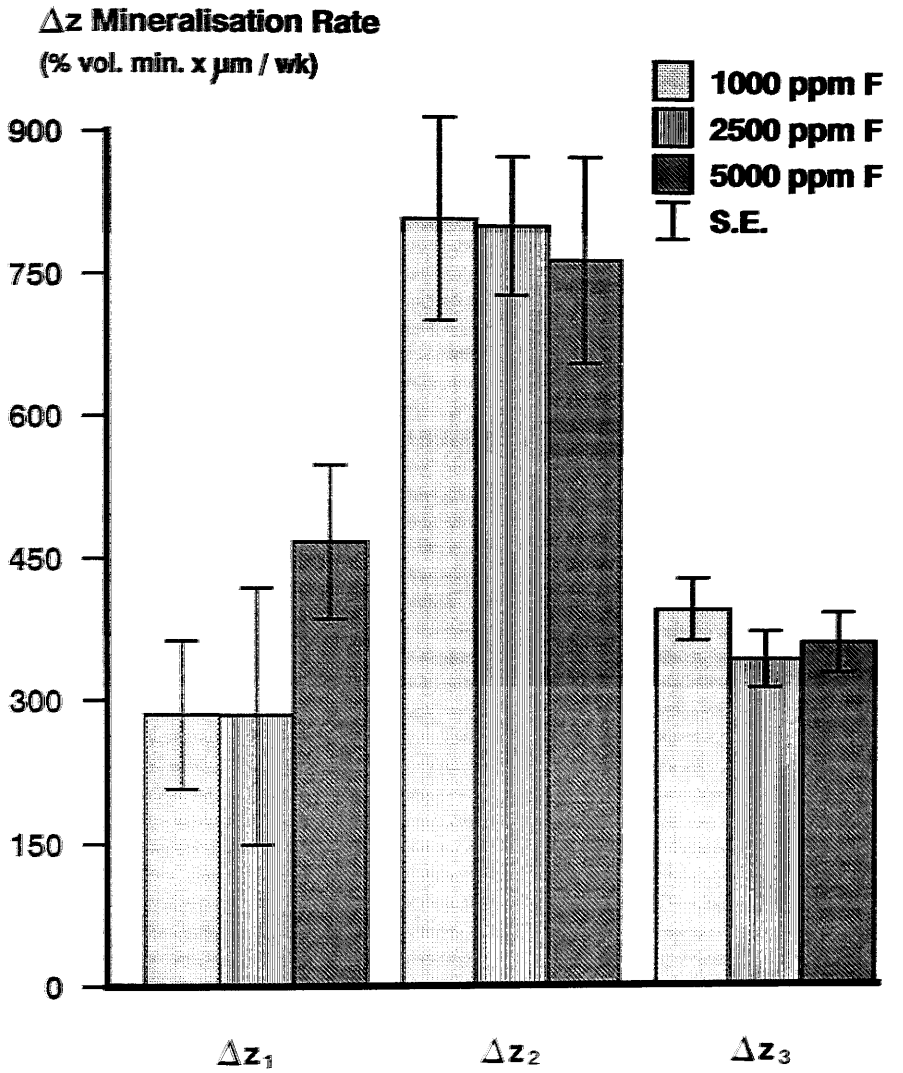


Figure 4.17 Histogram of the mean mineralisation rates for the Δz parameters for the three test dentifrices. (+ve rates indicate remineralisation).
Volunteer D, abraded (dentine) root lesions.

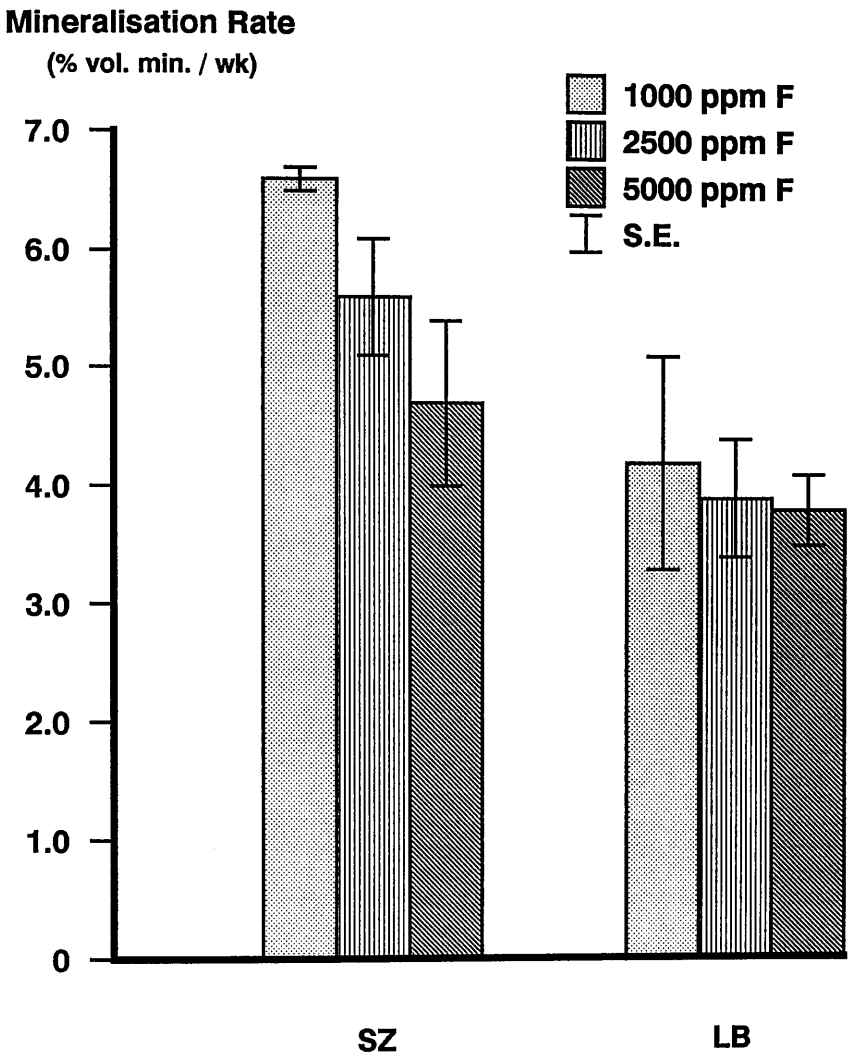


Figure 4.18 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the three test dentifrices. (+ve rates indicate remineralisation).
Volunteer D, abraded (dentine) root lesions.

4.4.9 Fluoride concentration effect on non - abraded root surface lesion for subject E (Figs. 4.19, 4.20, Tab.4.6).

In this subject, all Δz parameters (Fig. 4.19) showed remineralisation rates for the three fluoride concentrations (1000, 2500 and 5000) higher than the placebo and the mean remineralisation rate for 2500 ppm F^- paste was higher than for the 1000 and 5000 ppm F^- pastes.

For the Δz_1 parameter (Fig. 4.19), the mineral loss increased further when the lesions were exposed to the placebo. However, this decreased significantly with the fluoridated toothpastes, ($p < 0.001$). The mean remineralisation rates for lesions treated with 2500 ppm F^- paste were found to be significantly higher than those for lesions treated with either 1000 ($0.01 < p < 0.05$) or 5000 ppm F^- ($p < 0.001$).

For the Δz_2 parameter (Fig. 4.19), the remineralisation rates for the fluoridated dentifrices were significantly higher than for the placebo ($p < 0.001$). In addition, the remineralisation rates for 2500 ppm F^- paste were significantly higher than those of 1000 ppm F^- pastes ($0.001 < p < 0.01$).

For the Δz_3 parameter (Fig. 4.19), the 1000 and 2500 ppm F^- dentifrices significantly greater remineralisation than the placebo ($p < 0.001$). In addition, the 2500 ppm F^- paste was found to be significantly superior to both 1000 ($0.01 < p < 0.05$) and the 5000 ppm F^- dentifrices ($p < 0.001$). Also, 1000 ppm F^- dentfrice was significantly superior to 5000 ppm F^- dentifrice.

Table 4.6 Mean Mineralisation rates for the Δz, Surface Zone (SZ) and Lesion Body (LB) parameters. Volunteer E.

ppm F^-	n	Δz_1	Δz_2	Δz_3	SZ	LB
		(% Vol. min x μm/wk)			(% Vol. min. / wk)	
Non-abraded (cementum) lesions.						
0	8	-90.1 (72.5)	162.0 (60.1)	24.6 (34.3)	2.3 (0.3)	2.3 (0.3)
1000	7	170.3 (61.4)	467.0 (59.5)	212.7 (28.3)	4.2 (0.3)	3.4 (0.3)
2500	6	332.0 (44.4)	658.0 (54.7)	307.6 (36.5)	3.8 (0.5)	3.1 (0.2)
5000	8	144.3 (26.8)	527.1 (65.4)	99.4 (29.0)	4.6 (0.3)	1.9 (0.3)
Abraded (dentine) lesions.						
0	8	-52.1 (68.3)	407.5 (79.1)	-0.5 (63.3)	1.9 (0.5)	1.8 (0.4)
1000	6	146.9 (41.6)	457.3 (80.4)	204.8 (46.4)	3.1 (0.1)	2.7 (0.2)
2500	8	457.9 (36.4)	743.2 (38.1)	382.0 (26.1)	4.8 (0.3)	4.1 (0.2)
5000	6	116.7 (45.9)	534.4 (76.4)	176.5 (36.7)	3.7 (0.4)	2.2 (0.3)

n = number of lesions available for analysis.
Values in parenthesis are Standard Errors

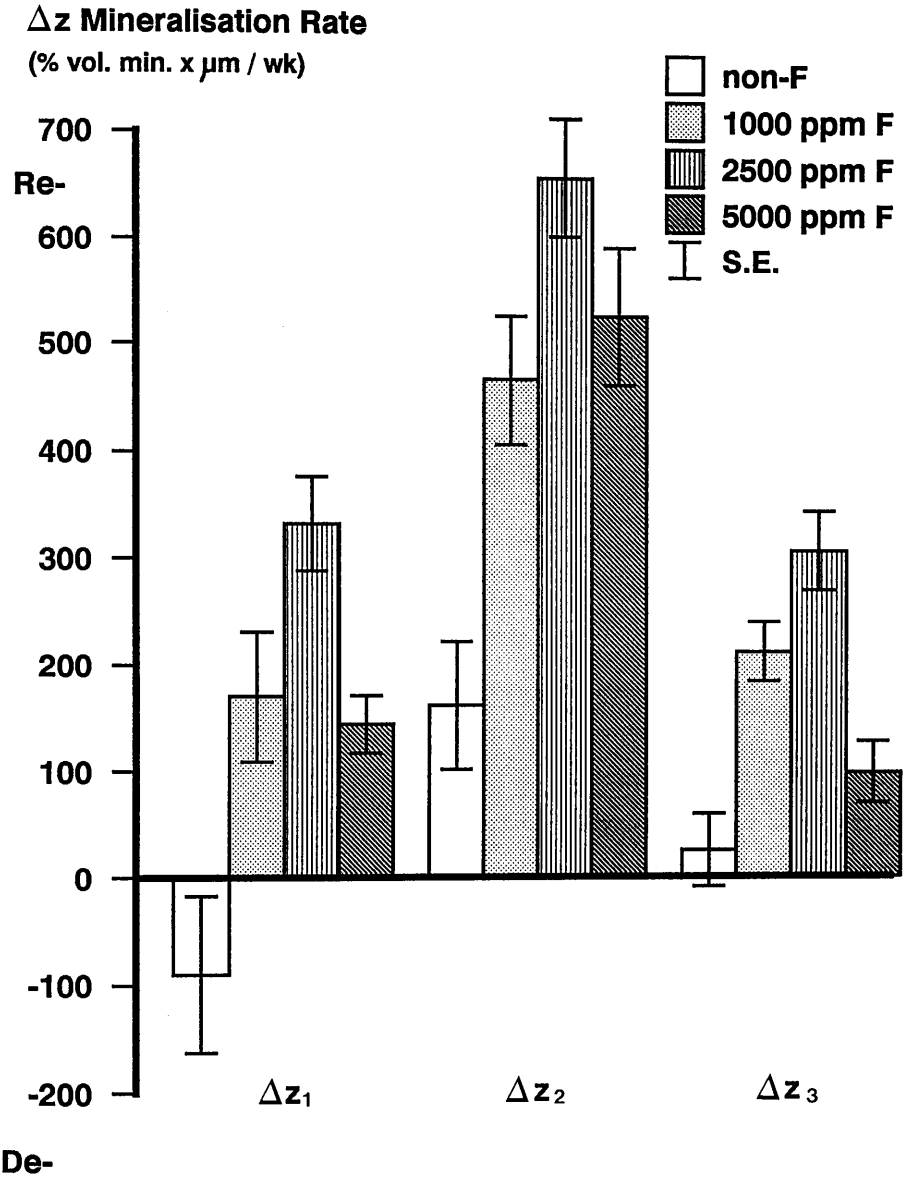


Figure 4.19 Histogram of the mean mineralisation rates for the Δz parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer E, non - abraded (cementum) root lesions.

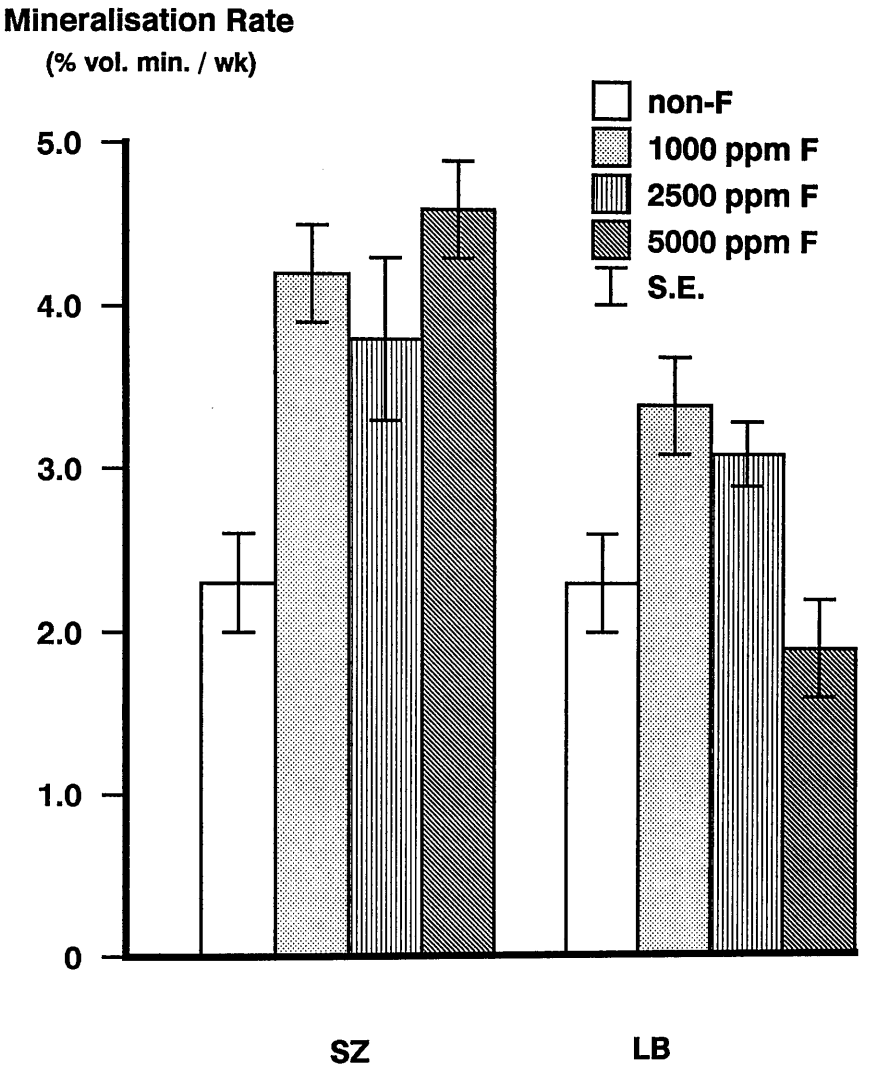


Figure 4.20 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer E, non - abraded (cementum) root lesions.

The mineral content of the surface zone (Fig. 4.20) increased with all pastes but this was significantly higher for the fluoride pastes than for the placebo. However, there were no significant differences amongst the effects of the three fluoride pastes.

The mineral content of the lesion body also increased with all pastes and this was significantly higher with both 1000 ($0.001 < p < 0.01$) and 2500 ppm F^- pastes ($p < 0.001$) than the placebo. However, the mean remineralisation rate for the 5000 ppm F^- paste was significantly lower than those for 1000 and for 2500 ppm F^- pastes ($p < 0.001$).

4.4.10 Fluoride concentration effect on abraded root surface lesion for subject E (Figs. 4.21, 4.22, Tab. 4.6).

Generally, for all parameters the 2500 ppm F^- treatment was superior to the other treatments. The details of results are as following:

For the Δz_1 parameter (Fig. 4.21), exposing lesions to placebo dentifrices resulted in demineralisation which was significantly different from the remineralisation resulting from exposing similar lesions to the three fluoride dentifrices ($p < 0.001$). Moreover, the results for the 2500 ppm F^- paste were significantly higher than those for the 1000 and 5000 ppm F^- pastes ($p < 00.1$).

For the Δz_2 parameter (Fig. 4.21), all lesions gained mineral when exposed to all four pastes. The 2500 ppm F^- paste was significantly superior to the placebo,

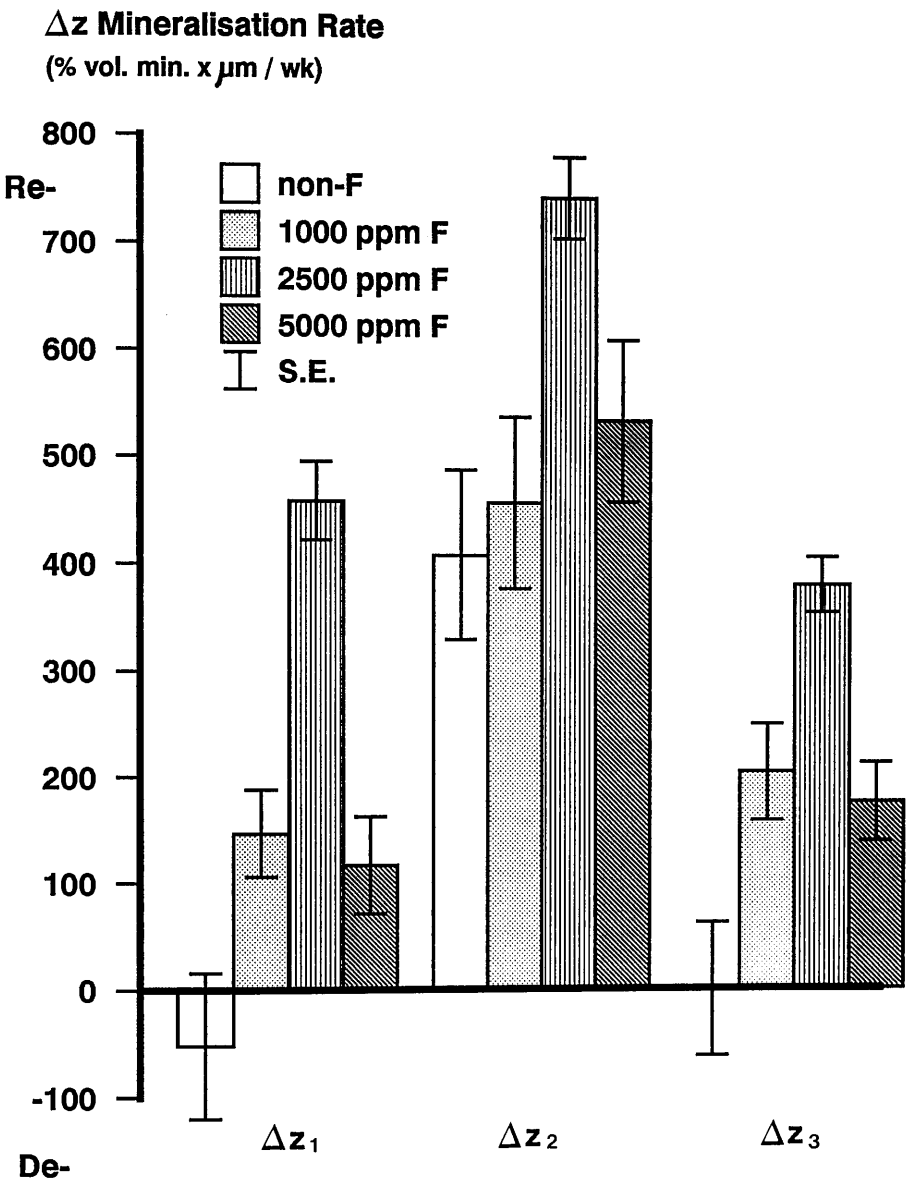


Figure 4.21 Histogram of the mean mineralisation rates for the Δz parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer E, abraded (dentine) root lesions.

the 1000 ($p < 0.001$) and the 5000 ppm F^- ($0.001 < p < 0.01$)) pastes.

The picture for Δz_3 parameter (Fig. 4.21), was similar to the Δz_2 parameter, with all treatments producing varying degrees of mineral gain. However, that of fluoride dentifrices was significantly greater than that of placebo treatment ($p < 0.001$). Moreover, the 2500 ppm F^- treatment was significantly superior to the 1000 and 5000 ppm F^- pastes.

The mineral contents of both surface zone and lesion body (Fig. 4.22) increased with each of the four treatments. For the surface zone, the remineralisation rates for the 2500, 5000 ppm F^- ($p < 0.001$) and 1000 pastes ($0.001 < p < 0.01$) were significantly higher than those for the placebo. The 2500 ppm F^- treatment was also significantly superior to the 1000 ($p < 0.001$) and to 5000 ppm F^- pates ($0.01 < p < 0.05$).

For the lesion body parameter (Fig. 4.22), the remineralisation rates achieved by treating lesions with 2500 ppm F^- paste were significantly higher than other dentifrices ($p < 0.001$). Also 1000 ppm F^- treatment showed mean remineralisation rates which were significantly higher than the placebo ($0.01 < p < 0.05$).

Comparisons between remineralisation of cementum and dentine lesions.

The only significant difference for the placebo lesions was for the Δz_2 parameter in which dentine lesions remineralised significantly better than cementum

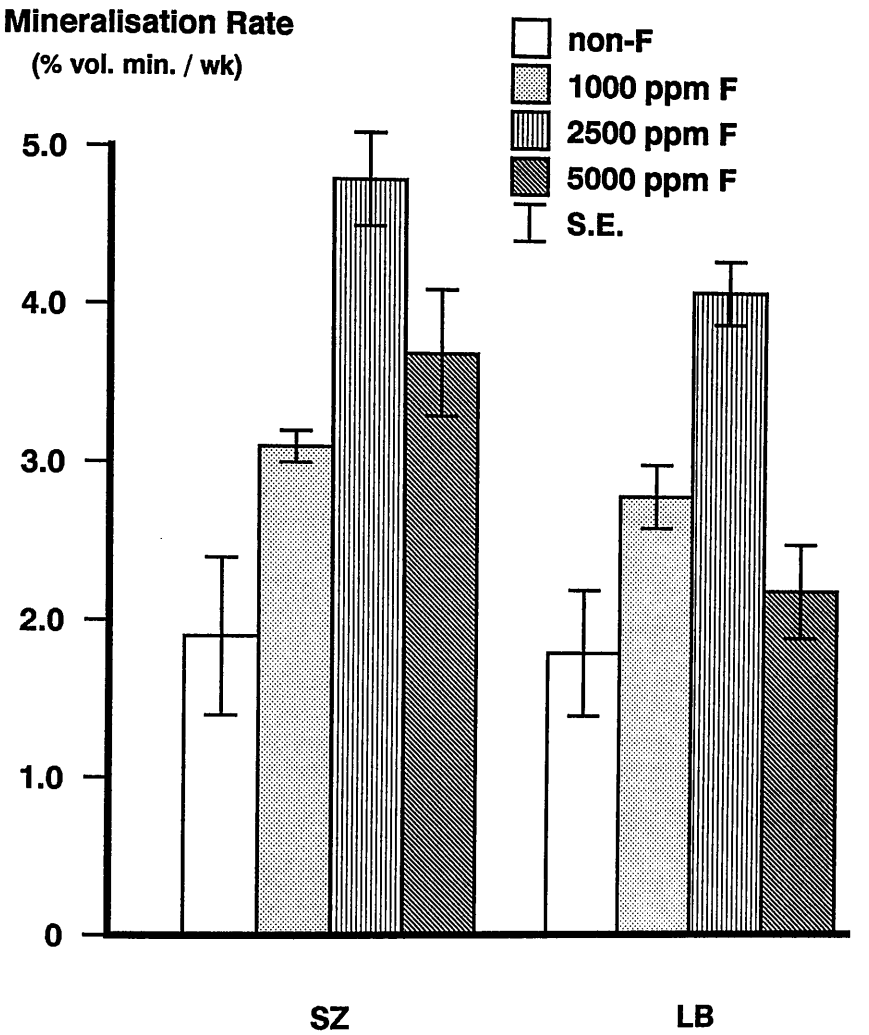


Figure 4.22 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer E, abraded (dentine) root lesions.

($0.001 < p < 0.01$).

For the 1000 ppm F^- paste treated dentine, lesions, remineralisation rates for the surface zone parameter were also significantly better than for cementum lesions ($p < 0.001$)

In addition, for the 2500 ppm F^- paste, remineralisation was significantly better for dentine than for cementum lesions for the Δz_1 ($0.001 < p < 0.01$) and lesion body ($0.001 < p < 0.01$) parameters. However, the surface zone of the cementum lesions remineralised significantly greater than dentine lesions ($0.01 < p < 0.05$).

For 5000 ppm F^- paste, the remineralisation rates for the surface zone, were significantly higher ($0.01 < p < 0.05$) for cementum than for dentine lesions. None of the other values was significantly different.

4.4.11 Fluoride treatment effect on non - abraded root surface lesion for subject F (Fig. 4.23, 4.24, Tab. 4.7).

For all parameters , exposing the lesions to the placebo dentifrice resulted in further demineralisation which was significantly different from the remineralisation effect of the fluoride treatments. Moreover, the remineralisation of the 5000 ppm F^- lesions was always higher than those for the other treatments.

For the Δz_1 parameter (Fig. 4.23), there was a definite concentration $\overset{\text{effect}}{\wedge}$ with each F^- paste producing significantly greater ($p < 0.001$) remineralisation than the pastes with lower F^- concentrations.

Table 4.7 Mean Mineralisation rates for the Δz, Surface Zone (SZ) and Lesion Body (LB) parameters. Volunteer F.

ppm F^-	n	Δz_1	Δz_2	Δz_3	SZ	LB
		(% Vol. min x μm/wk)			(% Vol. min. / wk)	
Non-abraded (cementum) lesions.						
0	8	-944.0 (117.5)	-839.3 (136.2)	-925.0 (103.6)	-0.1 (0.5)	-1.8 (0.2)
1000	7	132.9 (45.4)	300.1 (31.0)	88.2 (37.8)	4.9 (0.2)	3.2 (0.2)
2500	8	183.7 (75.8)	201.3 (30.1)	93.7 (25.4)	2.8 (0.3)	1.4 (0.2)
5000	8	453.0 (71.3)	752.7 (76.1)	346.3 (46.6)	6.0 (0.3)	3.0 (0.7)
Abraded (dentine) lesions.						
0	8	-301.8 (253.3)	-139.1 (278.7)	-335.1 (220.2)	-1.2 (1.0)	-2.4 (0.6)
1000	8	46.0 (48.2)	172.5 (85.4)	117.3 (53.4)	5.4 (0.4)	0.9 (0.5)
2500	8	-54.9 (40.0)	240.3 (37.9)	-54.7 (41.2)	3.1 (0.3)	1.5 (0.3)
5000	8	59.4 (58.8)	637.6 (56.7)	134.1 (31.0)	4.0 (0.4)	2.9 (0.4)

n = number of lesions available for analysis.
Values in parenthesis are Standard Errors

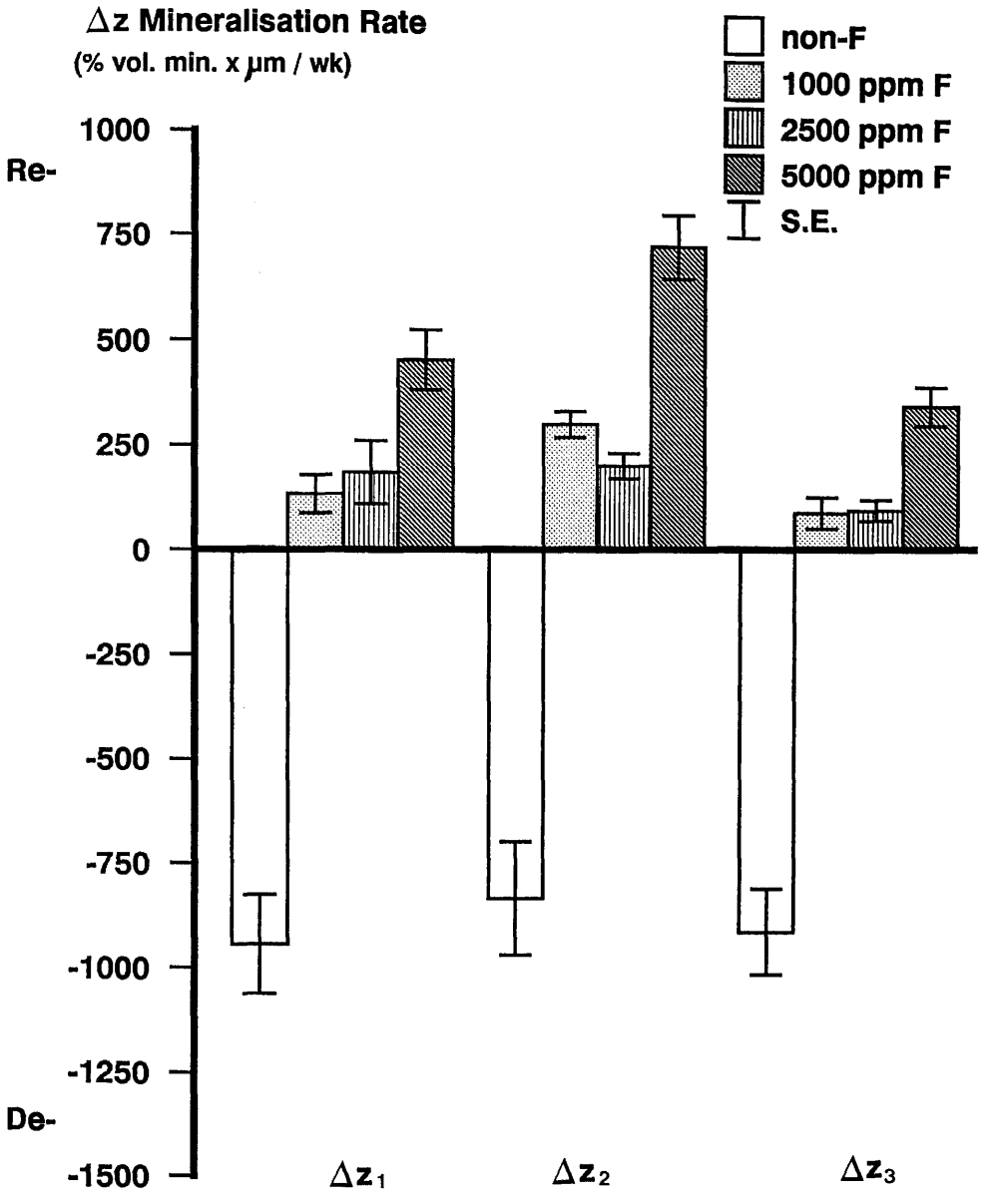


Figure 4.23 Histogram of the mean mineralisation rates for the Δz parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer F, non - abraded (cementum) root lesions.

For the Δz_2 parameter (Fig. 4.23), lesions demineralised further when exposed to the placebo treatment and in contrast to the remineralisation when similar lesions were exposed to the three fluoride treatments. However, among the fluoride concentrations, the remineralisation rates for the 5000 ppm F^- lesions were significantly superior to both 1000 and 2500 ppm F^- ($p < 0.001$). Also, the 1000 ppm F^- treatment was significantly superior to the 2500 ppm F^- treatment.

The picture for Δz_3 parameter (Fig. 4.23) was similar to the one above: i.e lesions demineralised further when subjected to the placebo dentifrice and remineralised when subjected to 1000, 2500 and 5000 ppm F^- treatments. However, the 5000 ppm F^- was found to be significantly superior to both 2500 and 1000 ppm F^- ($p < 0.001$). Also, 2500 ppm F^- was significantly superior to the 1000 ppm F^- ($p < 0.001$).

The surface zone and lesion body demineralised (Fig. 4.24) further after exposure to the placebo dentifrice whereas they remineralised significantly after the other three fluoride treatments ($p < 0.001$). For the surface zone, the mean remineralisation rate for the 5000 ppm F^- lesions was significantly ($0.001 < p < 0.01$) higher than for the 1000 and 2500 ppm F^- groups ($p < 0.001$). Also, for the same parameter, the 1000 ppm F^- values were significantly superior to the 2500 ppm F^- paste ($p < 0.001$). For the lesion body parameter, both the 1000 and 5000 ppm F^- remineralisation rates were significantly greater than the 2500 ppm F^- values, ($p < 0.001$).

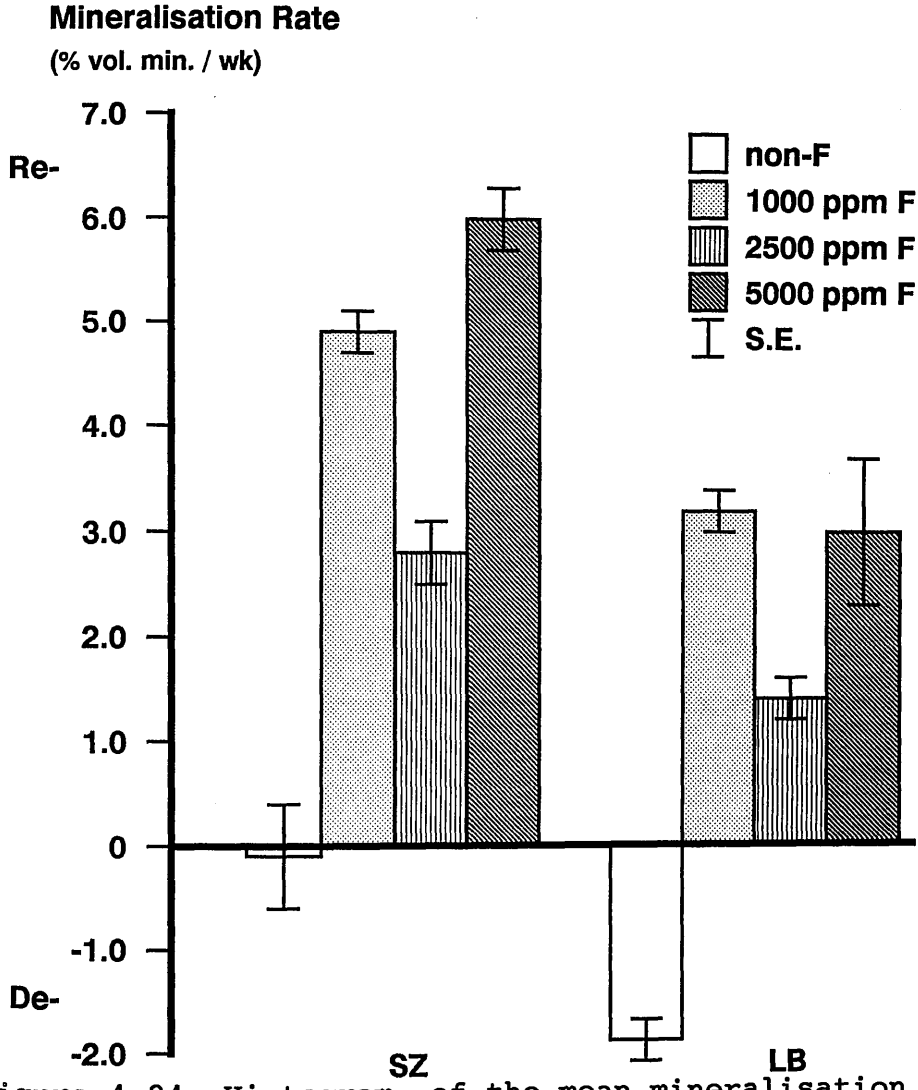


Figure 4.24 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer F, non - abraded (cementum) root lesions.

4.4.12 Fluoride concentration effect on abraded root surface lesion for subject F (Figs. 4.25, 4.26, Tab4.7).

For both Δz_1 and Δz_3 parameters (Fig. 4.25), lesions which were subjected to both placebo and 2500 ppm F^- demineralised significantly compared to lesions exposed to the fluoridated pastes, which remineralised, ($p < 0.001$). However, the demineralisation was significantly greater for the placebo than for the 2500 ppm F^- lesions ($p < 0.001$).

For the Δz_1 parameter (Fig. 4.25), although demineralisation occurred with the placebo and 2500 ppm F^- dentifrices, no significant differences were found to exist between the four dentifrices tested.

For the Δz_2 parameter (Fig. 4.25), lesions further demineralised when subjected to the placebo dentifrice and this was significantly different from the remineralisation which resulted with the 5000 ppm F^- paste, ($p < 0.001$). Moreover, the 5000 ppm F^- was found to be significantly superior to the 2500 and 1000 ppm F^- pastes ($p < 0.001$).

For the Δz_3 parameter (Fig. 4.25), lesions also demineralised further when subjected to the placebo dentifrice and this was significantly different from remineralisation which resulted when lesions were subjected to the 5000 ppm fluoride treatment.

The mineral content of both surface zone and lesion body (Fig. 4.26) increased when lesions were subjected to the placebo dentifrice, and this was significantly different from the remineralisation which resulted when

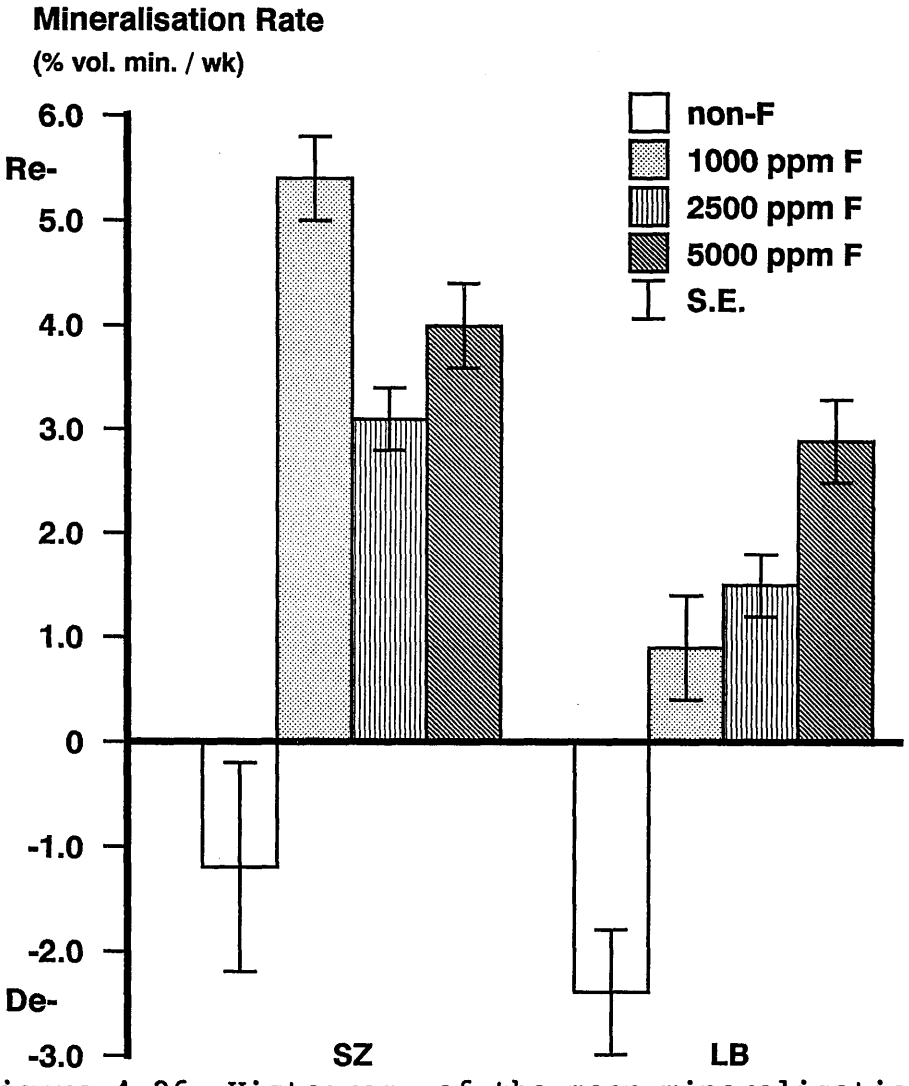


Figure 4.26 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test dentifrices. (+ve rates indicate remineralisation).
Volunteer F, abraded (dentine) root lesions.

similar lesions were subjected to the fluoride treatments. For the surface zone, the 1000 ppm F^- values were significantly superior to the 2500 ppm F^- ($p < 0.001$) and to the 5000 ppm F^- ($0.001 < p < 0.01$). For the lesion body, the 5000 ppm F^- was significantly superior to 1000 ($p < 0.001$) and to 2500 ppm F^- ($0.001 < p < 0.01$).

Comparisons between remineralisaion in cementum and dentine lesions.

All integrated mineral loss parameters showed that demineralisation which resulted from exposing lesions to the placebo dentifrice was significantly greater for dentine lesions than for cementum lesions ($0.001 < p < 0.01$). However, they were not significantly different for surface zone and lesion body parameters.

For the 1000 ppm F^- lesions, remineralisation was found to be significantly better for cementum than dentine lesions for Δz_1 and lesion body parameter.

There was no significant difference evident between cementum and dentine lesions for all parameters with 2500 ppm F^-.

For the 5000 ppm F^- paste, remineralisation rates were significantly higher for cementum lesions than for dentine lesions for all parameters except Δz_2 and lesion body ($p < 0.001$).

4.5 DISCUSSION.

The majority of remineralisation studies have been carried out on enamel lesions, and there is little information on *in situ* remineralisation of root caries.

Only in one *in situ* experiment, in which no microdensitometric measurements were attempted, has root caries been studied (Teranaka & Koulourides, 1987). The results indicated that the tested NaF mouthrinse (100 ppm F^-) was a contributory factor in increasing the resistance of the surface layer of the sound root specimens.

The *in situ* assessment of the cariostatic effect of different fluoride treatments on artificially created root subsurface lesions has not previously been investigated by a combined microradiographic and microdensitometric methods. The intra-oral appliance and the single section technique (*in situ* model) described earlier (Section 4.2) enabled such investigations to be carried out in the natural oral environmental under conditions which cannot adequately be duplicated *in vitro*. The major advantage of this *in situ* system was the ability to determine the mineral changes in each subsurface lesion throughout the experimental period thus obviating the need for a separate control sample with the consequent reduction in sensitivity to measure remineralisation. The main disadvantage of the single section technique is that great care is required to ensure the cut surfaces of the sections are varnished right to the edge of the section.

Moreover, while the fluoride concentration variable was controlled for all subjects, it was not feasible to regulate other inter-subject variables e.g cariogenic potential, salivary flow rate, diet, plaque or calculus formation which may be responsible for some of the differences observed between subjects.

The results reported in this Chapter demonstrated that fluoride dentifrices had a significant remineralising effect on both abraded and non-abraded root subsurface lesions, and showed that the remineralising effect of the saliva can be further enhanced by the availability of fluoride at the root - saliva interface. The degree of remineralisation for the fluoridated dentifrices was always significantly higher than that of the placebo ($p < 0.001$) except for subject C. There was no consistant concentration - dependent effect for all subjects. However, for the non-abraded root surface lesions, for all parameters, maximum remineralisation rates for subjects A and F were always correlated to the 5000 ppm F^- dentifrice. The same observation was also noticed for subject C for all parameters except for lesion body and for subject D except for surface zone. However, for subject B and E the trend was such that remineralisation rates increased with increasing fluoride concentration but decreased when 5000 ppm F^- was used.

For dentine lesions, the remineralisation rates for fluoridated dentifrices were always significantly greater than the placebo. No fluoride effect trend was noticeable and lesions responded differently to the

fluoride treatment among subjects, perhaps reflecting differences in host resistance to continued carious attack, or remineralising potential. Both subjects B and F showed fluoride concentration dependent effects upon remineralisation, although remineralisation rates, for some parameters for 1000 were better than that for 5000 ppm F^- pastes. Maximum remineralisation rates for subject A were achieved with 1000 ppm F^- dentifrice for all parameters except the lesion body. There was no trend at all in subject C, whereas in subject D remineralisation rates decreased with increasing fluoride concentration for all parameters except for Δz_1. The fluoride effect trend for subject E for dentine lesions was similar to that for cementum lesions in which remineralisation rates increased when fluoride was increased from 1000 and 2500 ppm but decreased when 5000 ppm was used.

The cause of the considerable inter-subject variability is not readily apparent, although factors such as sugar intake, salivary flow, plaque formation may be contributory. Although, the mechanisms of enamel lesion remineralisation might be different, the results reported here are consistent with those reported by Creanor & Strang, (1989). However, with the absence of clinical root caries prevention trials employing the same dentifrices as used in this study, no comparisons could be made and, therefore, no firm conclusion regarding the concentration effect could be drawn. This reflects the need for both root caries trials and more *in situ* experiments testing the same fluoride agents.

The demineralisation which resulted from exposing lesions to the placebo, was generally greater for abraded than non-abraded root surfaces. This situation might be explained by the absence of the indigenous fluoride rich layer from the abraded surfaces. Also, this may suggest that cementum may be more caries resistant than dentine. Roots were abraded mechanically in an attempt to attain a more standard (less variable) surface and to study the situation where root subsurface lesion start in dentine after loss of cementum following root surface exposure. It is possible that the increased demineralisation in dentine was due to the effects of mechanical preparation. Whether this would be observed in dentine exposed "naturally" remains to be determined.

4.6 CONCLUSIONS.

The results reported above indicated that non - fluoridated toothpastes are of little benefit in arresting demineralisation and should not be used.

For most parameters, maximum remineralisation was attained when 5000 ppm F^- was used (subjects A, C, D and F). However, maximum remineralisation was achieved with the 2500 ppm F^- dentifrice for Δz parameters in the two other subjects (B and C). The 1000 ppm F^- occasionally achieved maximum remineralisation rates for few parameters. Therefore, there was no definitive evidence to support the hypothesis that higher than 1000 ppm fluoride concentrations are better and could inhibit the root

caries process.

The subjective factors for e.g microbial flora, salivary flow, sucrose intake, and their influence on the remineralisation process might explain in part the inter - subject variability and should be taken into consideration in future experiments. However, the microbiological effects are dealt with in Chapter six.

Demineralisation was always greater for dentine than cementum lesions which might be attributed to the removal of the fluoride rich surface layer. Generally, remineralisation rates for cemental lesions were better than those for dentine lesions for the 5000 ppm F dentifrice, but the opposite was true for the 1000 ppm F dentifrice. Thus in the light of this finding a high (1000 - 5000 ppm) fluoride dose should be recommended for patients undergoing root planing.

Further experiments are required to establish if remineralisation can be improved further by increasing the frequency of lesion exposure to the fluoride treatment or by using a different fluoride agent for e.g NaF.

CHAPTER FIVE.
IN VITRO STUDIES.

5.1 INTRODUCTION.

Animal, epidemiological and clinical studies have demonstrated that fluoride has contributed to the reduction of root surface caries (Section 1.7). This reduction might have resulted from fluoride being incorporated in root surfaces either systemically during tooth development, or topically from frequent availablity or both.

In vitro Experiments have been designed (a) to study the rate of lesion progression and mineral dissolution (Arends *et al.*, 1987; Featherstone *et al.*, 1987), (b) to study the fluoride effect on both demineralisation and remineralisation (Wefel *et al.*, 1987) and (c) to determine the most effective fluoride agent in reducing root solubility in acids (Shannon, 1980; Tveit & Halse, 1982; Scholtanus, Schuthof & Arends, 1986).

The majority of *in vitro* experiments involved treating root surfaces with a fluoride agent and subsequently exposing it to an acid attack, of either an acidified gel or buffered solution (Shannon, 1980; Derand & Petersson, 1982; Al-Joburi & Koulouride, 1984).

In recent years there has been a trend to test dentifrices in an experimental situation where many aspects of the intra-oral environment are simulated (ten Cate, 1986). In this approach, teeth are subjected daily

to a demineralising period, a remineralising period in artificial saliva and to the test dentifrice (ten Cate, 1986) or fluoride solution (Damato, Strang & Stephen, 1988). This technique, "pH - cycling", has also been used with enamel to study the influence of either low concentrations of fluoride continually present in the remineralisation fluid (ten Cate & Duijster, 1982), or the effect of high concentrations of fluoride when applied in a short - term treatment (ten Cate & Duijster, 1983).

Although of proven value in enamel studies, pH - cycling has been used sparsely to study remineralisation of incipient root lesions. However, although not a true pH - cycling technique, Mellberg & Sanchez (1986) used slabs (blocks) of root dentine, after chemically removing cementum, to measure remineralisation. The slabs were placed for four days in 20 ml of pH 4.0 gels to form subsurface lesions, then treated by immersion in a 50% aqueous slurry of either an MFP dentifrice, or a placebo, for five minutes twice daily for ten days. They were stored in a remineralising solution between treatments.

From the above introduction and the literature review in Section (1.12) it can be stated:

1. Root surface resistance to acid attacks can be increased by increased fluoride uptake.
2. Few *in vitro* experiments have investigated the remineralisation of either subsurface lesions which show a distinct surface zone, or lesions with no surface zone.
3. Although pH cycling experimental designs proved

valuable in studies related to the fluoride effect on enamel remineralisation, surprisingly few experiments have been carried out on roots.

4. Different fluoride agents have been investigated to assess their effect on root solubility. Only in one experiment has the fluoride effect on root lesion remineralisation been investigated (Mellberg & Sanchez, 1986). However, fluoride concentration effect on remineralisation has not yet been reported.

5.2 AIMS AND OBJECTIVES.

The aims and objectives of this study were:

1. to develop a pH cycling model for studies related to root surface caries,
2. to study remineralisation of the incipient lesion in two root tissues, cementum and dentine,
3. to investigate the effect of different sodium monofluorophosphate solution concentrations namely placebo (< 0.03 ppm F^-), 1000, 2500 and 5000 ppm F^-,
4. to examine and compare the remineralisation behaviour of root surface lesions created by fluoridated and non-fluoridated solutions.

5.3 MATERIALS AND METHODS.

5.3.1 General procedures.

A total of nine teeth were employed in these experiments, the roots of which had not been exposed to the oral environment. They were obtained from the same sources and prepared in the same manner as described in Chapter 2. The cementum was removed mechanically from the surfaces of three teeth as detailed in Chapter 2. Two demineralising solutions were used to create the artificial lesions. The first contained 0.1 ppm F^- (see Table 3.1), and the second was a demineralising solution of the same composition, but without the addition of fluoride. Varnishing, window creation and immersion in the artificial caries system were carried out in the same fashion as described in Chapter 2. After immersion for ten days, the roots were then cut into sections and ground to a thickness between 130 - 200 μm. Experiments lasted for 5 weeks as with the *in situ* studies, and lesions were microradiographed at baseline, 2, 4 and 5 week intervals. The mineral content of the lesions was assessed using microdensitometric measurements as detailed in Chapter 2.

5.3.2 Groups and section allocation.

Sections were allocated to three main groups, each comprising of four subgroups. In Group (I), lesions were created in non-abraded root surfaces, using the non - fluoridated artificial caries system. Group (II) contained lesions which were created, using the

fluoridated demineralising solution, in non-abraded root surfaces, whereas Group (III) lesions were created, in abraded root surfaces, using the fluoridated demineralising solution. Each of the twelve subgroups received a fluoride solution treatment of either placebo (distilled water), 1000, 2500 or 5000 ppm. Eight sections with 32 lesions were allocated to each subgroup. Sections of the same tooth were distributed among the subgroups to eliminate any variation effect (see Chapter 3).

5.3.3 Experimental design (pH cycling).

In the pH cycling regime used in this investigation, sections were demineralised in the buffered solution no. 1 (Table 3.1) for 3 hours and stored in an artificial saliva (2 mM $CaCl_2$, 2 mM NaH_2PO_4 and 2 mM NaOH) for 20 hours and 55 minutes daily during a 5 week period, with fresh de- and remineralising solutions being used in each cycle. Daily, after the demineralising period, the specimens were immersed for 5 minutes into placebo (distilled water) or a monofluorophosphate solution of either 1000, 2500 and 5000 ppm concentration and then carefully rinsed with distilled water before placing them into the storing solutions. The monofluorophosphate powder used in these experiments was supplied by Unilever Research Port Sunlight, England.

5.3.4 Method of analysis.

Only those lesions which survived longer than a four week period without breakage, were included in the

analysis.

The lesion parameters (Δzs, SZ and LB) were plotted against time and a "least squares fit" carried out on the data. The resultant gradient was taken as a measure of the mineralisation rate for that lesion after being subjected to a particular fluoride solution treatment. Thereafter the mean slope and standard error of a group of lesions under the same fluoride solution treatment were calculated, and inter - group comparisons made.

5.4 RESULTS.

The means and standard errors of remineralisation rate values of all measured parameters (Δz_1, Δz_2, Δz_3, SZ and LB) for all 12 subgroups are given in Table 5.1, and are shown as histograms in Figs. 5.1 - 5.6.

For each subgroup of lesions, all fluoride treatments (i.e 1000 ppm, 2500 ppm and 5000 ppm) resulted in remineralisation rates significantly different from those of the placebo ($p < 0.001$). However, no remineralisation was achieved when lesions were subjected to a placebo (distilled water) solution. Indeed, all measured parameters showed varying degrees of demineralisation.

Concentration effect on Group (I) lesions.

In Group (I) lesions which were created, in non-abraded root surfaces, with the non - fluoridated

Table 5.1 Mean Mineralisation rates for the Δz, Surface Zone (SZ) and Lesion Body (LB) parameters.

ppm F^-	n	Δz_1	Δz_2	Δz_3	SZ	LB
		(% Vol. min x µm/wk)			(% Vol. min. / wk)	
Group I: (no fluoride in solution, non-abraded lesions)						
0	22	-59.6 (42.1)	-38.3 (46.5)	-146.9 (39.6)	-0.7 (0.1)	-0.9 (0.1)
1000	28	139.3 (19.0)	328.2 (19.9)	65.5 (11.4)	2.3 (0.3)	1.9 (0.2)
2500	27	208.5 (26.9)	530.5 (40.2)	141.3 (25.0)	3.0 (0.4)	2.4 (0.4)
5000	24	135.4 (12.5)	366.2 (26.1)	97.0 (11.6)	2.4 (0.3)	2.0 (0.2)
Group II: (0.1 ppm F^- in solution, non-abraded lesions)						
0	16	-171.9 (95.1)	-236.1 (112.1)	-251.7 (85.3)	-3.3 (0.5)	-3.1 (0.5)
1000	30	154.7 (17.0)	416.9 (25.5)	106.9 (12.2)	4.2 (0.2)	1.7 (0.1)
2500	31	162.4 (15.5)	342.1 (20.7)	115.2 (9.1)	3.7 (0.1)	1.8 (0.1)
5000	30	150.4 (14.2)	375.3 (21.6)	130.5 (12.0)	3.8 (0.2)	1.9 (0.1)
Group III: (0.1 ppm F^- in solution, abraded lesions)						
0	20	-252.4 (34.0)	-296.6 (50.4)	-322.6 (32.5)	-1.8 (0.4)	-1.7 (0.2)
1000	27	219.5 (28.4)	420.8 (30.4)	160.9 (22.1)	4.5 (0.3)	2.2 (0.2)
2500	24	95.9 (15.5)	259.1 (27.1)	80.4 (13.5)	1.4 (0.2)	1.0 (0.2)
5000	28	154.8 (17.2)	405.0 (20.0)	138.2 (13.6)	3.7 (0.2)	1.8 (0.1)

n = number of lesions available for analysis.
values in parenthesis are Standard Errors

demineralising solution (Figs. 5.1, 5.2) the highest remineralisation rate for all measured parameters was evident in lesions subjected to 2500 ppm F^-.

For the Δz_1 parameter (Fig. 5.1), the mean ($\pm$ S.E) remineralisation rate 208.5 (26.9) % vol. min. x μm / week, for the 2500 ppm F^- treated lesions was significantly higher than those achieved by the placebo, -59.6 (42.1) % vol. min. x μm / week, 1000 ppm F^- 139.3 (19.0) % vol. min. x μm / week and 5000 ppm F^- 135.4 (12.5) % vol. min. x μm / week with p values of < 0.001, < 0.01 and < 0.01 respectively. Also, remineralisation rates of both 1000 and 5000 ppm F^- - treated subgroups were significantly higher ($p < 0.001$) than the placebo.

A similar picture was seen in Δz_2 parameter (Fig. 5.1) in which remineralisation rate values were higher than the other two Δz parameters, reflecting the discrepancy in the sensitivity of these parameters. All tested fluoride concentrations resulted in remineralisaion rates which were significantly higher than that of the placebo solution. The concentration of 2500 ppm F^- was significantly superior to that of both 1000 and 5000 ppm F^- ($p < 0.001$).

The picture for Δz_3 (Fig. 5.1) was consistent with the other two parameters, in that all fluoride concentrations had significantly higher remineralisation rates than the placebo ($p < 0.001$). The mean remineralisation rate value for the 2500 ppm F^- subgroup was significantly greater than that of the 1000 ppm F^- subgroup ($p < 0.001$) and, although higher than the mean

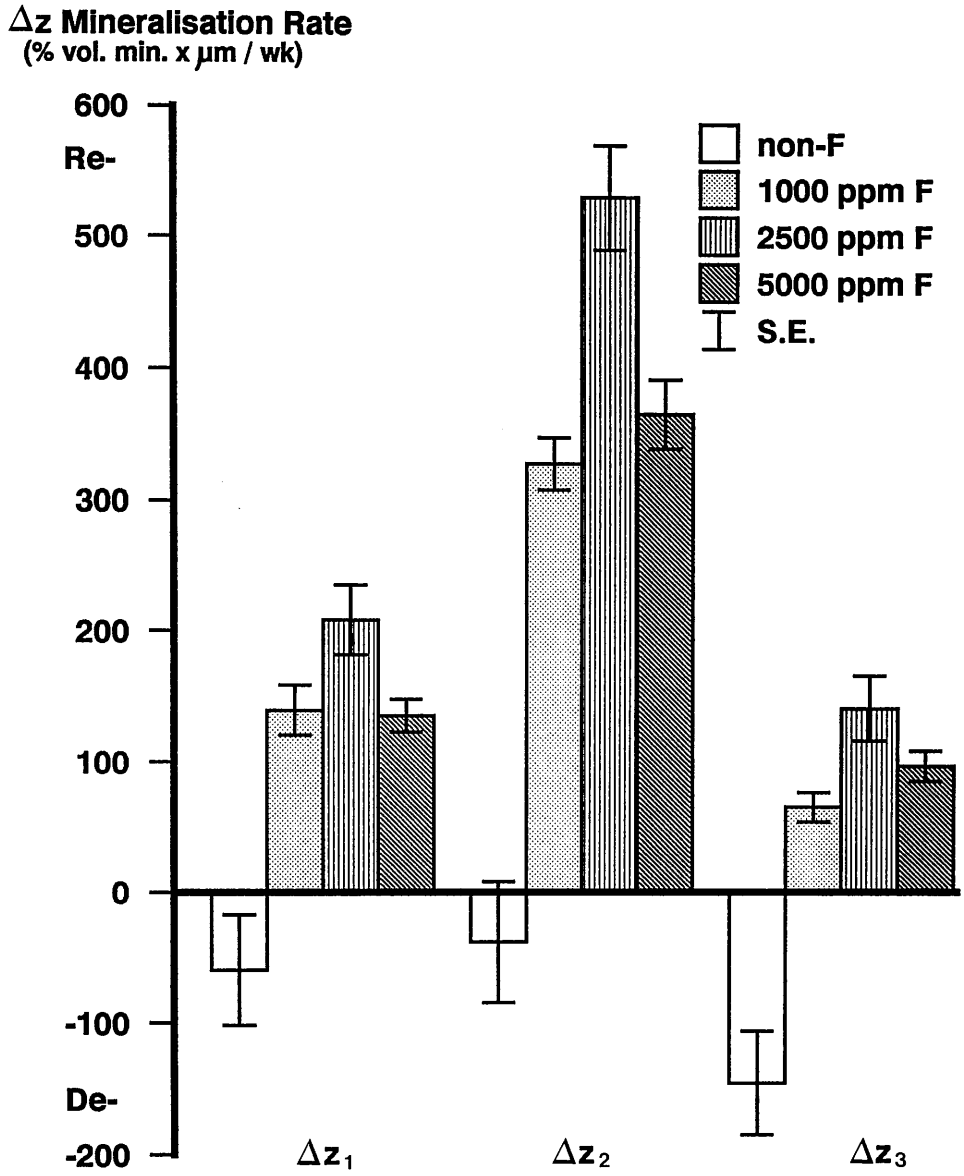


Figure 5.1 Histogram of the mean mineralisation rates for the Δz parameters for the four test solutions.
Group I. (no fluoride in solution, non - abraded lesions).

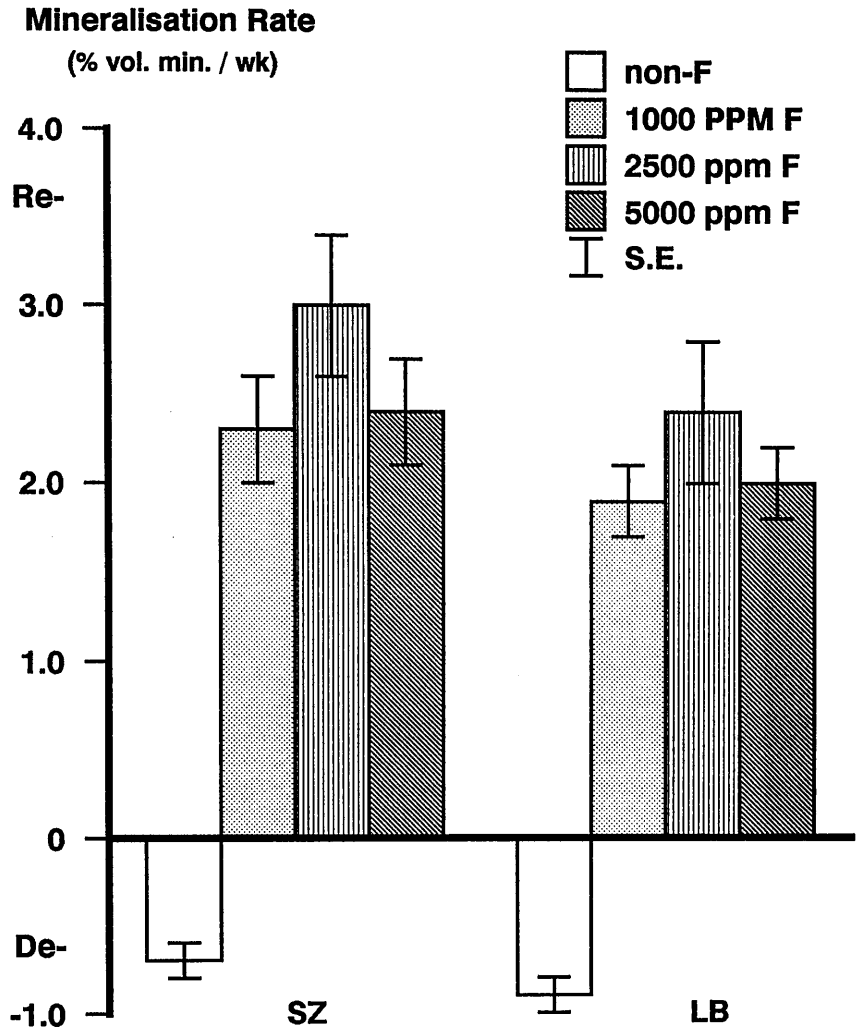


Figure 5.2 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test solutions. Group I. (no fluoride in solution, non - abraded lesions).

value for the 5000 ppm F^- subgroup, this was not statistically significant.

The mineral content of the surface zone (Fig. 5.2) demineralised further when subjected to the placebo solution, whereas significant ($p < 0.001$) remineralisation occurred with all fluoride concentrations. The surface zone mean ($\pm$ S.E) remineralisation rate for the 2500 ppm F^- subgroup was: 3.0 (0.4) % vol. min. / week, which was higher than the values for the 1000 subgroup of 2.3 (0.3) % vol. min. / week, and 5000 ppm F^- 2.4 (0.3) % vol. min. / week, although the differences were not significant.

The mineral content of the lesion body of this group (Fig. 5.2) showed a pattern similar to the other parameters. Here, again, the lesion body demineralised further when subjected to the placebo solution and remineralised as a result of fluoride solution treatments. Remineralisation was significantly greater with fluoride treatments, than with the placebo. Although not significant, there was a trend of higher remineralisation rates being achieved by the 2500 ppm F^- subgroup (2.4) % min. vol. / week against 1.9 and 2.0 % vol. min. / week for the 1000 and the 5000 ppm F^- subgroups respectively.

Concentration effect on Group (II) lesions.

In Group (II) lesions (Figs. 5.3, 5.4), which were created in non-abraded root surfaces with the demineralising solution which contained 0.1 ppm F^-, further demineralisation was observed when lesions were subjected to the placebo solution, whereas they

significantly gained mineral when treated with the three different fluoride concentrations ($p < 0.001$).

For the Δz_1 parameter (Fig. 5.3), treating lesions with 1000, 2500 and 5000 ppm F^- solutions resulted in remineralisation rates being significantly higher than those of the placebo subgroup ($p < 0.001$). However, none of these fluoride concentrations was significantly superior to the others.

For the Δz_2 parameter (Fig. 5.3), fluoride treatment resulted in significant ($p < 0.001$) remineralisation compared to placebo values in which demineralisation occurred. The fluoride solution concentration of 1000 ppm F^- was significantly superior to that of 2500 ppm F^- ($p < 0.01$), and this was the only significant difference amongst the three fluoride concentrations.

For the Δz_3 parameter (Fig. 5.3), fluoride treatment again produced significant ($p < 0.001$) lesion remineralisation compared to the placebo. Here, there was a trend, although not significant, of fluoride concentration effect on remineralisation. The fluoride treatment of lesions with 5000 ppm achieved a mean ($\pm$ S.E) remineralisation rate value of 130.5 (12.0), in contrast to 115.2 (9.1), 106.9 (12.2) % volume mineral x μm / week, (Table, 5.1), achieved by 2500 and 1000 ppm F^- respectively, but differences were not significant.

The surface zone (Fig. 5.4) remineralisation was qualitatively similar to that of the Δz_2 parameter, with the fluoride subgroups being significantly higher than the

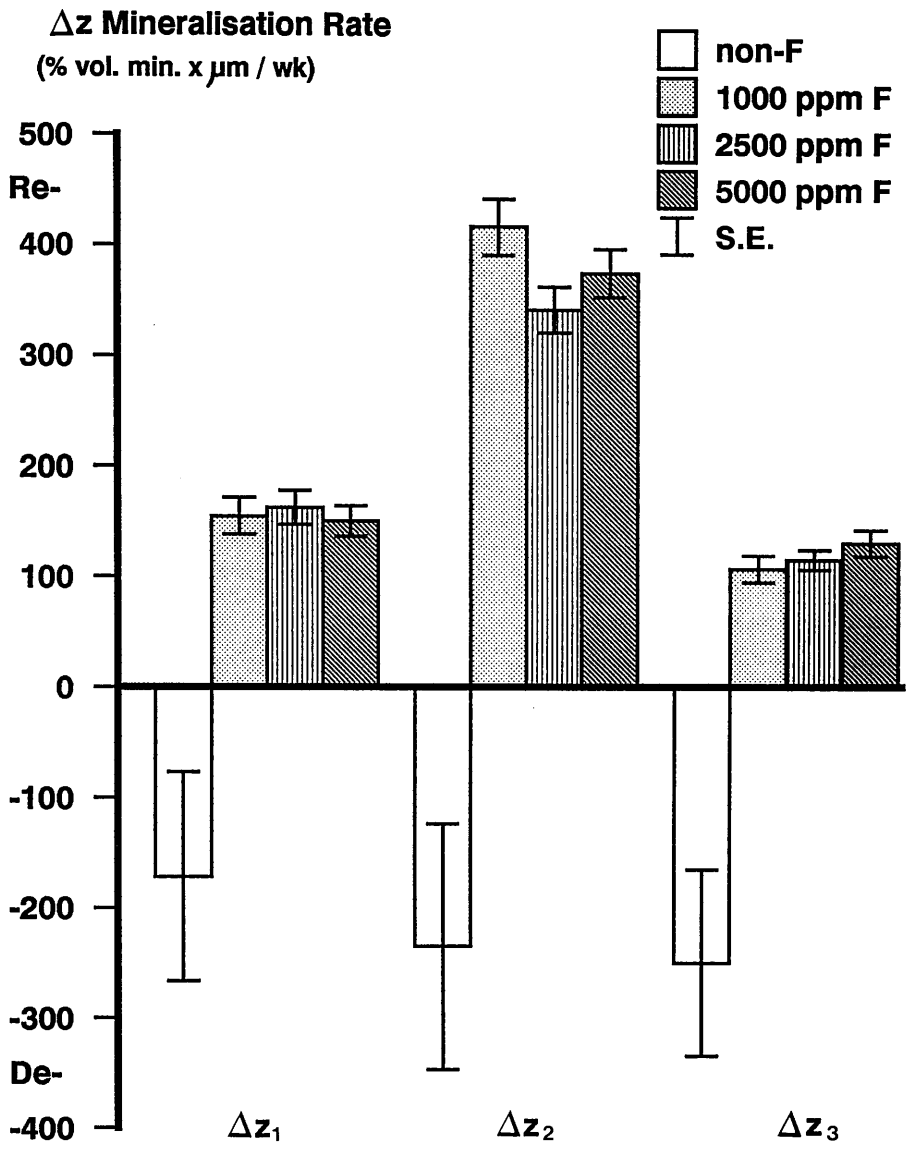


Figure 5.3 Histogram of the mean mineralisation rates for the Δz parameters for the four test solutions.
Group II. (0.1 ppm F^- in solution, non - abraded lesions).

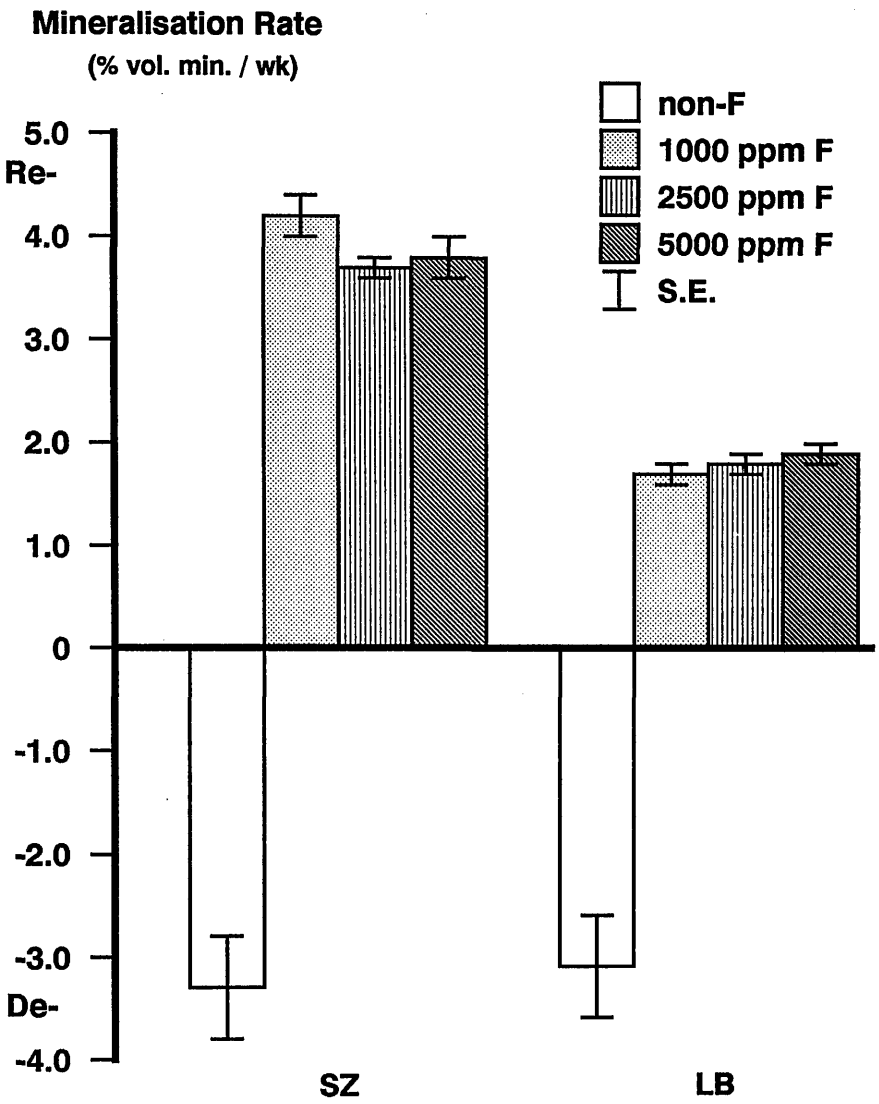


Figure 5.4 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test solutions. Group II. (0.1 ppm F^- in solution, non - abraded lesions).

placebo subgroup ($p < 0.001$). The surface zone remineralisation rate values for the 1000 ppm F^- group were significantly higher than the 2500 ppm F^- group ($0.01 < p < 0.05$).

However, the picture with respect to the lesion body parameter (Fig. 5.4) was similar to that of the z_3 in which all fluoride subgroups tested produced remineralisation which was significantly different from the demineralisation which occurred with the placebo ($p < 0.001$). Also, there was a trend, although again not - significant, of increased remineralisation with increased fluoride concentration. For the 5000 ppm F^- subgroup, the mean lesion body remineralisation rate was : 1.9 % vol. min. x week as compared to 1.8 and 1.7 % vol. min. / week for the 2500 and 1000 ppm fluoride concentrations respectively.

Concentration effect on Group (III) lesions.

Group (III) lesions (Figs. 5.5, 5.6) were created in abraded root surfaces by using the same demineralisation solution as that of Group (II). Exposing lesions to the placebo solution during the pH-cycle resulted in demineralisation of these lesions, whereas remineralisation occurred in lesions which were exposed to different fluoride concentrations. Among the three fluoride concentrations here, the lowest (1000 ppm) resulted in mean remineralisation rates which were significantly higher ($p < 0.001$) than those of the 2500 ppm F^- subgroup in all parameters (Figs.5.5, 5.6) and from

the 5000 ppm F^- for the Δz_1 ($p < 0.01$), surface zone ($p < 0.05$) and lesion body ($p < 0.05$) parameters. The 2500 ppm F^- concentration resulted in remineralisation rates which were significantly lower than those of the 1000 or 5000 ppm F^- subgroups.

For the Δz_1 parameter (Fig. 5.5), lesions exposed to all fluoride solution concentrations signficantly remineralised as compared to those exposed to the palcebo solution ($p < 0.001$). Both the 1000 and 5000 ppm F^- subgroups were significantly superior to that of 2500 ppm F^- ($p < 0.001$). In addition the 1000 ppm F^- group data was also significantly superior to the 5000 ppm F^- subgroup values ($0.01 < p < 0.05$).

For the Δz_2 parameter (Fig. 5.5), lesions remineralised significantly after being exposed to fluoride, whereas they demineralised after exposure to the placebo ($p < 0.001$). Here, the 1000 and 5000 ppm F^- solutions were both superior in remineralising lesions to the 2500 ppm F^- subgroups ($p < 0.001$). However, remineralisation rates in lesions exposed to 1000 and 5000 ppm F^- were not significantly different.

The picture for the Δz_3 parameter (Fig. 5.5) resembled the two above. Here also, lesions exposed to both 1000 and 5000 ppm F^- concentrations were remineralised significantly better than when exposed to 2500 ppm F^-. In addition, exposing lesions to the placebo solution resulted in further demineralisation. This was significantly different from the remineralisation observed in lesions treated with fluoride ($p < 0.001$).

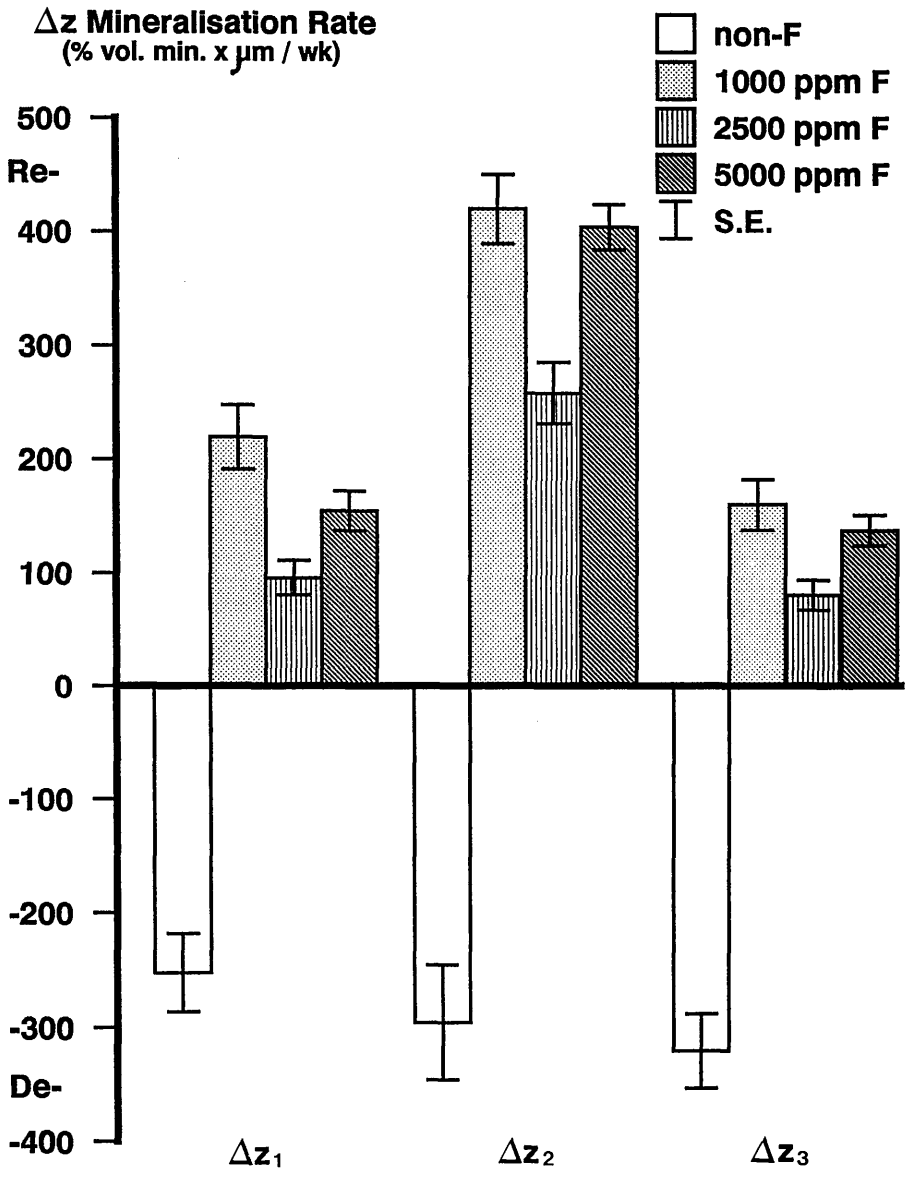


Figure 5.5 Histogram of the mean mineralisation rates for the Δz parameters for the four test solutions.
Group III. (0.1 ppm F^- in solution, abraded lesions).

The mineral content in both the lesion body and surface zone (Fig. 5.6) demineralised further when lesions were exposed to the placebo solutions again being significantly different from the fluoride - treated remineralisation ($p < 0.001$). In both these parameters, the remineralisation rates were significantly higher after the 1000 and 5000 ppm F^- treatment than after the 2500 ppm F^- treatment ($p < 0.001$). Also, lesion remineralisation was significantly better after being treated with the 1000 than with the 5000 ppm F^- concentrations ($0.001 p < 0.01$).

Inter - group comparisons.

When remineralisation rates of the three main groups were compared, for a measured parameter, no significant trend was noticed but the following facts were observed:

(1) For the Δz_1 parameter, demineralisation rates were significantly higher in Group (III) lesions which were created in abraded cementum / dentine, ($p < 0.001$), than in Group (I) or (II) lesions. Remineralisation rates of lesions subjected to 1000 ppm F^- were significantly higher in Group III (dentine) lesions than those in Group II (cementum), or Group (I) lesions which were produced by non - fluoridated demineralising solution. The latter were remineralised significantly more than those of Group (III) lesions when exposed to the 2500 ppm F^- solution ($p < 0.001$). Also, cementum lesions remineralised more than dentine lesions under the influence of the 2500 ppm F^- concentration. However, no significant differences were

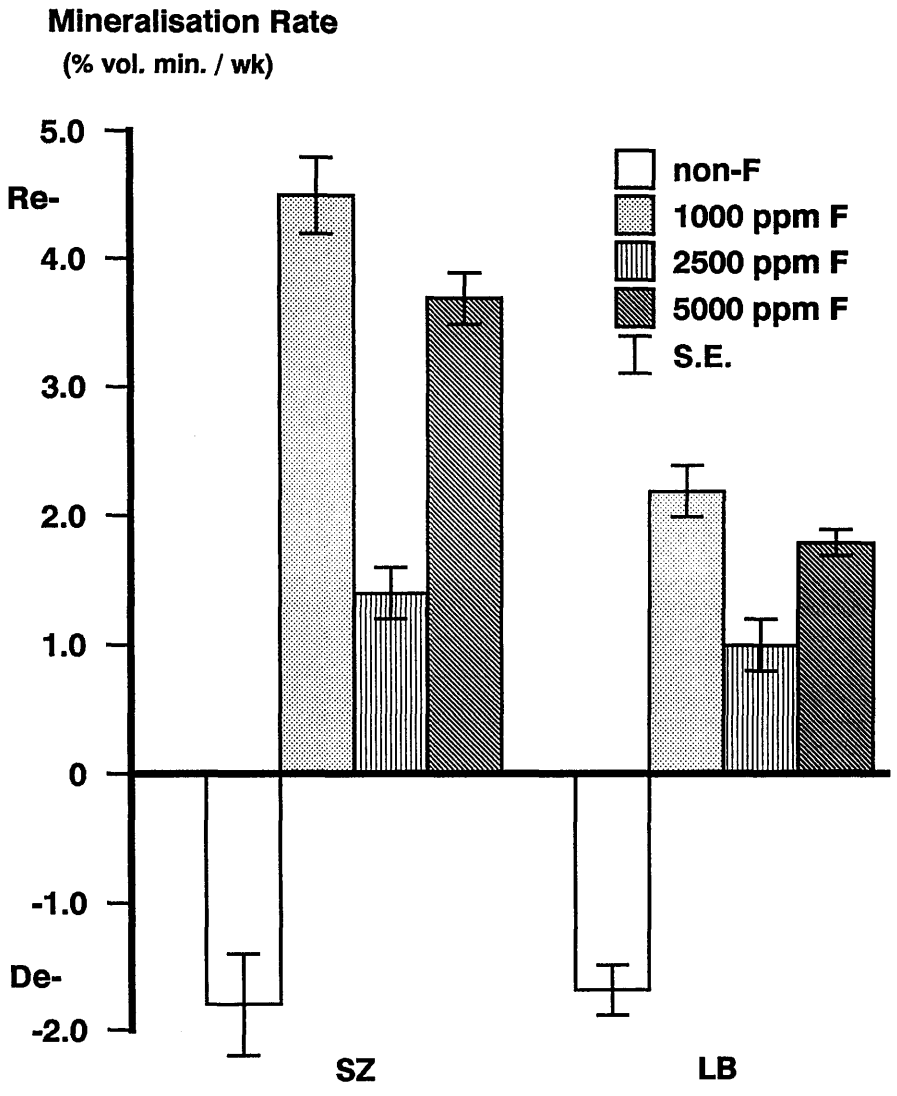


Figure 5.6 Histogram of the mean mineralisation rates for the Surface Zone (SZ) and Lesion Body (LB) parameters for the four test solutions. Group III. (0.1 ppm F^- in solution, abraded lesions).

found to exist between the groups when lesions were treated with the 5000 ppm F^- solution.

(2) For the Δz_2, demineralisation in Group (III) lesions was significantly higher ($p < 0.001$) than in Group (I) lesions when both lesion types were subjected to placebo solution. Also, demineralisation in Group (II), cementum, lesions was significantly higher than in Group (I) lesions ($p < 0.01$). However, remineralisation rates in lesions exposed to 1000 ppm F^- were significantly greater in both dentine and cementum lesions than in Group (I) lesions ($p < 0.001$), although no significant differences were found to exist between remineralisation rates in cementum and dentine. Remineralisation rates were superior ($p < 0.001$) in Group (I) lesions, after those lesions were exposed to 2500 ppm F^- than in the case of dentine (Group III) or Group (II) lesions. Also, these rates were superior ($p < 0.01$) for Group (II) as compared to Group (III). No significant differences were found to exist between the the three main groups when lesions were treated with 5000 ppm F^- solutions.

(3) For the z_3 parameter, demineralisation was also significantly higher in dentinal than in Group (I) lesions ($p < 0.001$), but was not significantly different from that obtained in Group (II) lesions. However, remineralisation rates in dentine lesions subjected to the 1000 ppm F^- concentration were significantly higher than those in Groups (I) ($p < 0.001$) and (II) ($0.001 < p < 0.01$). Also, remineralisation rate was significantly higher for the same concentration in lesions of Group

(II), than in those of Group (I). However, the mean remineralisation rate for the 2500 ppm F^- subgroup were significantly higher in Group (I) than in Group (III) ($p < 0.001$), and Group (II) ($0.001 < p < 0.01$) lesions. Again no significant differences were found to exist between remineralisation rates of lesions which were subjected to 5000 ppm F^- in the three main groups.

(4) The demineralisation of the surface zones in Group (II) lesions was greater than for Group (I) lesions as was the mineral content of Group (III) lesions with probability values of < 0.001 and < 0.01 respectively. However, with regards to remineralisation rates, they were significantly higher ($p < 0.001$) in both the cementum (Group II) - and dentine (Group III) - prepared lesions, than in Group (I) material, when those lesions were exposed to 1000 ppm F^-. In contrast, the surface zone of Group (I) lesions gained significantly more mineral ($p < 0.001$) when exposed to 2500 ppm F^- than did those of both Group (II) and Group (III) lesions. However, remineralisation rates in the cementum and dentine lesions' surface zones were not found to be different. Also, exposing Group (II) and Group (III) lesions to a fluoride solution of 5000 ppm, resulted in the remineralisation rates of their surface zones being significantly higher ($p < 0.001$) than in Group (I) lesions. Remineralisation for dentinal lesions was not significantly different from that for cemental lesions.

(5) For the lesion body parameter, Group (II) lesions demineralised significantly more ($p < 0.001$) than

did Group (I) and (III) lesions. Also Group (III) lesions demineralised significantly more than did Group (I) lesions ($p < 0.001$). The mineral acquired by the lesion body in those exposed to the 1000 ppm F^- solution was significantly higher in Group (III) than Group (II) lesions. For the 2500 ppm F^- lesions, the lesion body in Group (I) specimens remineralised significantly more than did dentinal ($p < 0.001$) or cemental lesions ($0.001 < p < 0.01$). The mineral acquired by the lesion body in cementum preparations was significantly higher ($p < 0.05$) than that acquired by dentine lesions. The remineralisation rates of the lesion body, in the main groups, were not significantly different after exposure to the 5000 ppm F^- solution.

5.5 DISCUSSION.

As was noted in the introduction to this chapter, few *in vitro* experiments have been carried out to investigate remineralisation of the incipient caries lesion in root surfaces. In the experiments reported in this chapter, remineralisation of subsurface lesions in two tissues, cementum and dentine, was investigated, the latter obtained by mechanically removing cementum from root surfaces. The objective here was to attain a less variable surface, since mineral content in the cementum varies between surfaces which have and have not been exposed to the oral environment (see Section 1.3). Moreover, cementum might be removed from root surfaces

soon after the latter becomes subjected to abrasion processes (Section 1.1). However, histological evidence has shown that caries starts in the cementum of root surfaces (Section 1.3) which suggests that both tissues might be available for a caries attack at a given time.

Although fluoride is available to the community in different commercial dentifrices, surprisingly the prevalence of root surface caries shows no decrease (see Section 1.2). Most of these dentifrices contain approximately 1000 ppm F^-, therefore, it could be speculated that such a concentration might not be high enough to reduce substantially the disease prevalence. In the experiments reported in this chapter, therefore, the remineralisation effects of three different concentrations (1000, 2500 and 5000 ppm F^-) were investigated in an attempt to demonstrate any significant concentration benefits.

The experiments reported here were designed to simulate the natural environment and be compatible with the *in situ* experiments reported in Chapter 4. As the technique of "pH-cycling" is a powerful tool to evaluate caries preventive agents and has been used successfully to study enamel caries (see Section 5.1), it was adopted for the experiments reported. The chosen timespan of de - and remineralisation intervals (3 h vs 21 h) bears good agreement with daily practice although *in vivo* demineralisation occurs over several short phases of 30 to 40 minutes, rather than during one extended period. However, at present, it is not known whether several short

demineralisation cycles throughout the day will result in different de- and remineralisation efficacies (ten Cate, 1986). Moreover, five minutes, immersion of the lesion in fluoride solutions is not inconsistent with toothbrushing and the time taken by the oral fluid for fluoride clearance (ten Cate & Duijster, 1986).

During data handling there was difficulty of finding a fixed anatomical mark to align the upper horizontal line on the lesion image of the BBC computer screen (see Fig 2.7). Thus the operator had to rely on visual judgement using a sequence of graphic prints of the same lesion's four microradiographs, taken at 0, 2, 4 and 5 week intervals. Moreover, some difficulties were experienced with respect to the analyses of results, particularly in Group (I) lesions which showed no surface zone. Hence, no surface maximum point existed for these specimens and, in some instances, it was either at the same level or even overlying that of the minimum mineral point. Also, some of the lesions showed a flat, initial slope since the surface layer was completely eroded making it extremely difficult to establish good alignment of the outermost mineral and maximum mineral points. The normalisation point also had to be placed carefully since mineral content decreases towards the pulp. As a result it was always placed as near to the surface as was possible.

It is not possible to make direct comparisons between results reported here and those in the literature, due to the differences in design and methodology employed. Experiments reported to date were designed to study either

the progression rate of the subsurface lesion and mineral dissolution, or to examine remineralisation and the prevention of further mineral loss (Arends et al., 1987; Featherstone et al., 1987). While the effect on root solubility of different fluoride agents has been investigated (see Section 1.10.4) few attempts have been made to determine the action of these agents on subsurface lesion remineralisation.

In the present studies, a placebo and three different fluoride concentrations were compared. Exposing lesions to the placebo resulted in further demineralisation which was significantly higher in dentinal (Group III) lesions than in the other two, Groups (I) and cementum (Group II). This might have occurred as a result of the removal of the root surfaces outer layers which may be more caries - resistant than the deeper layers. However, such superficial levels were reported to have a higher fluoride content than the deeper layers (Murakami *et al.*, 1987).

All the fluoride concentrations investigated in this study resulted in significant remineralisation of the lesions for all parameters studied. Remineralisation rates were highly significantly different from those resulting from placebo solution treatments ($p < 0.001$). This finding is in general agreement with data from Mellberg & Sanchez, (1986) who showed that remineralisation of subsurface lesions which had no surface zone, was higher in a monofluorophosphate group than in a placebo group.

With lesions being subjected to the fluoride

treatment for five minutes each day, there was no significant fluoride concentration effect evident in any of the measured parameters, for any lesion group. This might indicate that the treatment frequency of a lesion is an important remineralisation factor, and suggests the need for further experiments in this field.

Rates of remineralisation were found to be significantly higher in lesions with a surface zone, than in those with no surface zone. This is hardly surprising and could be attributed to the fact that the integrity of the root surface tissue was lacking, since it was eroded. Hence it would seem that such integrity should preferably be preserved to act as a meshwork into which remmineralising material could be deposited. Although, mineral was laid down on the outermost region of the concavity which resulted from root surface erosion, this should not be expected to restore completely the lost surface because, *in vivo*, this layer might be easily removed by mechanical stresses e.g toothbrushing. Moreover, the nature of this observed mineral, and its resistance to further acid attack, is unknown and also worthy of future study. In 12% of the lesions which had no surface zones, a hazy matrix "cloud" was seen in each lesion's concavity (Fig. 5.7). This matrix might contribute to lesion remineralisation by acting as a meshwork into which mineral is seeded. However, a further attempt failed to obtain these lesions in higher proportions. This hazy matrix is thought to be a part of the root organic material which was washed away while

Figure 5.7 A microradiographic picture of a lesion before remineralisation indicating a hazy matrix "cloud" (arrow).

cutting, grinding and handling sections during microradiography procedures.

Two types of lesions were employed here, one of which showed a distinct surface zone as shown in Fig. 3.2 and another in where no surface zone was visible (Fig 3.2). As a result, the latter was used to investigate remineralisation behaviour in specimens with no surface zone and to determine the extent of any mineral deposition on the concavity which resulted from outer root surface erosion.

A disadvantage of these experiments is their incompatibility with the natural oral environment which contains, in addition to the minerals in saliva, proteins, enzymes, microorganisms and epithelial cells. Furthermore, it is at a more or less constant temperature under normal physiological conditions. In addition, root surfaces are in direct relation to gingival crevicular fluids a factor not taken into account in these laboratory investigations.

Nonetheless, these studies have assisted with the understanding of the fluoride effect on root caries subsurface lesion remineralisation, by permitting the control of different variables and focussing on any concentration effect alone. Also, they have demonstrated the remineralisation behaviour of lesions with no surface zone and shown the importance of maintaining the surface tissue if root surface contour to be preserved.

5.6 CONCLUSIONS.

The pH cycling technique was a useful method to control all variables and test a possible fluoride concentration effect. Fluoride has been shown to contribute to significant remineralisation of the subsurface lesions as compared to the demineralisation which occurred with exposure to the placebo.

Demineralisation occurred significantly more in dentinal than cemental lesions.

There was no specific fluoride concentration effect on remineralisation. While remineralisation rates in Group (I) were significantly superior in the 2500 ppm F^- subgroup as compared to those for other two subgroups, they were significantly inferior in the same subgroup to Group (III), where 1000 ppm F^- was significantly superior. However, in cementum (Group II) lesions, remineralisation rates of the 1000 ppm F^- subgroup lesions were significantly higher than the 2500 ppm F^- Group for the surface zone and Δz_2 parameter. Generally, in the 5000 ppm F^- subgroup, remineralisation rates were lower than for the other two subgroups in the main three groups.

Generally, remineralisation rates for the dentinal lesions group were not significantly different from those of the cemental lesions groups.

Finally, remineralisation rates in lesions with surface zones were generally higher than those which were created with a non-fluoridated demineralising solution and were without surface zones.

CHAPTER SIX.
MICROORGANISMS ASSOCIATED WITH THE INITIAL HUMAN ROOT SURFACE LESION.

6.1 INTRODUCTION.

Root surfaces of the teeth are normally protected by healthy gingival tissues, but, periodontal disease, excessive mechanical hygiene procedures or periodontal surgery can lead to gingival recession and thus the exposure of the cementum to oral fluids (Nyvad & Fejerskov, 1982; Banting, 1986, Scheister, 1987). Cementum may then become subjected to a variety of mechanical, chemical and pathological processes e.g scaling, polishing, root planing, abrasion, erosion, periodontitis and caries, any of which might result in loss of cementum and exposure of dentine. Initially the root surface becomes colonised by microorganisms and in time caries may be initiated, depending on complex interactions involving diet, plaque bacteria, host tissues and oral hygiene (Newbrun *et al.*, 1984). The lesion first develops in cementum, and usually extends into dentine (Arends *et al.*, 1987).

In contrast to enamel caries relatively little attention has been paid to the microbiological aspects of root caries.

However, based on animal studies, it has been suggested that a unique microflora might be involved, which is not necessarily the same as that responsible for

enamel caries (Keyes & Jordan, 1964). This hypothesis has not been validated by studies in human root surface caries (Jordan, 1986).

The following sections will discuss the normal microflora on intact root surfaces and bring together the available knowledge relating to the microbiology of root surface caries. Models used for inducing root surface lesions in both human and experimental animals will be reported. However, a comprehensive review in Section 1.7, was given including studies in experimental animals.

6.1.1 The microflora on exposed intact root surfaces.

The main bacterial types observed on exposed intact cementum and dentine (Brown *et al.*, 1983; Ellen *et al.*, 1985a, 1985b) are fairly comparable to those found on smooth enamel surfaces (Marsh & Martin, 1984). Thus one would expect to find approximately two thirds Gram positive and one third Gram negative bacteria. The predominant cultivable genera are Gram positive *Streptococci* and *Actinomyces*; while the Gram negatives are *Veillonella*, *Neisseria* and *Bacteroides*. The number of *Strep. mutans* varies while *Lactobacillus* spp. are usually present in low concentration (Edwardsson, 1987).

6.1.2 Root surface caries creation by microbial models.

There is lack of agreement between investigators on the microorganisms responsible for root caries (see Section, 1.7). To investigate this problem a variety of laboratory models have been used.

Human root surface caries-like lesions were consistently produced *in vitro* using *Strep. mutans* in one experiment; and using *Strep. mutans* and *Lactobacillus casei* in another (Clarkson *et al.*, 1984). Clinical caries like lesions have also been produced *in vitro* by incubating sound root surfaces with a mixture of *Strep. mutans*, *Actinomyces viscosus* and *Actinomyces naeslundii* for six weeks (Katz *et al.*, 1987).

Studies on experimental animals have shown that there are several microorganisms capable of initiating root caries. Human isolates of *A. naeslundii* and *A. viscosus* induced periodontal disease and root surface caries in hamsters and gnotobiotic rats (Socransky *et al.*, 1970; Jordan *et al.*, 1972). Root caries has also been produced using strains resembling *Strep. salivarius* in rats, and with *Strep. mutans* in gnotobiotic rats (Gibbons *et al.*, 1966). More recently, Firestone *et al.*, (1987) has induced caries in rat root surfaces previously exposed by gingivectomy and inoculated with *Strep. mutans* and *A. viscosus*.

In situ experiments have been used recently to study the microorganisms associated with enamel demineralisation (Macpherson, 1988). Here, an intra - oral appliance, on which enamel sections and slabs were mounted, was used to study enamel demineralisation after three weeks' plaque accumulation. This model had the advantages of using the individuals' own plaque and exposing the test specimens to the oral environment, the latter being very difficult to duplicate *in vitro*.

However, there appears to have been no reports using an *in situ* model for investigating root surface caries.

In human *in vivo* studies *A. viscosus* has been isolated from virtually all plaque samples of sound cementum and from lesion sites (Jordan & Hammond, 1972; Syed *et al.*, 1975). In a longitudinal study, Ellen *et al.* (1985b) isolated *A. viscosus* from every subject with exposed intact root surfaces, with other *Actinomyces* strains also being found. *Strep. mutans* was recovered from all plaque samples collected from the root surfaces of human teeth (Syed *et al.*, 1975; Sumney & Jordan, 1974; Billing *et al.*, 1985; Ellen *et al.*, 1985a, 1985b; Keltjen *et al.*, 1987). In fact, Ellen *et al.*, (1985a) suggested that its detection on an exposed cement surface could be used as an indication of high caries risk.

In only one study (Ellen *et al.*, 1985a), have lactobacilli been found to be associated with root caries. However, their data were too sparse and too variable to draw any firm conclusions. Also, studies in gnotobiotic animals infected with different *Lactobacillus* species have failed to produce root surface lesions (Rosen *et al.*, 1968; Fitzgerald *et al.*, 1981). Therefore, it would appear that coronal and root caries may involve different species of bacteria (see Section 1.7).

6.1.3 Conclusions and aims.

In the light of the above discussion, it can be concluded that:

1. The identity of the microorganisms definitely

responsible for root caries has not been agreed yet. Although, a strong association between root caries and both *Strep. mutans* and *Actinomyces* spp. has been reported in the literature (*vide supra*), this was based on cross-sectional experiments, from which no definite conclusions could be drawn, and on longitudinal study data which were "....too low and too variable..." (Ellen *et al.*, 1985a) with standard deviations being greater than the means.

2. Although *in vitro* models are helpful in studying dental caries, they suffer from the disadvantage that they do not utilise the oral environment.

3. *In situ* experiments for studying the microorganisms associated with root demineralisation have not been attempted.

Therefore, the experiments reported in this chapter were designed to answer the following questions.

1. Can three week old plaque produce demineralisation in root surfaces which have not been exposed previously to the oral environment?

2. Does sucrose influence the composition of plaque on root surfaces and on subsequent demineralisation?

3. What species of microorganism are isolated from plaque collected from sound cementum and carious cementum?

6.2 MATERIALS AND METHODS.

6.2.1 Section and slab preparation.

Teeth used in these experimentswere prepared in the same manner as in section (2.2.2). Using pumice and a rubber cup, roots were polished briefly to remove periodontal remnants. A saw microtome was used to cut transverse sections from the cervical third of the root to a maximum thickness of 350 μm. Sections were then hand - ground to a final thickness of 130 - 200 μm which was found to allow adequate microdensitometric measurements, (see Chapter 3). Sections were then microradiographed as detailed in Chapter (2). Two sections were allocated to each experiment, one to be used on either side of the appliance. Each section was coded using a graphite pencil with a number corresponding to the sequence in which the section was cut.

After cutting the sections, four slabs were cut from the same root, two for each side of the appliance. The thickness of the slab was reduced to permit a clearance between the slab surface and gingivae when the appliance was worn in the mouth. Slabs were marked with identification letters, A, B, C, D.

6.2.2 Varnishing procedures.

Using a stereomicroscope, (10x magnification), four window sites, each 1.5 - 2.0 mm long, were marked on the natural surface of the section. They were also identified by marking the cut surface immediately adjacent

to the windows with the letters D, M, B, L. The cut surfaces were then covered with a coat of red nail varnish. The first coat was allowed to dry before another coat was applied. After adequate drying of the second coat, all of the section periphery (cemental surface) except the window sites, was covered carefully with nail varnish. Thus each section had four small cementum areas which were not covered with nail varnish.

In the case of root surface slabs, all cut surfaces were covered with nail varnish leaving only the natural cementum surface exposed. Both the sections and slabs were fixed with nail varnish on to the trough of the appliance (Fig. 6.1), and a diagram of their layout was recorded for consultation while sampling plaque from the window sites and slab surfaces.

6.2.3 Volunteer and experimental protocols.

Two volunteers participated in this set of experiments. Both received a scale and polish, with non-fluoride paste, and the teeth of the lower arch were flossed immediately before the appliance was inserted in the mouth. The appliance, containing one section and two slabs on each side, was worn for three weeks, only being removed from the mouth twice daily for toothbrushing using a non-fluoride tooth paste. Subjects were also instructed to clean the appliance on these occasions with brush and water, but the trough area was to be avoided. The volunteers were advised to floss between the teeth once per day in all areas of the mouth, paying

Figure 6.1 The intra - oral appliance with two slabs and one varnished section mounted on the trough area.

particular attention to the lower molar region. The normal diet of the subject was maintained in each experimental period. For sucrose application, the appliance was removed nine times daily at one hourly intervals during the waking period, and four drops of a sterile 10% w/v sucrose solution was applied to the trough area on each occasion. After one minute the fluid was allowed to drain from the appliance which was then replaced in the mouth without rinsing. A sterile plastic dropper bottle was used to store and dispense the sucrose solution.

At the beginning of each run, volunteers were instructed as follows: (1) to use only the non-fluoride paste supplied. (2) to fill the same trough of the appliance with the supplied sucrose, every hour for nine times daily and, (3) not to brush or disturb the plaque which had accumulated in the troughs. Two runs, each of three weeks, were carried out per volunteer, and sucrose treatment was applied to one side of the appliance in the first run and to the other side in the second run. At the end of the three week period, the appliance was collected from the volunteer and immediately plaque was sampled from both sections and slabs.

6.2.4 Plaque sampling from sections and slabs.

At the end of an experimental period, the appliance was removed from the mouth and saliva gently washed from the trough area using a pasteur pipette containing 5 ml of sterile physiological saline. The excess saline was removed by carefully applying sterile cotton wool pellets

to the edge of the trough area. The diagram of the sections layout prepared at the start of the expermiment (see section 6.2.2) was consulted and any plaque overlying each window was removed accurately using a sterile excavator (No. 243, Ash, England), and collected in a labelled sterile plastic bijou bottle containing 1 ml of sterile Anaerobic Blood Broth (ABB) (Gibco-Europe, Paisley, Scotland). The plaque on each slab surface was removed and collected in the same manner.

6.2.5 Plaque dispersion and dilution.

Each plaque sample was dispersed immediately in the ABB by sonication for 15 s at a setting of 1.5 (Ultrasonic Sonicator, Heat Systems Ultrasonics, Plainview, N.Y.), followed by vortex mixing for 30 s (Fisons Scientific Apparatus, Leicestershire, England). Thereafter, 10-fold dilutions from neat to 10^{-6} were carried out in ABB using an Eppendorf micropipette (BDH Laboratory Suppliers, Glasgow, Scotland) with 15 s vortex mixing between each dilution.

6.2.6 Plate inoculation and culturing.

Fifty microlitres were taken from each, 10^{-3} to 10^{-6}, dilution of every sample and were inoculated on to 7.5% Trypticase Soy Blood Agar (Gibco-Europe, Paisley, Scotland) supplemented with 1% vitamin K and haemin using a spiral plater (Model D, Don Whitley Scientific Ltd., West Yorkshire, England) . In addition neat, 10^{-1} and 10^{-2} samples of plaque were inoculated in the same way on to

Mitis Salivarius Agar (Difco, Surrey, England) supplemented with 20% sterile sucrose (BDH Chemicals Ltd., Poole, England) and 20 units bacitracin (Sigma Chemical co. Ltd, Poole, England) per 100 ml, (MSB), and on to Rogosa SL agar (Difco, Surrey, England).

The blood agar plates were incubated at 37° C for five days in an anerobic cabinet (Don Whitley Scientific Ltd., West Yorkshire, England) using an atmosphere of 85% N_2, 10% H_2, 5% CO_2. At this time the colonies present on a selected area of the plate were removed for subculture as described below and the plate incubated anaerobically for a further five days. Any colonies which appeared on the selected area during this time were also subcultured. The MSB and Rogosa plates were incubated at 37° C in an atmosphere of 5% CO_2 in air for three days before counts were performed.

6.2.7 Identification of isolates from Blood Agar Plates.

A blood agar plate with discrete colonies was selected for each plaque sample and stamped with a grid (10 cm Spiral Systems Manual Counting Grid, Don Whitley Scientific Ltd., West Yorkshire, England) which divided the surface into zones representing known volumes of inoculum. To obtain pure cultures, an area containing 30 - 50 colony forming units (cfu) was chosen, and each colony present in this area was subcultured on to a separate blood agar plate and incubated in the anaerobic cabinet until growth occured. The tests detailed below were then carried out on each isolate.

Gram staining.

Colonies were removed from the plate using a sterile wire loop and emulsified in a loopful of sterile distilled water on a glass microscope slide. The smear was allowed to dry and was then heat-fixed by passing through a bunsen flame. Thereafter. the following procedure was carried out: (1) bacteria on the slide were stained, for 1 minute with a 0.5% w/v solution of crystal violet. (2) the slide was then rinsed under running tap water, and (3) covered with Gram's iodine solution (Iodine 5 g, KI 10 g in 1 L distilled water) for a further minute before (4) the smear was decolourised with acetone (M & A Pharmachem Ltd., Bolton, England) and, (5) counterstained with a 5% v/v solution of carbol fuschin for 2 - 3 minutes. The smear was then examined using an Olympus Microscope (Tokyo, Japan) at a magnification of 1000 times and the morphology and staining characteristics noted. All the above chemicals, with the exception of acetone, were supplied by Clin-Tech Ltd, London, England.

Atmospheric growth conditions.

Each isolate was incubated aerobically, in 5% CO_2 and under anaerobic conditions in order to determine the atmospheric requirement necessary for growth. Thereafter the appropriate biochemical tests were performed for identification. This, together with Gram staining characteristics and colonial and cultural morphology, was used for organism identification.

Slide agglutination test.

This was carried out on the catalase positve, facultatively anaerobic Gram positive cocci, using the Roche Diagnostica Staph-Rapid Test (Roche products Ltd, Welwyn Graden City, Egland). Colonies of the isolate were removed from a blood agar plate using a wire loop and mixed with a drop of sensitised erythrocyte suspension and a drop of control erythrocyte suspension on a microscope slide. At the same time, a positive control using a type culture of *Staphylococcus aureus*, was also tested. The slides were gently rocked to and fro, and the test was considered postive if the sensitised erythrocyres clearly agglutinated within 15 s, whilst the control erythrocytes remained finely dispersed.

Oxidase disc test.

The identification of bacteria belonging to the family *Neisseriaceae* was based on microscopic appearance, positive or negative catalase production, and on a positive oxidase disc test (A/S Rosco, Taastrup, Denmark). Colonies of the isolate were removed from the plate using a sterile dry swab and suspended in 3 ml of sterile distilled water in a plastic bijou. A disc was then added and the bijou hand-shaken; a purple colour within 30 s indicated a positive reaction.

Catalase production.

The test for catalase production was performed using a 3% solution of hydrogen peroxide (BDH Chemicals

Ltd., Poole, England). Colonies of the isolate were removed from the blood agar plate using a wire loop and added to a drop of the hydrogen peroxide solution on a microscope slide. If bubbles appeared within 10 s the test was considered positive, and if no visible reaction took place, the test was recorded as negative.

API 20 strep.

This system was used to identify catalase negative, facultatively anaerobic Gram positive cocci. The system combines 20 biochemical tests and is used to demonstrate enzymatic activity and the fermentation of sugars by streptococci. The tests were carried out as follows:- (1) a 24 hour blood agar culture of the unknown microoganism was grown at 37o C under anaerobic conditions. (2) All colonies were collected from the plate using a sterile swab, and a dense suspension of the isolate was made in 2 ml of sterile distilled water. (3) Aliquots of the suspension were then distributed into the wells of the first half of the test strip as directed by the manufacturer. (4) The remaining suspension was transferred to an ampoule containing the API 20 strep Medium and the new suspension was distributed into the remaining cupules which were then overlaid with mineral oil. Thereafter the strip was incubated at 37o C in 5% CO2 in air. The results were read at 4 and 24 hours, following the manufacturer's instructions (Fig. 6.2), and the profile obtained was entered into a computer (Apple Computer Inc., California, USA) programmed with the API

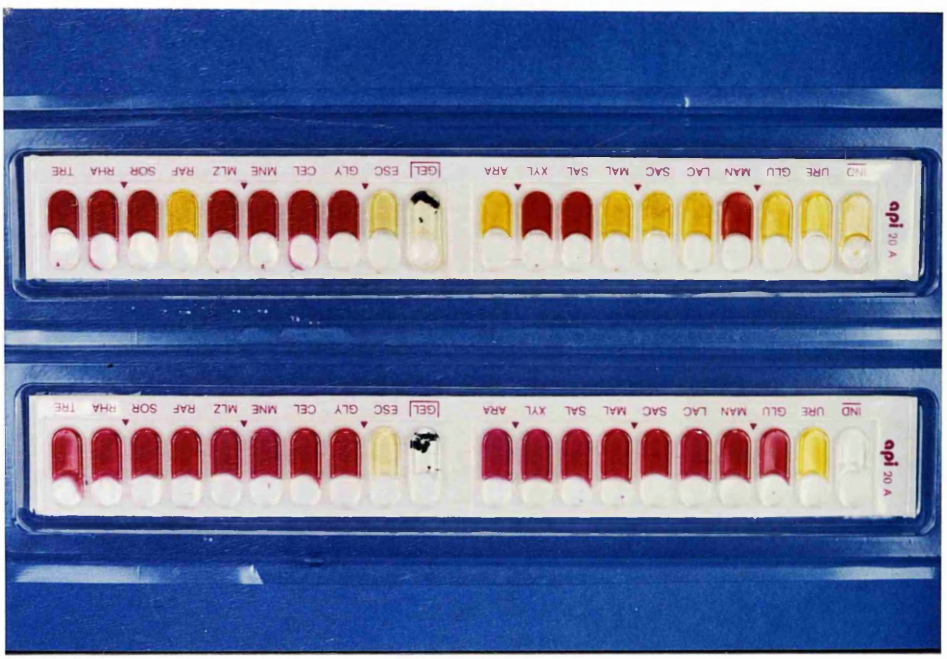


Figure 6.2 API strips following inoculation of bacterial suspensions.

identification disc. Thus the streptococci were identified to species level. If the computer regarded any profile as "doubtful" or as showing poor discrimination between species, the purity of the culture was checked, and the test repeated. If the same profile was obtained, the organism was reported at the genus level in a descriptive manner e.g. facultatively anaerobic streptococcus. Streptococcal species identified as Strep. sanguis II and Strep. mitis, using the API 20 Strep System, were classified under Strep. oralis (Schmidhuber, kilpper-balz & schleifer, 1987).

API 20 A.

The API 20 A system was used to identify anaerobic Gram negative rods and to aid in the identification of Gram positive rods when results of tests using the Minitek system (vide infra) were equivocal. The API system consists of cupules containing dehydrated substrates. Colonies of the test organism were removed from the blood agar plate and inoculated into the API 20 A medium to produce a heavy suspension. Aliquots of this were then added to the cupules following the manufacturer's instruction, and the test strip incubated in an anaerobic cabinet at 37o C for a minimum of 24 hours. Appropriate reagents were then added and the colour changes noted. A biochemical profile was obtained for the bacterium and was entered into the Apple computer programmed with the API identification disc as described above.

Minitek anaerobic system.

The Minitek anaerobic system was used for the differentiation of the strictly anaerobic Gram positive and negative cocci, the facultative and strictly anaerobic Gram positive rods, and on occasions, the Gram negative rods if the discrimination between genera was poor when using the API 20 A system. Discs impregnated with the test substrates were individually dispensed into wells of the Minitek plate. A dense suspension was produced by mixing the test bacterium collected from the pure culture and the Minitek anaerobic broth. Using a plastic pipette a standard drop of this suspension was dispensed into each well. After a minimum of 24 hours anaerobic incubation, biochemical reagents were added to the wells and colour changes were noted. The biochemical profile of the organism was obtained and compared with the manufacturer's differentiation tables for identification. When the identification of gram-positive rods had a confidence level of under 75%, and the purity of the culture had been checked, the isolate was inoculated into the API 20 A system (API systems SA Montalieu-Vercieu, France). If doubt remained at the species level, the organism was reported at the genus level.

Details of other laboratory procedures used to identify the isolates are:

X and V disc test.

This test was used for the differentiation of

facultatively anaerobic Gram negative cocco-bacilli. Colonies were removed from the blood agar plate using a sterile swab and were suspended in sterile water which was then inoculated on to a nutrient agar plate (Gibco-Europe, Paisley, Scotland), deficient in both X and V factors. Discs containing X, V, or both X and V (Mast Laboratories, Merseyside, England) were applied to the surface of the agar and the plate incubated in 5% CO_2 at 37° C for a minimum of 24 hours. The growth pattern around the discs was used to differentiate *Haemophilus* species and *Eikenella corrodens*.

On a few occasions, isolates were reported as "unidentified" due, either to insufficient growth for biochemical tests to be performed, or to poor discrimination in biochemical profiles.

The organisms identified from each plaque sample obtained from a section were expressed as percentage of the total cfu.

6.2.8 Enumeration and identification of Streptococcus mutans and Lactobacillus species.

On each MSB and Rogosa plate, the number of cfu with similar macroscopic colonial morphology was counted. This was carried out for each of the dilutions where colonies were present, and the mean calculated. Two representatives of each colonial type in each sample were identified using the API 20 A Strep system for MSB plates isolates and the anaerobic Minitek System for isolates from Rogosa plates. The number of *Strep. mutans* and

Lactobacillus spp. present were calculated and expressed as a percentage of the total cultivable flora count which was obtained from the blood agar cultures for each sample.

6.2.9 Microradiographic and microdensitometric methods.

In order to detect any mineral loss from the window sites on the root surface sections, they were microradiographed and assessed microdensitometrically at the end of each run as described in Chapter 2. The parameters measured were the surface zone (SZ) and the lesion body (LB), in units of percent volume mineral, and total mineral loss (Δz) in units of % vol mineral x μm.

Quantification was made by substracting the final values from base - line values for each parameter. The values quoted in the results reflect the net mineral loss, and are expressed as SZ, LB and Δz_1. The results of the Δz_1 were divided into three groups, with Δz Group I containing sites experiencing demineralisation of less than 250 units; Group II containing sites of 250 - 999; Group III comprising those sites where demineralisation exceeded 1000 % vol min x μm.

6.3 RESULTS.

6.3.1 Normal plaque conditions.

Shown in Table 6.1 are the microbiological results for the normal plaque (NP) which formed on the root surface of the sections under normal conditions for both volunteers. It is evident that Gram positive cocci were

recovered from all the specimens in both volunteers, with *Strep. oralis* being isolated from all samples.

Apart from Gram positive bacilli, microorganisms isolated from the normal plaques in both subjects A and B, were approximately similar in their mean percentages distribution.

The mean percentages of Gram positive cocci were 38.3 in subject A, and 36.4 in subject B, and were identical (0.1) for *Strep. mutans* in both volunteers.

Strep. oralis and *Strep. sanguis* dominated the positive cocci group. In subject A, the mean percentage for *Strep. oralis* was 22.6 with a range between 2.5 - 41.5, and for *Strep. sanguis* it was 13.3 and a range values between 0 - 39.4. Mean percentages values in subject B, were 17.5 (a range value between 2.3 - 42.9) for *Strep. oralis*, and 15.5 (with a range of 0 - 46.6) for *Strep. sanguis*.

Gram negative cocci were isolated from all samples obtained from subject A, and from 75% of those obtained from subject B, but were in higher numbers in subject A (mean % 21.0) than in subject B (mean % 6.5). *Veillonella* predominated the negative cocci isolates and were more numerous in subject A than in subject B with mean percentages values of 20.3 and 5.8 respectively. *Neisseria* species were also occasionally identified in this group.

Gram positive bacilli were isolated from all samples and the mean percentages were greater in subject B (52.2) than in subject A (27.7). The predominant species in this group was *A. viscosus* which were again higher in

subject B (43.7) than in subject A (22.7). *Lactobacillus* spp. were also more evident in subject B than in subject A with mean percentages values of 7.2 and 2.8 respectively. Among the species which were also identified in this group were, *Actinomyces naeslundii, Actinomyces odontolyticus, Actinomyces israeli* and *Actinomyces myeri.*

The mean proportion of Gram negative bacilli in the plaque samples was 12.2 for subject A, and only 4.6 for subject B. Bacteroides were predominant in this group with mean percentages values of 0.4 for subject A and 2.2 for subject B. *Capnocytophaga, H. parainfluenzae* and *Fusobacterium* were also identified in this group.

6.3.2 Sucrose plaque conditions.

The microbiological results for the plaque which formed on the root surface at windows under sucrose treatment conditions for both volunteers, are given in Table 6.2.

Generally, the isolates of plaque samples collected from subject A were higher in numbers than those from subject B, except for Gram positive bacilli which were greater in subject B than in subject A.

In both volunteers, Gram positive cocci and bacilli again dominated the plaque isolates of this plaque which had been exposed to extra-oral sucrose applications nine times daily. Gram positive cocci were recovered from all plaque samples collected from both subjects A and B.

The mean percentages distribution for Gram positive cocci were higher in subject A (43.2) than in subject B

(29.2). *Strep. mutans* were isolated from 25% of the samples in subject A, with a mean percetages value (0.02) and from 62% of the samples in subject B, with a mean value (0.06) being slightly lower than that of subject A.

There was a clear trend of differences between *Strep. sanguis* spp. isolates with mean percentages distribution being higher in subject A (17.9) than subject B, (6.3). However, the mean percentages distribution of *Strep. oralis* were approximately the same, being 22.5 and 22.6 for subjects A and B respectively, and the isolation frequency was exactly the same (100%).

Gram negative cocci, which were dominated by Veillonella, had a mean of 10.6% for subject A, and 3.5% for subject B. No *Neisseria* spp. were recovered from any isolates of this group.

The mean percentages of Gram positive bacilli were higher in subject B than subject A, and were 63.9 and 33.9 respectively.

Actinomyces spp. and *Lactobacillus* spp. were higher in subject B than subject A. The mean percentages distribution of *Actinomyces* spp. was 50.2 in subject B aginst 26.2 in subject A, and for *Lactobacillus* spp. they were 8.0% and 1.7% for subjects B and A respectively. Among *Actinomyces* spp. which were identified were *A. viscosus, A. naeslundii, A. israeli, A. odontolyticus* and *A. meyeri*. Also noted more, *L. fermentum, Arach. propionica* and *L. acidophilus*.

Gram negative bacilli spp. were higher in subject A than subject B. *Bacteroides* spp. had a mean value of 1.0%

and not detected in plaque samples obtained from subject A. However, *Fusobacterium* and *H.parainflue* spp. were isolated from plaque samples collected from subject A, and they were also isolated from subject B.

6.3.3. Effect of sucrose treatment on root section plaque microflora.

The combined percentage distributions of predominant cultivable microflora obtained from plaque samples under normal and sucrose treatment conditions for both subjects amalgamated are shown in Table 6.3.

Results of the sugar treated data are approximately similar to those obtained from the normal plaque, with only Gram negative cocci being significantly different ($0.01 < p < 0.05$, analysis of variance).

The mean percentages of Gram negative cocci ranged from 13.8% in normal plaque and 7.1% in the sugar treated plaque (SP). The mean percentages of *Veillonella*, which is the most predominant organism in this group, was (13.8) higher in subject A than that of (7.1) B.

6.3.4 Root slab plaque microflora under normal and treatment conditions.

A total of 16 slabs (8 per volunteer) were used in the four experimental runs. The microbiological results of the predominant isolates obtained from plaque collected from the slabs surfaces under normal and sucrose treatment conditions are given in Table 6.4.

Microorganisms isolated from normal and sugar

Table 6.3 % Predominant cultivable plaque microflora isolated from root sections under normal and sucrose treated plaque for both Subjects A and B.

	F	Mean	(S.D)	Median	Range
(a) Normal Plaque					
+ve cocci	16/16	37.3	(17.5)	34.2	14.9 - 73.8
S. mutans	8/16	0.05	(0.05)	0.05	N.D. - 0.1
S. sanguis	9/16	14.4	(16.9)	6.6	N.D. - 46.6
S. oralis	16/16	20.0	(16.3)	15.2	2.3 - 42.9
-ve cocci	14/16	13.8	(12.4)	10.4	N.D. - 46.7
Veillonella	12/16	13.0	(13.1)	10.4	N.D. - 46.7
+ve bacilli	16/16	40.1	(24.6)	29.6	6.7 - 84.3
Actinomyces	16/16	33.2	(20.6)	27.6	6.7 - 74.4
Lactobacillus	13/16	5.0	(7.4)	2.3	N.D. - 29.6
-ve bacilli	13/16	8.4	(8.3)	5.0	N.D. - 27.5
Bacteroides	4/16	1.3	(3.0)	N.D.	N.D. - 11.4
(b) Sucrose Treated Plaque					
+ve cocci	16/16	36.2	(18.9)	36.0	1.3 - 64.5
S. mutans	7/16	0.04	(0.05)	N.D.	N.D. - 0.1
S. sanguis	11/16	12.1	(11.4)	11.4	N.D. - 35.5
S. oralis	16/16	22.6	(14.7)	25.0	1.3 - 46.9
-ve cocci	11/16	7.1	(8.2)	4.4	N.D. - 29.0
Veillonella	11/16	7.1	(8.2)	4.4	N.D. - 29.0
+ve bacilli	16/16	48.9	(25.7)	49.9	8.6 - 93.4
Actinomyces	16/16	38.2	(23.3)	31.4	8.6 - 93.4
Lactobacillus	13/16	4.9	(6.2)	1.6	N.D. - 17.9
-ve bacilli	13/16	5.0	(6.9)	3.2	N.D. - 25.8
Bacteroides	3/16	0.5	(1.3)	N.D.	N.D. - 4.8

F = Isolation Frequency
N.D. = Not Detected

treated plaque showed few differences. However, Gram positive cocci spp. were higher in normal than in treated plaques. Meanwhile, the mean percentages of *Strep. mutans* spp. were exactly (0.1) the same under normal and sucrose treated plaques, they were slightly higher in normal (16.0) than sugar (12.0) plaques for *Strep. sanguis*, and were 8.6 against 4.7 for *Strep. oralis*.

Gram negative cocci spp. showed a trend of treatment effect with a mean percentages being lower in the sugar treated plaque (3.0) than normal plaque (7.8). The same microorganisms which were isolated from section plaques (*vide supra*) in this group were also isolated from slabs plaque.

The Gram positive bacilli spp. were slightly higher in the normal plaque than treated one, but *Actinomyces* spp. were higher in treated than normal plaques with a mean percentages value of 56.7, 54.6 respectively. In contrast the mean of *Lactobacillus* spp. was higher in normal (7.3) than sugar treated (1.8) plaques.

Gram negative spp. were higher in treated than normal plaque with means being 10.3% against 7.8% respectively. However, *Bacteroids* were higher in normal than sugar treated palque with mean values of (3.2%), (0.9%) respectively.

No particular trend was evident when comparing the plaques collected from sections with that from slabs, although the mean percentages of targetted microorganisms i.e *Actinomyces* and *Strep. mutans* were generally higher in slabs than sections plaque (Tables 6.3, 6.4).

Table 6.4 % Predominant cultivable plaque microflora isolated from root slabs under normal and sucrose treated plaque for both Subjects A and B.

	F	Mean	(S.D)	Median	Range
(a) Normal Plaque					
+ve cocci	8/8	27.4	(15.4)	26.0	8.3 - 57.7
S. mutans	7/8	0.1	(0.03)	0.1	N.D. - 0.1
S. sanguis	6/8	16.0	(13.7)	19.2	N.D. - 36.5
S. oralis	6/8	8.6	(7.8)	7.8	N.D. - 21.2
-ve cocci	5/8	4.3	(4.2)	4.4	N.D. - 11.1
Veillonella	5/8	4.1	(4.2)	3.6	N.D. - 11.1
+ve bacilli	8/8	60.6	(17.2)	61.3	23.1 - 77.2
Actinomyces	8/8	53.1	(21.1)	54.6	9.6 - 77.2
Lactobacillus	6/8	7.3	(9.5)	1.6	N.D. - 21.6
-ve bacilli	7/8	7.8	(4.9)	8.9	N.D. - 13.5
Bacteroides	4/8	3.2	(3.6)	1.9	N.D. - 8.1
(b) Sucrose Treated Plaque					
+ve cocci	8/8	18.8	(11.4)	18.3	6.7 - 43.9
S. mutans	6/8	0.1	(0.05)	0.1	N.D. - 0.1
S. sanguis	7/8	12.0	(14.4)	6.6	N.D. - 43.9
S. oralis	4/8	4.7	(6.8)	1.0	N.D. - 18.5
-ve cocci	5/8	3.0	(2.8)	3.4	N.D. - 6.7
Veillonella	5/8	3.0	(2.8)	3.4	N.D. - 6.7
+ve bacilli	8/8	57.2	(13.2)	72.2	47.0 - 84.3
Actinomyces	8/8	60.6	(11.1)	56.7	46.7 - 73.8
Lactobacillus	6/8	1.8	(2.9)	0.1	N.D. - 6.5
-ve bacilli	8/8	10.3	(9.4)	6.5	1.5 - 26.6
Bacteroides	2/8	0.9	(1.6)	N.D.	N.D. - 3.9

F = Isolation Frequency
N.D. = Not Detected

6.3.5 Effect of treatment conditions on root demineralisation.

The mean difference in demineralisation parameters Δz_1, SZ and LB between baseline and the end of three week experimental period for each subject under both normal and treatment conditions are given in Tables 6.5.

Minimal demineralisation was observed in sections of both subjects under normal plaque conditions, with the z mean values ranging from 92.6 in subject A, to 1386.7 in subject B, the mean total, for both subjects combined, being 743.6 % vol. min. x μm.

Although, demineralisation levels were slightly higher in sucrose than normal plaques, they were not found to be significantly different using analysis of variance Table (6.7)

Surface zones and lesion bodies in both subjects showed no significant difference in between normal and sugar - treated plaques. The Δz values, lesion bodies and surface zones were greater in subject B than in subject A.

Thirty two root surface sites were exposed to both normal and sucrose treatment conditions in both subjects. Of these 78.1% lesions developed surface demineralisation. Two lesions have shown the characteristic features of a subsurface lesion, with a surface layer overlying the lesion body. The remaining lesions showed various degrees of demineralisation.

6.3.6 Relationship between plaque microflora and total mineral loss under normal and sucrose conditions.

Integrated mineral loss (Δz) was placed into three

Table 6.5 Mean difference in demineralisation parameters between baseline and end of the experiment for root sections exposed to normal (NP), sucrose treated (SP) plaques and (NP + SP) combined.

	NP	SP	NP + SP
Subject A			
z*	92.6	137.2	114.9
SZ	6.11	4.01	5.06
LB	7.32	6.31	6.82
Subject B			
z*	1358.0	1386.7	1372.4
SZ	11.36	14.36	12.86
LB	13.19	13.28	13.53
Subjects A + B			
z*	725.3	762.0	743.6
SZ	8.74	9.18	8.96
LB	10.32	9.80	10.03

* positive values indicate demineralisation

Units: Δz % Vol. Mineral x μm.
SZ % Vol. Mineral
LB % Vol. Mineral

groups namely: 0-250, 250-1000, and > 1000. Results revealed that 10, 13, and 9 lesions fell into groups 1, 2 and 3 repectively.

The percentage distribution of the predominant cultivable microflora associated with each Δz group for both normal and sucrose conditions is given in Table 6.6.

Results revealed that Gram positive cocci and bacilli were significantly ($0.01 < p < 0.05$) associated with demineralisation (Table 6.7) with mean percentages values decreasing from Group I to Group III for Gram positive cocci and increasing from Group I to III for Gram positive bacilli. *Strep. sanguis* and *Strep. oralis* mean counts were significantly ($0.01 < p < 0.05$) associated with increased demineralisation whereas *Strep. mutans* spp., although detected in each group, represented only a very small proportion with no significant Δz association.

The mean proportion of Gram negative cocci in the three groups was generally low, and decreased in the group representing the greatest mineral loss with *Veillonella* following the same pattern. *Neisseria* spp. were not associated with Group I, but were associated with Group II and III patterns, with mean propotions values being 0.5 and 0.6 respectively.

Actinomyces spp. mean percentages were the highest for both subjects among all flora isolates. They were significantly ($0.01 < p < 0.05$) associated with all Δz_1 groups and their means correlated positively with increased mineral loss i.e from Group I towards Group III. However, although not significant, there was a trend of

Table 6.6 The mean percentages of each cultivable plaque microflora associated with Δz* groups. I. (0 - 25). II. (250 - 1000). III. (> 1000).

	Δz Group I n=10	II n=13	III n=9
+ve cocci	45.2 (13.4)	39.2 (17.0)	23.8 (17.9)
S. mutans	0.03 (0.05)	0.03 (0.05)	0.09 (0.03)
S. sanguis	19.2 (13.0)	10.4 (12.6)	10.8 (17.2)
S. oralis	23.0 (17.1)	27.3 (14.3)	10.8 (9.4)
S. salivarius	0.7 (2.2)	N.D.	0.3 (0.8)
ANO2 Strep	0.2 (0.5)	0.5 (1.7)	N.D.
Staph / Micrococcus	N.D.	0.2 (0.9)	0.2 (0.7)
Other +ve cocci	2.1 (3.4)	0.6 (1.2)	1.7 (3.9)
-ve cocci	13.6 (10.3)	11.5 (13.5)	5.3 (5.0)
Veillonella	13.6 (10.3)	11.1 (13.8)	4.7 (5.3)
Neisseria	N.D.	0.5 (1.7)	0.6 (1.9)
+ve bacilli	28.2 (14.69	41.7 (23.5)	66.7 (21.9)
A. naeslundii	7.1 (8.7)	16.3 (10.6)	13.3 (16.1)
A. viscosus	1.7 (2.6)	5.6 (18.1)	34.5 (28.2)
A. odontolyticus	11.8 (14.3)	5.3 (8.1)	8.8 (8.5)
A. meyeri	2.5 (7.1)	1.6 (4.6)	0.2 (0.7)
Other Actyn.	0.2 (0.5)	1.7 (6.3)	N.D.
Lactobacillus	2.3 (3.5)	4.1 (5.3)	9.0 (9.6)
Arachnia	2.6 (6.1)	3.9 (10.7)	1.7 (4.2)
Propionibacterium	N.D.	N.D.	0.2 (0.7)
Eubacterium	N.D.	0.2 (0.9)	N.D.
Other +ve baccilli	N.D.	1.2 (3.0)	N.D.
-ve bacilli	8.4 (10.0)	7.3 (7.8)	4.1 (3.4)
Bacteroides	0.2 (0.7)	0.8 (1.6)	1.9 (3.8)
Fusobacterium	5.3 (9.3)	2.4 (7.1)	1.0 (1.6)
Capnocytophaga	2.0 (4.7)	2.2 (3.4)	0.5 (1.0)
Haemophilus	0.5 (1.1)	1.6 (2.6)	0.5 (1.0)
Other -ve baccilli	N.D.	0.2 (0.9)	N.D.

N.D. = Not Detected
* units - % Volume mineral x μm.

Table 6.7 Relation between isolated microorganisms and measured demineralisation parameters. Levels of significance (Analysis of variance).

Isolated organism	Δz	SZ	LB
+ve Cocci	*	NS	NS
Strep. mutans	NS	NS	NS
Strep. sanguis	*	NS	NS
Strep. oralis	*	NS	NS
-ve Cocci	NS	NS	NS
Veillonella	NS	NS	NS
+ve Bacilli	*	NS	NS
Actinomyces	*	NS	NS
Lactobacillus	NS	NS	NS
-ve Bacilli	NS	NS	NS
Bacteroides	NS	NS	NS

NS: Not Significant.
* : $p < 0.05$

Lactobacilli association with increased demineralisation.

Sucrose treament seemed not to influence demineralisation of root surfaces since significant differences between treatment and integrated mineral loss values (Δz_1) lesion body and surface zone were not proven.

6.4 DISCUSSION.

This *in situ* model has the advantage of utilising the natural oral environment which could not adequately be duplicated *in vitro*. It also facilitated the determination of demineralisation and the associated microflora. Longitudinal, *in vivo*, studies are probably the ideal experimental design to investigate the microbial aetiology of root surface caries. However, since these studies rely on the development of clinically evident lesions they usually fail to furnish any information about the subsurface lesion. In constrast, this model produced data regarding plaque microorganisms which could be closely related to the initial demineralisation of root surfaces.

Root surfaces which had not been exposed to the oral environment were used. This eliminated any variations of root surface mineral since it is known that the mineral content of exposed root surfaces differ (generally higher) from that of the non-exposed surfaces (Furseth & Johansen, 1968). Cementum was not removed from the specimens in order to mimic the situation *in vivo* where the initial lesion usually starts with this tissue and progresses to dentine. Moreover, the use of abraded root

surface (dentine) would be exposed to the criticim that the dentine of an extracted tooth is a dead tissue which is not identical to the vital dentine in a living tooth.

Although root surface caries may occur in both adults and the elderly (Section 1.2), young adult subjects were investigated because they volunteered to participate, possessed comfortable, well-fitting appliances, and could be trusted to carry out the experimental protocol accurately (especially sucrose applications). A 10% solution of sucrose was applied to plaque since this concentration was found to be within the range of normal sugar physiological levels in the oral cavity and was reported to be not significantly different from 5% solution (Macpherson, 1988).

In the microbiological procedures, selective media were used to assist in the identification of those bacteria which comprised only a small proportion of the plaque flora i.e *Strep. mutans* and *Lactobacillus* Spp. which would not be isolated otherwise.

Demineralisation was measured, in addition to surface zone and lesions body, by Δz_1 which was a very reliable parameter since there was no shrinkage problem which was of common occurence in lesions produced by the artificial caries system and used for experiments reported in Chapter 4 and 5.

As has been pointed out in Section 1.4, there is lack of agreement between researchers on organisms resposible for root surface caries.

Studies with experimental animals revealed that

several types of oral bacteria could induce root caries. *Actinomyces* spp. caused periodontal destruction and root caries (Jordan & Keyes, 1964; Socransky *et al.*, 1970; Jordan *et al.*, 1972; Crawford *et al.*, 1977). Similarly, implantation of pure cultures of *Strep. mutans* in gnotobiotic rats resulted in root surface caries (Gibbons *et al.*, 1966; Crawford *et al.*; 1977).

Human *in vivo* cross-section experiments have shown that *Strep. mutans* was significantly found more often in soft caries than sound root surfaces, but were equally available on hard carious and sound cementum surfaces (Keltjens *et al.*, 1987). They were also isolated from some of the plaque samples collected from root surfaces exhibiting root caries (Sumney & Jordan, 1974; Syed *et al.*, 1975).

From carious root surfaces in humans, *Actinomyces* spp. have been isolated frequently and in some studies were present in large numbers (Jordan & Hamond, 1972; Sumney & Jordan, 1974; Syed *et al.*, 1975; Hill *et al.*, 1977).

In human longitudinal investigations, *Strep. mutans* were isolated from 70% of plaque samples collected from carious lesions, and *Actinomyces* spp. and *Lactobacillus* spp. were isolated from all, and 70% of plaque samples respectively (Ellen *et al.*, 1985b). Furthermore, *Actinomyces* spp. were found to be the predominant flora on sound cementum and were lower in number on established lesions. However, *Strep. mutans* and *Lactobacillus* were the dominant organisms on established lesions (Billings *et al.*,

1985).

It would thus appear that there is convincing evidence that *Actinomyces* spp. are associated with the disease but their role has not yet been demonstrated. Although it has been suggested that *Strep. mutans* is also associated with root caries, the evidence is not convincing since they were isolated less frequently, and in low numbers. Lactobacillus spp. have been isolated from plaques collected from lesion sites but it is not known if this has any pathological role to play in the disease process. Nonetheless, microoganisms resembling the genus *Arthrobacter* have been collected from deeper parts of carious root lesions (Sumney & Jordan, 1974).

It must be emphasised here that no direct comparisons can be made between the results of experiments reported here and those of published data, because of different experimental design and methodology. In the studies reported in this chapter, microorganisms isolated from normal plaque were dominated by Gram positive cocci and bacilli. At the species levels *Strep. oralis* and *Strep. sanguis* dominated the cultivable flora, and *Actinomyces* were the most predominant and frequently isolated microorganisms from all plaque samples. The isolation of *Strep. sanguis* in relatively higher numbers, might not be of any significance since this organism is usually considered as a member of the normal oral cavity flora and is one of the first organisms to colonise the tooth (Syed *et al.*, 1975).

The isolation frequency of Strep. mutans was high

but the mean percentages values were relatively lower than those of other cultivable microorganisms. This as probably explained in part by the use of selective media, although it may also be related to *Strep. mutans* being associated more with soft than hard lesions, as *in vitro* studies have shown that *Strep. mutans* produced root caries of leathery consistency when they were incubated with a mixture of *Strep mutans* and *A. viscosus* (Katz *et al.*, 1987).

Actinomyces spp. dominated both normal and sugar treated plaques and were associated with groups representing higher demineralisation (Table 6.6). This is in general agreement with results from experimental animal studies where root caries has been created by inoculating the root surfaces with *Actinomyces* species (Socransky *et al.*, 1970; Jordan *et al.*, 1972; Firestone *et al.*, 1987). It is also in agreement with many cross - section experiments in which workers have isolated, *in vivo, Actinomyces* from root caries sites (Jordan & Hamond, 1972; Sumney & Jordan, 1972; Syed *et al* ., 1975). Therefore, one cannot rule out that they play a role in the carious process. However, doubt remain about their role in root caries since, in longitudinal studies, *Actinomyces* species have been isolated from both carious and sound cementum sites. Indeed, they were found in higher numbers on sound than on the carious cementum (Billings *et al.*, 1985).

There was no significant association between *Lactobacillus* and higher demineralisation values. This result is in contradiction with *in vitro* studies in which

root surface caries was produced with *Lactobacillus Casei* inoculation. This supports the theory that the microbial aetiology of coronal caries is different from that of root surface (Banting & Ellen, 1976).

Comparisons between sucrose treated and normal plaques showed little difference and sucrose had little influence on root demineralisation measured parameters, z_1, Sz and LB. This might indicate that the bacterial indigenous acid are of more importance than the exogenous acids.

It was originally planned that demineralisation in slabs would also be studied but, due to difficulties of cutting and grinding those slab sections, being too thin and fragile, this aim was abandoned. However, the slabs demonstrated that the microflora isolated from both sugar-treated and normal plaques which accumulated on thin surfaces (sections) were almost the same as those on larger surface areas, slabs, (Tables, 6.3, 6.4).

6.5 CONCLUSIONS.

The following conclusions may be drawn from the results reported in this Chapter:

1. Three week old plaque produced demineralisation in non - abraded root surfaces which had not been exposed to the oral cavity. The degree of demineralisation varied and in two occasions the characteristic features of a subsurface root caries lesion were seen.

2. Sucrose had no significant influence on either

the composition of the plaque or on the root surface demineralisation.

3. Generally, the results support the theory that both coronal and root caries are of different aetiologies.

4. *Actinomyces* spp. were significantly associated with root surface demineralisation and, therefore, might be important in the initiation of the disease.

5. Although *Strep. mutans* was isolated from all specimens, their numbers were generally low. While, their role cannot be ruled out, it is possible that they become more important in the later soft stage of root caries. It would thus be interesting to see if artificial inoculation of root surfaces could produce softening.

7. More experiments are needed in this field to obtain further data which could lead to definite conclusions, since the experiments described here, involved two volunteers only.

CHAPTER SEVEN.

GENERAL DISCUSSION AND CONCLUSIONS.

The aims of this project as mentioned in Section 1.2 were:

1. to establish an artificial caries system which can produce repeatedly standard subsurface root lesions;
2. to examine *in situ*, the remineralisation of such artificial lesions using fluoridated and placebo dentifrices;
3. to investigate remineralisation of the artificial lesion *in vitro*, using fluoridated and placebo solutions; and
4. to carry out a microbiological study to determine the extent of demineralisation in sound root surfaces exposed to three week old natural plaque; and to identify associated microorganisms.

With respect to the first aim, the buffered solution system was chosen to produce root subsurface lesions as the ingredients are adequately controlled and, unlike the undialised gels, this system suffers little from the impurity disadvantage. Fluoride was required in the demineralising solution to produce the characteristic features of the natural subsurface lesion namely: a microradiopaque surface layer overlying a radiolucent area. Non - fluoridated solutions resulted in root surface erosion at the sites exposed to these solutions. As little as 0.1 ppm F^- contributed to the production of excellent

subsurface lesions which were found to allow accurate microdensitometric measurements. In addition, a series of preliminary investigations were performed to standardise the microradiographic / microdensitometric techniques as little was known about their application to root remineralisation studies.

To investigate the remineralisation effect of fluoridated dentifrice in the natural environment, the intra - oral appliance of Creanor and coworkers, (1986) was modified and used with the single section technique. This system allowed the assessment of mineral changes throughout the experimental period and eliminated the need for a control as each lesion acted as its own control. In addition the single section technique was also used in the *in vitro* studies reported in Chapter 5.

In these experiments, the error magnitude was probably greater than that reported for enamel studies and this situation was due to differences in the nature of both tissues. In addition to lesion sites, mineral was deposited on the sound part of root surfaces. This situation caused a difficulty during microdensitometric procedures, in that placing horizontal lines at baseline images on a root surface before treatment would be inconsistent when placed again after treament as the original point would have shifted due to mineral deposition. This difficulty was minimised by taking prints of lesions at baseline and comparing these with subsequent post - treatment images. However, since remineralisation of root lesions was substantially greater than that

reported for enamel, the error discussed had insignificant influence on the results.

The results of the *in situ* study demonstrated that the fluoridated toothpastes were significantly better in remineralising root subsurface lesions than the non-fluoridated placebo. Regarding the fluoride concentration effect, inter - subject variations were evident. Maximum remineralisation rates were achieved when using the 5000 ppm F^-, but this was only significantly different from the others on some occasions. Although these variations are not readily explained by the results of the *in situ* investigation, they might be attributed to the following factors which were not taken into consideration in the intra - oral model:

1. Realiability: although all volunteers agreed to carry out the experimental protocol, this factor remains subjective and controlled by the volunteer's comfort with the appliance. There is no doubt that on some occasions appliances were not worn during social events as some volunteers expressed concern about halitosis which they believed was due to the plaque accumulation on the trough area.

2. Oral hygiene and sucrose intake: because of their close association with the dental profession, the volunteers employed in these experiments were dentally motivated. Therefore, they were expected to have relatively less plaque and have more control of their diet, particulary with regard to sugar intake. Thus, with such motivation maximum remineralisation could have been

achieved with the 1000 ppm F^- paste which left no room for improvement with higher doses.

3. Monofluorophosphate dentifrice: this dentifrice has been reported to be more effective in the acidic environment where fluoride would be liberated and become ionised. It follows that those subjects who had more acidic attacks and used regularly the toothpastes provided achieved theoretically better remineralisation than other volunteers. Thus remineralisation results could be explained in part by varied dietary habits.

4. The higher fluoride concentration might have resulted in blockage of the microchannel via which ionised mineral is seeded into the surface or body of the lesions and thus allowing little more mineral to pass through.

5. Finally toxic effects of the highly fluoridated toothpaste on the microbial flora should be given more attention and is worth further investigation. With high fluoride concentrations such as those obtained with the 5000 ppm dentifrice, it is thought that microbial activity is dimininished and the efflux of fluoride from the plaque to the lesion is extremely reduced because plaque is less acidic in nature in the absence of microbial activity.

Unfortunately no root caries clinical trial using the same fluorides is available for comparisons.

The pH cycling study has obviated the disadvantages of the above variables but suffered from the disadvantage of working in artificial conditions. The results of these experiments were consistent with results of the *in situ*

study in that they demonstrated greater remineralisation potential of fluoridated compared to placebo solutions. Generally, remineralisation rates were more for the *in situ* than for the pH cycling experiment and this could be attributed to the following factors:

(1). The fluoride exposure time was twice daily for the *in situ* study and once daily of five minutes duration for the *in vitro* experiment; (2). the demineralisation period in the pH cycling experiment was for three continuous hours whereas that in the oral cavity usually occurs over different period and would not reach three hour period in the poorest oral hygiene standards. Moreover, salivary clearance of acids reduces the demineralistion period *in situ*. (3). In the oral environment the demineralisation and remineralisation effects on the lesion would be occurring at the same time whereas that in the pH cycling experiment were occurring in separate events; and (4), the absence of the microflora might have resulted in the decreased remineralisation observed. Whether low fluoride availability in demineralising solutions over long periods would achieve better remineralisation results than higher fluoride levels available for short times, is a question which remains unanswered.

In the light of the remineralistion behaviour of the lesions which had no surface zones, it is believed that remineralisations (healing) could occur in two ways: 1. By seeding mineral into the demineralised root tissue, which is now mainly organic material, in a manner

resembling healing by 'first intention' and; 2, by depositing mineral and thus filling the eroded root (concavity) in a manner like healing by 'second intention'. This theory emphasises the necessity to preserve the organic matrix of the root tissue during demineralisation either *in vivo* or experimentally. Therefore, attention should be paid particulary on how, in the event of demineralisation, to preserve the organic matrix and protect it against proteolytic or mechanical destructive activities.

The results of the microbiological studies demonstrated that actinomyces was significantly associated with demineralisation of the sound cementum surfaces. Both *Strep. mutans* and lactobacilli played no significant role in demineralisation. This is in agreement with published reports that coronal and root caries are of different aetiologies. Treating the natural plaque with sugar made no change in the plaque composition and played little effect on demineralisation. It is believed that the indigenous acid of the flora is of more importance for demineralisation than the exogenous acid. More experiments are needed in this field to establish the microbiological aetiology of this disease and attention should be focussed particularly on the microorganisms' proteolytic effect on the organic matrix of sound and carious teeth. Also, of further interest will be to assess the depth penetration of microorganisms by histopathological methods. Sampling the deeper parts of the lesions might reveal the identity of microorganisms at the lesion front.

Finally, the microradiographic / microdensitometric methods used to determine changes in mineral content, proved to be a useful technique for the studies described in this thesis. However, its major disadvantage is that it assumes the mineral deposited is hydroxyapatite. It does not give any information on the structure and nature of the deposited mineral. It would, therefore, be worthwhile to examine such mineral using X-ray diffraction and electron probe techniques.

Appendix I - Instructions to volunteers.

The *in situ* experiment protocol.

1. The appliance should be worn at all times except where detailed below.
2. In this experiment I wish you brush twice per day with the coded dentifrice supplied in the following manner:

a. Remove the appliance. If you wish, you may clean it but leave the trough area with the experimental sections undisturbed. Clean the lower lingual aspects of your teeth with only brush and water. The lower teeth can be flossed etc at this point.

b. Insert the appliance. Extrude a half inch of toothpaste onto brush and with the appliance in place clean all other areas for 2 minutes.

c. Merely spit out excess paste at the end of the two minutes, and do not rinse.

3. I would prefer that you used no other dentifrice and other fluoride supplement. If, however, you feel that you must use a dentifrice at other times, a non-F^- will be supplied. Use a separate brush and remove the appliance while you brush your teeth. Rinse out thoroughly afterwards before replacing the appliance.
4. At the end of each week, remove the appliance and carefully clean the experimental trough.
5. I will let you know when we require the appliance back for analysis.

Appendix II - Formulation of Dentifrices.

	FORMULATION OF TOOTHPASTE	
	NaMFP	Placebo
Abrasive (Alumina):	50%	50%
Humectant (Sorbitol):	27%	27%
Thickener (SCMC):	0.85%	0.85%
Therapeutic Agent: (NaMFP):	0.76%, 1.9%, & 3.8%	NIL
Flavouring Agents & Water:	added to make 100%	

SCMC = Sodium carboxymethylcellulose

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