THE INFLUENCE OF SALIVA ON THE DYNAMICS OF THE EARLY ENAMEL LESION

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ABSTRACT

The work presented in this thesis has aimed to address the effect of saliva on the early enamel lesion using an intra-oral model. Preliminary work on model validation was undertaken and demonstrated the ability of stimulated salivary flow, by means of sorbitol gum chewing, in a fluoridated environment, to increase remineralisation of artificial enamel lesions to a greater extent than fluoride alone. Additional work demonstrated the ability of the model to distinguish between fluoridated and non-fluoridated environments. However, a definitive three phase intra-oral trial failed to demonstrate any significant difference between fluoridated and non-fluoridated environments or any significant beneficial effect of salivary stimulation, by means of sorbitol gum chewing, in a non-fluoridated environment. Further analysis of this data suggested this was a result of a small baseline lesion size and that larger lesions would perhaps have reacted in such a manner as to demonstrate significant differences between protocols.

Additional work to determine plaque and salivary fluoride levels, the ability of saliva to clear substrate from the appliance test site and the reaction of plaque within the appliance trough to various stimuli, yielded some interesting results.

Plaque and salivary fluoride levels tended to fall, in some cases significantly, over a 4 week washout period when subjects changed from a

fluoridated to a non-fluoridated dentifrice. The rate of clearance by saliva of substrate from the appliance trough was much faster than anticipated. This work demonstrated the ineffectual protection provided by the appliance trough against high salivary flow rates in this area of the mouth. Methodologies were also devised to assess the ability of plaque within the appliance trough to produce organic acid in response to a sucrose stimulus and to determine the ability of saliva to buffer the pH of such acid. With the limited amount of data available, it would appear that a reduced ability of the appliance trough plaque to produce acid and an increased ability of saliva to buffer a reduced plaque pH were associated with significant lesion remineralisation.

This work has demonstrated the versatility of this model and the potential to demonstrate some of the effects of saliva on artificial lesion remineralisation given lesions which display an appropriate ability to respond to a given protocol. Further work is required to determine the nature of the response of lesions with different characteristics to different intra-oral protocols, particularly in a non-fluoride environment.

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PUBLICATIONS

The following are (a) papers presented by the author, (b) in association with the author at Scientific meetings and (c) papers published in association with the author related to the work contained in this thesis.

(a)

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(b)

Creanor SL, Hall AF, Gilmour WH, Strang R, Foye RH. Non-fluoridated dentifrice and enamel remineralisation *in situ*. *Caries Research* 1994;28:208. (Paper presented at 41st ORCA meeting, Cork, Eire, 1994)

(c)

Creanor SL, Strang R, Gilmour WH, Foye RH, Brown J, Geddes DAM, Hall AF. The effect of chewing gum use on *in situ* enamel lesion remineralisation. *Journal of Dental Research* 1992;71:1895-1900.

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DECLARATION

This thesis is the work of the author, and associates under the direct supervision of the author.

CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION AND AIMS

Few human diseases have been the subject of as much research as dental caries. Research undertaken to date has determined many of the aetiological and modifying factors of the disease process. Cariologists are now able to discuss at length, many of the biological and chemical mechanisms involved in the initiation and progression of dental caries. These advances in research have had clinical application to the extent that dental caries is now an entirely preventable disease. The effect of fluoride, probably the most intensively researched preventative methodology in Medicine and Dentistry, has been largely responsible for reducing the incidence of the disease. Such advances in the knowledge of prevention of dental caries have been seen within a period of little more than a generation. In many western societies dental caries is no longer feared as a harbinger of pain, ending in the inevitable extraction of the tooth. Dental caries is now seen, in many cases, as an occasional finding on routine dental examination which can be remedied by the re-establishment of a vigourous preventative strategy or the timely placement of a small restoration.

The effect of fluoride on dental caries is one factor in the dynamics of this disease process. Fluoride is often an extraneous agent, utilised in the fight against caries. Recent attention has been focused on the effect of endogenous

factors such as saliva. Whilst early observations have shown the ability of saliva to heal early dental caries, little work has subsequently been undertaken to quantify this effect.

Methods of observation of the caries process have also changed over the past 30 years. The use of caries models has permitted great advances in the understanding of the disease process. A new generation of caries models uses the oral cavity as a laboratory, simulating as closely as possible the conditions under which caries occurs.

The aims of this thesis were to investigate the relative effects of stimulated salivary flow and fluoride on mineral changes within artificial carious lesions using an intra-oral model. In addition, the ways in which saliva may bring about such effects were studied by careful recording of salivary composition, the rate of salivary flow and the effect of saliva on dental plaque. This work has been carried out by adapting existing methodologies to observe the effect of fluoride and saliva on dental caries and instituting new ones to measure salivary flow and record plaque/saliva interactions.

1.2 DEFINITION OF DENTAL CARIES

Dental caries may be defined as a dynamic process of mineral transfer out of and into dental hard tissues. Dental caries is initially a sub-surface phenomenon, occurring as a result of the collective effects of an overlying microbial deposit, a substrate to stimulate acid production, and time.

There are several principle factors involved in the initiation and progression of the disease process. Such factors may be divided into primary and secondary factors.

1.3 FACTORS IN DENTAL CARIES INITIATION AND PROGRESSION

Factors in dental caries initiation and progression are divisible into primary and secondary types. A primary factor is one without which the disease process cannot occur. Keyes (1960) discusses the three primary factors responsible for initiation and progression of dental caries. This work has classically been presented as three overlapping circles representing the tooth, the oral flora and a bacterial substrate. Caries initiation and progression only occurs in the presence of all three factors. Secondary factors in caries initiation and progression are discussed by Nikiforuk (1985). They may be defined as those factors which modify the primary factors, in relation to the timing of onset and rate of progression of the disease. Secondary factors include the

microbiological composition of the dental plaque, the biochemical make up of the tooth and the type and pattern of substrate use. Other secondary factors such a salivary physiology and biochemistry are also important. Furthermore, individual, intraoral physiological characteristics that determine, and control, the equilibria of calcium, phosphate and/or other species may, in future, prove to be vital in the understanding of caries aetiology and progression.

1.3.1 The Oral Flora

The work of van Leeuwenhoek (1674, referenced from Mellville & Russell, 1983) was fundamental in determining that the oral cavity, and specifically the teeth, could support bacterial colonies. Miller (1883) made significant advances in caries aetiology when he reported that fermentation of starch and sugar foods by dental plaque could produce organic acids which would cause dissolution of enamel and dentine. It was not until the study by Orland *et al.* (1954) that bacteria were established as an essential factor in the initiation and progression of dental caries *in vivo*.

Orland *et al.* (1954) developed a technique of raising germ free rats, delivered by Cesarian section and fed with sterile food. Germ free rats were compared with control rats with a normal oral flora. Both groups of animals were fed the same diet. At the end of the experimental period, examination of molar teeth showed carious lesions in the control group but no lesions in the germ free rats. This study was the first to show that caries could not be initiated *in vivo*

in the absence of bacteria.

1.3.2 The Bacterial Substrate

The presence of a bacterial substrate *per se* constitutes a primary factor in caries initiation and progression whereas the type and timing of substrate use constitutes a secondary factor. For the purposes of studying dental caries it is perhaps important to define a substrate as a substance metabolised by plaque bacteria to produce organic acids. Studies to illustrate the effect of bacterial substrate *per se* on dental caries *in vivo* are scarce. However, studies of the children of Hopewood House, Bowral, Australia (1952-1961) do illustrate this point.

The Hopewood House studies of the 1950's and 1960's examined a group of children following a strict dietary regime. Harris (1963) described the diet as, "Notable for the almost complete absence of refined carbohydrate and the presence of minimal animal protein". Both the studies of Sullivan & Harris (1958) and Harris (1963) compared the dental health of the children of Hopewood house to data from a survey of State School children by Barnard in 1956. The children of Hopewood House had significantly less caries than those children in the State Schools. Whilst the results of these studies may be due, in part, to the timing of food intake, the major difference between the two populations was the presence of bacterial substrate metabolised by plaque bacteria to produce organic acids.

1.3.3 The Tooth

The presence of a tooth is an obvious primary factor in the initiation and progression of dental caries as it is the medium in which caries occurs. The physical and biochemical composition of the tooth constitute a secondary factor and will be discussed later.

1.3.4 Microbial Composition Of Dental Plaque

Orland *et al.* (1955) inoculated germ free rats at 31 days with enterococcus bacteria and demonstrated that dental caries could be produced by a single strain of bacteria. This initiated a whole series of experiments where different bacteria were examined for their ability to induce dental caries. The physiological properties of caries inducing species of bacteria were identified. These properties included an ability to adhere to the tooth surface, the production of organic acid, toleration of an acidic environment and the production of extracelluar polysaccharides. To date, *Streptococcus mutans* has been found consistently to produce more carious lesions than any other bacterial species (Marsh & Martin, 1984) .

1.3.5 Physical and Biochemical Characteristics Of The Tooth

Studies have shown a relationship between tooth morphology, enamel

composition, and caries susceptibility. These are now discussed.

The pattern of caries development around the mouth varies from tooth to tooth; molar teeth having the highest incidence of caries (Carlos & Gittleschon, 1965). These findings are supported by data from the United Kingdom Dental Health Survey of 1988 (Todd & Lander, 1991). Caries incidence in the oral cavity may also be classified according to site. Backer Dirks (1965 and 1966) concluded that free, smooth surfaces of the teeth were the most caries resistant, interproximal surfaces were of intermediate resistance and pits and fissures were the least resistant to dental caries.

Variation in caries incidence between different teeth has been shown to be related to tooth morphology. Bossert (1933 and 1937) was able to correlate the cuspal incline with the presence or absence of caries in the fissure. The steeper the cuspal incline the greater the likelihood of caries in the fissure. No details were given of how caries was diagnosed and it is possible that caries may have increased fissure depth due to loss of hard tissue which would artificially increase the cuspal gradient. These findings are supported by the studies of Malherbe & Ockerse (1944) and Schiller & Wolcott (1961).

The biochemical composition of the tooth in relation to caries susceptibility has been the subject of much research. The surface layer of dental enamel is more highly mineralised than the underlying enamel (Glas, 1962; Angmar *et al.*, 1963). The concentration of calcium and phosphate also tends to decrease

from the surface towards the amelodentinal junction (Robinson *et al.*, 1971). Fluoride levels are highest at the enamel surface and decrease towards the amelodentinal junction (Robinson *et al.*, 1982). Different teeth contain variable levels of fluoride and it has been difficult to determine if this has any effect on enamel caries susceptibility (Chow, 1990). The effect of fluoride in dental enamel is discussed at length later in this chapter.

The carbonate content of enamel mineral has been proposed as a determining factor for enamel caries susceptibility (Little & Brudevold, 1958). Gron *et al.* (1963) showed that synthetic apatite was more soluble in a solution with a high carbonate content and suggested that apatites which contain carbonate may be more soluble than those which do not. Hallsworth *et al.* (1973) used a microdissection technique to show that the "primary translucent zone" of white spot lesions contained less carbonate than adjacent normal enamel. They concluded that carbonate is preferentially lost at the initial stages of enamel caries. The increase in carbonate towards the amelodentinal junction has been proposed as one reason for the penetration of enamel lesions (Robinson *et al.*,1982).

1.3.6 Type And Pattern Of Substrate Use

The Vipeholm study (Gustafsson *et al.*, 1954) was designed to determine the effect of variable carbohydrate levels on the incidence of dental caries in 436 individuals over a five year period. The authors studied patients at the

Vipeholm Hospital for the mentally deficient outside Lund in Sweden. All participants were placed on a basic diet, judged to be adequate in all respects except caloric content. Caloric content was increased by the addition of either fat or sugar in various forms at various times. Such additions to the basic diet formed the different protocols throughout the course of the study. Four different protocols were tested. These were:-

1 Basic diet with fat (Control group)

2 Basic diet with sugar (300g) in solution at meals

3 Basic diet with sugar (50g) in bread (345g) with afternoon coffee only, for first two years, then four times per day, with all meals, for the second two years

4 Basic diet with sugar, in the form of sweets, consumed between meals. This group included subjects eating chocolate, caramel and 8-toffee and 24-toffee groups.

The results of this study have been summarised by Nikiforuk (1985), who concluded that caries levels increased with raised dietary sugar intake. Moreover, levels were increased further when sugar was consumed between meals and when sugar was retained at the tooth surface or had a reduced rate of clearance. Withdrawal of such foodstuffs from the diet reduced caries activity. However, caries still occurred in subjects maintaining a low refined sugar and carbohydrate diet.

Bibby (1990) suggested that the case for the dominant role of sucrose is

incomplete. He argued that the use of sucrose by primitive people was often accompanied by the use of wheat flour, that rationing of sugar during wartime was also accompanied by rationing of flour and that the amount of sugar consumed by the subjects in the Vipeholm study did not parallel the caries incidence observed. Woodward & Walker (1994) analyzed data for sugar consumption and dental caries prevalence in 90 countries and were unable to find any evidence of a sugar-caries relationship in the data from the 29 industrialised countries. Furthermore, they suggested that the other aspects, such as exposure to fluoride, should also be taken into account when making recommendations for caries control at the population level. Whilst these arguments are pertinent, there is still a case to be made for the greater influence of sucrose on dental caries than other dietary factors.

1.3.7 Salivary Physiology

Saliva has a crucial role to play through its interactions with plaque, its role in substrate clearance from the oral cavity and its buffering capacity against pH reduction. The effects of saliva on enamel caries will be discussed in greater detail later in this chapter.

1.3.8 Other Host Factors

Host systemic pathology can affect dental caries in a variety of ways. Certain specific pathological conditions may bring about changes within the oral

environment that alter patient susceptibility to dental caries. Such conditions include Cystic Fibrosis where mean salivary pH and buffering capacity are elevated and dental caries experience is low (Kinirons, 1985). Children with Chronic Renal Failure also exhibit low caries susceptibility. Analysis of a group of patients with Chronic Renal Failure by Obry *et al.* (1987) demonstrated much higher salivary levels of urea compared to a control group. An increase in urea levels would tend to counteract the effects of organic acid production by the action of bacteria on fermentable carbohydrate. Any illness that requires pharmacological agents prepared with a sugar-containing formula to increase palatability, will increase the patient susceptibility to dental caries (Roberts & Roberts, 1981).

1.3.9 Interaction Between Primary And Secondary Factors

No one factor in the caries process can act alone. Plaque composition is determined by the nature of the surface it colonises. The morphology of the tooth surface (fissure or smooth surface) will determine the amount of plaque accumulation. The amount, type and frequency of substrate will determine the nature of plaque metabolism. The presence of saliva will determine substrate clearance and buffering capacity within the oral cavity. Finally, host immunity or pathology may influence caries susceptibility through plaque modifications, salivary interactions or by increasing plaque substrate.

1.4 PATHOGENESIS OF ENAMEL CARIES.

The pathogenesis of enamel caries involves several distinct processes. Acid production must first occur within dental plaque. The acid must diffuse through plaque to gain access to the enamel via interprismatic channels. Once within the enamel, protonation occurs at specific sites on the apatite lattice. Acid/mineral interactions result in the establishment of variable concentration gradients between the interior of the enamel and the enamel surface. Changes in concentration gradients and alterations in saturation conditions with respect to different species are responsible for the subsurface demineralisation seen in early enamel caries.

1.4.1 Acid Production By Plaque

The production of acid by dental plaque creates local undersaturated conditions with respect to calcium phosphates in the fluid at the enamel surface (Lagerlof, 1983) resulting in mineral dissolution. Miller (1883) showed that organic acids could be produced by plaque microorganisms, when exposed to either sugar or starch. Furthermore, he showed that such acid could decalcify dental enamel.

To determine which organic acids were being produced, sophisticated analytical techniques were required. Gilmour & Poole (1967) used gas liquid chromatography and were able to identify the production of lactic acetic and

propionic acids from "membrane plaque" undergoing glucose fermentation *in vitro*. Geddes (1975) used a combination of enzyme techniques and gas liquid chromatography to analyze plaque before and after *in vivo* exposure to sucrose and glucose. She identified lactic acid and several other organic acids and concluded that higher levels of organic acid were found in plaque after sugar fermentation. The types of organic acid identified were the same for both resting plaque and that analyzed after a sucrose challenge. However, the levels of lactic acid were much higher in plaque challenged with sucrose. The amount of sucrose given as a challenge did not affect the amount of acid produced immediately by the plaque. Levels of acid after thirty minutes, however, were found to be much higher when substrate levels were increased.

1.4.2 Plaque Permeability To Organic Acid

The production of acid within dental plaque is often remote from its site of action at the plaque /enamel interface. Acid is required, therefore, to diffuse through plaque to reach the enamel surface. Plaque permeability to various organic acids has been addressed by Dibdin (1981) who suggested that acid diffusion through plaque will be affected by variation in acid molecular weight. Furthermore, there is a possible reduction in rate of diffusion due to monovalent negative charge. The model used by Dibdin applies only to young plaque *in vitro*. Stagnation sites such as fissures and approximal areas will have greater plaque thickness, greater plaque maturity and density, and possibly more pronounced permselectivity due to extracelluar polysaccharide.

Each of these factors is likely to reduce the rate of acid diffusion to the plaque/enamel interface.

1.4.3 Diffusion Of Acid Into Enamel

The detection of raised levels of lactic acid in plaque after carbohydrate consumption caused investigators to conclude that lactic acid was primarily responsible for the initiation and progression of the carious lesion. This assumption was challenged by two advances in research. Firstly, Featherstone *et al.*, (1979) demonstrated that the non-ionised form of an organic acid was the predominant diffusing species into dental enamel. This was contrary to the previously held belief that an acid diffused into enamel in its ionised form. Secondly, the work of Geddes (1975) and Geddes & Weetman (1983) has shown that levels of acetate diminish at about the pH minimum of the Stephan curve. This has been ascribed to uptake of acetic acid by enamel, indicating a potential role for this acid in caries initiation and progression.

Featherstone & Rodgers (1981) assessed the effect of acetic, lactic and other organic acids on the formation of the artificial carious lesion. They showed that lesion progression was a function of the unionised acid concentration and the acid dissociation constant. Acetic acid was thought to be as important as lactic acid in its contribution to lesion progression. The authors explained their findings as follows. The difference in pK values between acetic and lactic acid indicates more acetic acid will be present in its unionised form than lactic acid

at any given pH. As a result, acetic acid will diffuse into enamel faster than lactic acid. As acid diffuses into enamel, it will dissociate into H^+ ions and acid anions which will attack the enamel crystallites. Acid dissociation is determined by the strength of the acid. Lactic acid will dissociate more readily than acetic acid, although it will not diffuse into enamel as fast. Diffusion of ionic calcium and phosphate out of enamel, and hence the rate of lesion formation, will be the direct result of acid dissociation. These findings are in agreement with those of Edgar (1982).

1.4.4 Initial Mineral Loss In Enamel Caries

It is apparent from several studies that initial mineral loss results in a morphological change in the enamel surface. Arends *et al.*, (1982) have suggested tentatively that the initial site of attack can be at existing defects on the enamel surface, especially the perikymata or defects due to the Tomes process of the ameloblast. These effects have been described variously as:-

i enlargement of the interprismatic spaces (Thylstrup *et al.*, 1982).

ii the development of micro-fissures due to mineral loss and subsequent merging of Tomes process defects on the enamel surface (Haikel, 1982).

iii the formation of microchannels with no apparent prismatic relationship (Goldberg *et al.*, 1981).

The overall effect of this morphological change in the enamel surface is to produce a surface softening (Arends & ten Bosch, 1986), which has been

described on lesions produced *in vitro* (Arends & ten Cate, 1981) and *in vivo* (Dijkman, 1986a; Ogaard, 1986).

The initial change in the surface morphological and physical characteristics of enamel has been associated with well documented chemical changes. Hallsworth (1973) and Robinson *et al.* (1982) have demonstrated the preferential removal of magnesium and carbonate with the inception of the caries process. Magnesium and carbonate ions accumulate in the fluid within the intercrystalline spaces before migrating down a concentration gradient towards the enamel surface. Further mineral loss is due to the protonation of phosphate groups within the apatite lattice (Weatherell *et al.* , 1982). The degree of protonation will eventually render the lattice structure unstable. Such instability is overcome by lattice dissolution or conversion to a more stable lattice structure. Whilst phosphate group protonation is taking place, there will be a resultant charge imbalance within both the apatite lattice and any fluid in the intercrystalline spaces. Such charge imbalances are resolved by loss of calcium from the lattice and also by the loss of some protonated phosphate groups that are less strongly bound (Weatherell *et al.*, 1982).

Subsequent mineral loss with lesion progression is a sub-surface phenomenon resulting in the formation of a surface zone overlying an area of subsurface demineralisation (Arends & Christofferson, 1986). The surface zone was described as a porous, but mineral rich, zone which forms after initial surface softening and, once formed, changes thickness slowly in comparison with the

demineralisation period. In addition the fluoride level in saliva appeared to be the main reason for the development of the surface zone rather than fluoride in the surface layers of the enamel (Arends & Christoffersen, 1986).

Arends & Christoffersen (1986) have discussed four possible explanations for the formation of the surface zone. These are summarised in Table 1.1, taken from the text of their paper.

Table 1.1

Theories of surface zone formation in enamel lesions

Principle	Main Proponents	Basic Mechanism	Problems
Outer surface protection by adsorbed material	Gray 1977 Gray and Francis 1963 Francis *et al.* 1973	The outer enamel surface is insoluble due to the presence of protective agents (Fluoride, salivary proteins, polyphosphates). Dissociated and undissociated acid molecules penetrate deep into enamel and mineral subsequently dissolves out of lattice.	Does not account for initial surface softening phenomena seen *in vivo*. Find fluoride and proteins also invade lesions in substantial amounts.
Gradients in porosity/solubility - a mathematical model.	Van Dijk *et al.*, 1979	This model describes the diffusion of calcium, phosphate and both dissociated and undissociated acid into and out of lesion as well as the dissolution reaction of the mineral. The model assumes reactions in the aqueous environment achieve equilibrium very fast and that the rate of mineral dissolution is proportional to the concentration difference between the solution in the enamel pores and the solution at the surface of the enamel. The authors concluded that the formation of a surface layer could be explained by gradients in the solubility product of enamel, the enamel porosity and the rate constant of the dissolution reaction.	Surface zones form in cases where none of these gradients are present - eg lesion formation in pressed apatite.

Table 1.1 (Contd.)

Theories Of Surface Zone Formation In Enamel Lesions

Principle	Main Proponents	Basic Mechanism	Problems
Dissolution /precipitation mechanisms	Margolis & Moreno, 1985 Margolis *et al.*, 1985	Dissociated and undissociated acid diffuses into enamel resulting in apatite phase transitions to form dicalcium diphosphate dihydrate and fluorapatite. This formation suggests a flow of mineral from the inner enamel to the surface layer	This model cannot be used to predict how sub-surface lesions will progress with time
Outer surface protection combined with a dissolution/precipitation mechanism	Featherstone, 1977 Featherstone *et al.*, 1979	Protective agents (pellicle) first absorb to the outer surface of the enamel Subsequent dissolution/ precipitation	Does not account for surface softening Cannot predict lesion progression ? role of fluoride

1.4.5 Later Mineral Loss

Robinson *et al.*, (1982) suggested the mechanism of later tissue destruction in enamel is not simple dissolution, rather it reflects both the composite nature and the chemical composition of the tissue. The composite nature of the tissue is such that dissolution of ions from the crystallite surface initially involves a relatively large crystallite surface area in relation to the intercrystalline space. Ions from the crystallite surface in solution in the intercrystalline fluid will, therefore, be at very high concentrations, creating a steep concentration gradient between the intercrystalline fluid at the advancing front of the lesion and the fluid at the enamel surface. As crystallite dissolution proceeds, crystallite surface area will decrease and intercrystalline fluid volume will increase, reducing the concentration of ions in the intercrystalline fluid and hence the concentration gradient between lesion fluid and the enamel surface. This creates a situation where migration of ions from the lesion front is more rapid than migration from the body of the lesion, thus promoting lesion penetration. The increase in magnesium and carbonate concentrations towards the amelodentinal junction (Weatherell *et al.*, 1968; Robinson *et al.*, 1981) will accelerate lesion progression in this direction.

1.4.6 Summary

Caries initiation within enamel is dependent on preferential dissolution of the magnesium and carbonate rich areas of the crystal lattice followed by

protonation of phosphate groups with subsequent calcium release and disturbance of apatite structure. Differential degrees of lesion fluid saturation with calcium and phosphate will result in mineral removal via the enamel surface along concentration gradients, or mineral re-deposition within other areas of the developing lesion. The kinetics of ion movement within carious enamel are responsible for the development of the various histological zones seen on imbibed ground sections of carious lesions.

It is very important to appreciate that the events described do not occur in isolation. Moreover, they are not a series of finite steps to an inevitable conclusion. Dental caries is a dynamic process and mechanisms exist where, in some cases, demineralisation can be reversed. This will be discussed in the next part of the chapter.

1.5 REMINERALISATION

1.5.1 Definition Of Remineralisation

Remineralisation has been described by Arends & Gelhard (1982) as, "the deposition of mineral in enamel defects". Such mineral should be similar to sound human enamel and defects include subsurface lesions, etched enamel and surface softened enamel.

1.5.2 Early Observations Of Remineralisation

Remineralisation is not a new phenomenon. It was described by Head (1910) who demineralised dental enamel by leaving it in a segment of orange for two days. This produced, "a smooth white, translucent area of decalcification." Subsequently, the tooth was placed in a sample of the author's saliva for two weeks. After this time, Head observed, "the white spot of decalcification had almost, if not entirely, disappeared". Furthermore, Head went on to suggest that this may be due to, " a sort of recrystallization within the enamel substance".

1.5.3 Remineralisation *In Vivo*

In vivo studies of remineralisation of enamel caries are much less common than *in vivo* studies of demineralisation. Generally, the studies that do exist can be divided into two types. These are observations of naturally occurring caries and observations of artificially created caries. Studies of naturally occurring caries are less common than studies of artificially created caries. One study of naturally occurring caries is particularly noteworthy. Baker Dirks (1966) monitored the dental health of 90 children from the age of 7 through to 15 years. During this time he observed proximal lesions on standardised bitewing radiographs with variable rates of progression. This observation led him to conclude that the caries process may become arrested. Furthermore, he observed the resolution of white spot lesions on 37 out of 72 buccal surfaces

of teeth that could not be accounted for by a change in diagnostic criteria. From this observation, he concluded that remineralisation must have taken place. Other studies of this type have not been as comprehensive and have been reported to highlight the benefit of diagnostic and monitoring techniques (Pitts, 1986). Studies designed to show the feasibility of creating caries at specific sites in the mouth (Bunting *et al.*, 1926; Nygard-Ostby *et al.*, 1957; Hals & Simonsen, 1972) spawned several studies of remineralising factors for artificially created enamel caries. Of note is the study by von der Fehr *et al.* (1970b) who created white spot lesions *in vivo* in 6 dental students who refrained from oral hygiene procedures for 23 days and rinsed with a sucrose solution nine times daily. Subsequent daily rinsing with a 0.2% NaF solution in conjunction with restitution of oral hygiene procedures resulted in apparent resolution of the lesions after about two months. A similar result was described by Loe *et al.* (1972) using the same fluoride rinse regime. Other studies have demonstrated remineralisation in the absence of fluoride and include those of Holmen & Thylstrup (1986), Artun & Thylstrup (1986) and Holmen *et al.* (1987). In each of these studies remineralisation was demonstrated upon restitution of tooth brushing to previously protected sites. The authors commented that the clinical observation of lesion regression was primarily a result of surface abrasion and some redeposition of mineral and supported this conclusion by scanning electron microscope studies.

1.5.4 Remineralisation *In Vitro*

Remineralisation of dental caries *in vitro* has been shown by many workers. Such work has provided a significant insight into the process of remineralisation but does not necessarily reflect the *in vivo* situation. Some studies of note include those of Silverstone & Poole (1968) who used saliva and calcifying fluids to remineralise naturally occurring and artificially created enamel lesions and ten Cate & Arends (1977) who used an *in vitro* model to determine the kinetics of enamel remineralisation, the nature of the mineral deposited and its site of deposition. The advantages and disadvantages of *in vitro* models were discussed by White (1992). They are applicable to *in vitro* studies of remineralisation and are summarised in Table 1.2.

Table 1.2

Advantages and disadvantages of *in vitro* models to study remineralisation

Advantages
1 Generally inexpensive 2 Not time consuming 3 Permit careful control of conditions to enable:- - modifications to model sensitivity - repeat measurements and standardization of testing - measurement of effect of a single variable with others held constant 4 Potential to study effects on variable materials (Eg bovine or human enamel, dentine etc)
Disadvantages
1 Cannot simulate the real 'caries event', only general thermodynamic and kinetic effects occurring during lesion formation and remineralisation. 2 Cannot adequately simulate microbiological or specific salivary effects on caries. 3 Often require highly polished or flattened surfaces for analysis 4 May use non-human enamel 5 Difficulty in studying 'active systems' which rely on interactions between plaque, saliva and mineral. 6 Difficult to simulate intra-oral conditions such as topical application and salivary clearance.

1.5.5 Remineralisation *In Situ*

In vivo observations of remineralisation require either prolonged observation of a population (Baker Dirks, 1966) or the creation of lesions on vital teeth and the subsequent evaluation of a remineralisation protocol (von der Fehr, 1970a). These methods are either time-consuming or invasive. In addition, the potential disadvantages of *in vitro* models may also make them unsuitable to study remineralisation for the reasons stated in Table 1.2. In an effort to overcome these difficulties, researchers have attempted to combine attributes of each of these models to form an *in situ* model. Intra-oral *in situ* models use a medium in which caries can be easily observed within the oral cavity. Artificial lesions may be created in human enamel and placed in the oral cavity whilst the subject follows a remineralisation protocol. The advantages and disadvantages of *in situ* models to study dental caries are discussed in more detail later in this Chapter. Notable work using *in situ* models to demonstrate remineralisation includes that of Koulourides *et al.* (1974) who were able to show that fluoride could be preferentially taken up by pre-softened enamel which could result in enamel rehardening. Several workers have shown fluoridated dentifrices to be capable of remineralisation of artificially created lesions (Creanor *et al.*, 1986b; Wefel *et al.*, 1986; Gelhard *et al.*, 1979) and further work has been undertaken to show a fluoride dose response (Stephen *et al.*, 1992; Mellberg *et al.*, 1994). The remineralising effect of saliva on artificial enamel lesions *in situ* has been described in a pilot study by Leach *et al.* (1986).

1.5.6 Methods For Detection Of Remineralisation

Various methods exist for the assessment of the remineralisation process. These methods are discussed in Chapter 2 but are mentioned briefly here for continuity.

Visual examination of the tooth surface has been undertaken by von der Fehr *et al.* (1970) where resolution of white spot lesions was observed *in vivo*. This method does not enable quantification of remineralisation. Silverstone & Poole (1968) used polarised light microscopy with sections imbibed in various media. Such methods of analysis are destructive, requiring the creation of sections but are not quantitative in terms of mineral determination (Arends & ten Bosch, 1992). Koulourides (1980) and others have used the Knoop hardness test on various areas of the carious lesion. Once again, this tends to be a destructive method of analysis. Quantitative microradiography is also destructive as sections are required for analysis. However, the advent of the single section technique within an *in situ* appliance (Creanor *et al.*, 1986a) permits pre- and post-treatment of the same lesion. Microradiographic techniques also exist which permit examination of blocks of material or even whole teeth (Arends & ten Bosch, 1992). Other methods such as light scattering, iodine absorbtiometry, iodide penetration and ion probe analysis have also been described and are reviewed by Arends & ten Bosch (1992).

1.5.7 Summary

The events occurring during remineralisation are complex and are dependent upon local conditions prevailing at the time. Local conditions include the presence of a supersaturated solution of calcium and phosphate at the enamel / plaque interface. Concentration gradients may then be established between fluid within the lesion and the oral cavity. The additional presence of fluoride will continue to ensure such concentration gradients remain in favour of remineralisation. A further pre-requisite for enamel lesion remineralisation is the presence of partially demineralised crystallites to act as nuclei for mineral deposition (ten Cate, 1990). Such mineral deposition may be complicated by the presence of organic material (Featherstone, 1984) and limited by concentration gradients between lesion fluid and the oral cavity resulting in preferential deposition of mineral within the surface layer (ten Cate, 1990). A knowledge of the effects of diet, salivary flow and fluoride on remineralisation should permit dentists to advise patients on better preventative strategies. Many of these factors are discussed further later in this Chapter.

1.6 FLUORIDE AND ENAMEL CARIES

1.6.1 Historical Account

The effects of fluoride on enamel were first noted by Eager (1902) who reported an acquired condition affecting the enamel of teeth in Naples, Italy.

He suggested this may be connected with the water supply as the incidence decreased when the supply was changed. Independently, McKay & Black (1916) reported a condition of enamel with an "opaque, paper-white appearance, mottled with normal spots and clouded areas". The condition only affected people who had been raised in the area of prevalence or who had spent time there during the period of enamel formation. The population studied was noted to have fewer carious cavities than elsewhere. Further studies by McKay & Black (1925) described other well defined geographical locations where such mottled enamel was endemic. Several initial theories were put forward but were shown to be unfounded. A connection between mottling and water was established in the town of Bauxite where the water supply had been changed some years previously. Churchill (1931) found high levels of fluoride here and suggested that this was the cause of enamel mottling. In the UK, Ainsworth (1933) described a similar condition affecting the town of Maldon in Essex. Once again he noted a reduced incidence of caries and found the water supply to contain between 4.5 and 5.5 ppm fluoride. The commissioning of Dean's studies (1933, 1934 and 1936) by the US Public Health Department established those areas with a water supply containing 1.0ppm fluoride had minimal mottling and a significantly lower caries prevalence.

1.6.2 Effect Of Fluoride On Caries *In Vivo*

Early *in vivo* research took place with the introduction of fluoride into the

water supply at a level of 1.0 ppm in the town of Grand Rapids, Michigan. Arnold *et al.* (1953) observed a 50 per cent reduction in caries incidence after six years. There followed a large amount of research on water fluoridation which showed a reduction in dental caries at 1.0 ppmF. Other methods of fluoride delivery were proposed and there is now voluminous literature on *in vivo* trials involving fluoride. There follows a brief overview of the recent literature on various fluoride delivery systems.

The effect of water fluoridation at an optimal 1.0 ppm fluoride has been discussed by Murray (1993) in a review of 113 studies in 23 countries. The modal caries reduction is quoted as 40-49% in primary teeth and 50-59% in permanent teeth. Differences in design of studies and diagnostic criteria may have affected the results obtained. The data presented confirmed the importance of continuous residence within a fluoridated area to achieve maximal benefit and demonstrated the important pre-eruptive effect of fluoride. Several studies were presented where water fluoridation had been terminated. In all cases, a subsequent increase in caries increment was noted (Stephen *et al.*, 1987a; Attwood & Blinkhorn, 1988).

The majority of fluoride in the Western World is consumed in the form of fluoridated dentifrices. König (1993) has suggested that water fluoridation can no longer be primarily responsible for the reduction in caries seen in the West. He argues the advent of increasing personal compliance as a result of increasing social desirability of a healthy mouth means more people are using

a fluoridated dentifrice. There has been no reduction in the cariogenic challenge in the West, yet caries prevalence is still decreasing, a fact that König ascribes as, "nearly entirely due to fluoride contained in toothpastes". Concentrations of fluoride in dentifrices are generally in the range 1000-1500 ppm fluoride. Stookey *et al.* (1993) discussed the difficulty of quantitating the effects of fluoridated toothpastes given the large number of fluoride sources affecting populations. However, mean data from 23 clinical trials using 1000-1200 ppm fluoride toothpastes show a reduction of between 24 and 44% compared with placebo dentifrices. Trials to determine the clinical effectiveness of different levels of fluoride in dentifrices have proved difficult to interpret due to differences in protocol (Konig, 1993). Research has been directed recently at the relative efficacy of different fluoride systems in dentifrices, specifically the sodium fluoride and sodium monofluorophosphate systems. Stookey *et al.*(1993) compared the relative effects of 20 sodium fluoride versus sodium monofluorophosphate head-to-head clinical trials. Meta-analysis of the results showed superior efficacy of the sodium fluoride systems in the region of 5 - 10% DMFS compared to monofluorophosphate systems. However, the consensus position statement from an international scientific assembly on the comparative anticaries efficacy of sodium fluoride and sodium monofluorophosphate dentifrices held at Guy's Hospital, London (Garcia-Godoy, 1993) stated the work by Stookey *et al.* (1993) "does not reflect an accurate view and should be called into question". Furthermore,"the assembly agreed that the published clinical studies available to date support the conclusion that sodium fluoride and sodium monofluorophosphate at

similar concentrations in commercially available dentifrices across the world provide equivalent anticaries effectiveness". The large amount of data studied and the complex nature of the statistical analysis would suggest any difference between sodium fluoride and sodium monoflurophosphate dentifrices was relatively difficult to determine. On an individual basis, and with a well motivated patient, the clinical efficacy of one fluoride formulation over another is probably not detectable.

Fluoride may also be supplied in the form of tablets, drops, professionally applied gels and varnishes, or mouthrinses. Stephen (1993) discussed variable reductions in caries increment from 20 to >80 per cent as a result of the use of fluoride drops and tablets. It was suggested that such variability in outcome represented a problem of compliance. Recommendations for use of fluoride tablets and drops in regions where the water supply contains less than 0.3 ppm fluoride included the use of a non fluoridated dentifrice for the first six years of life. The question of pre-natal fluoride was discussed and data from a recent study (Bawden quoting Leverett, 1992) suggest no significant benefit, in terms of caries reduction. Fluoride drops were of benefit to both primary and permanent dentitions with maximal benefit being derived if taken pre-eruptively.

Wei & Yiu (1993) have reviewed the literature on topical fluoride gels. For professionally applied gels there appears to be a reduction in caries experience of around 25%. Furthermore, there would appear to be a carry-over protective

effect from such products after their use. Fluoride-containing gels are also available for home use and contain lower amounts of fluoride in comparison with professionally applied gels. Literature is lacking as to the efficacy of such preparations.

Fluoride varnish and mouthrinses have been reviewed by Petersson (1993). Fluoride varnish contains high levels of fluoride (approximately 5%) and is said to supply fluoride more efficiently than other methods (Petersson, 1993). Varnishes inhibit enamel demineralisation very effectively and have resulted in a 50-70 per cent reduction in fissure caries with even higher values being reported for proximal surfaces (Petersson, 1993). The concept of a low potency, high frequency or a high potency, low frequency mouthwash was considered ideal (Council on Dental Therapeutics, 1975). The effectiveness of 0.05% and 0.2% NaF mouthwashes, on caries reduction, has been estimated, through clinical trials, at between 25 and 50 per cent. Other developments have included the incorporation of chlorhexidine into mouthwashes to reduce plaque levels (Luoma *et al.*, 1978).

Fluoride may also be used to supplement certain dietary components, notably salt and milk. Kunzel (1993) reported that fluoridation of salt, with sodium fluoride, at levels of between 250 and 350 mg/kg resulted in caries inhibition comparable to that of water fluoridation. Maximal benefit is derived when fluoridated salt is used from birth. Fluoridated milk, at levels of between 1.0 and 2.5 ppm fluoride, has also reduced caries incidence (Kunzel, 1993).

Problems with such trials have included the age groups used and inconsistent consumption. Children involved are often of school age and little pre-eruptive effect can be derived in such circumstances. Milk is only given on school days and this has caused problems in interpretation of the results.

Other fluoride strategies have included sugar fluoridation, on the basis that fluoride is provided at the time of demineralisation, as well as fluoridation of beverages and mineral water. Results indicate such strategies are difficult to evaluate because of the individual nature of consumption of such foods (Kunzel, 1993).

1.6.3 The Effect Of Fluoride On Caries *In Vitro*

In vivo research is often considered a "Gold Standard" in caries research, However, it is often easier to determine component parts of an overall effect seen *in vivo* with the use of an *in vitro* system. Many of the mechanisms by which fluoride exerts such effects have been elucidated with the use of *in vitro* systems. The advantages and disadvantages of *in vitro* models presented in Table 1.2 are also applicable to *in vitro* systems to determine the effects of fluoride on enamel caries.

In vitro research on the effects of fluoride on the caries process is possibly even more extensive than *in vivo* research. Any attempt at division of such work will inevitably prove to be artificial although it is perhaps useful to discuss the

results of *in vitro* research and how they have contributed to our understanding of the effects of fluoride on the caries process at various sites within the oral cavity. Such sites include:-

1 fluoride within the tooth

2 fluoride in solution including fluoride in lesion fluid, plaque fluid and saliva.

Fluoride incorporated within the enamel of the tooth has the effect of reducing enamel solubility in acid. Such an effect has been recognised by Brown *et al.* (1977) and ten Cate & Duijsters (1983) and was thought to be a way of increasing enamel resistance to dental caries. However, studies by Mellberg *et al.* (1985) and Reteif *et al.* (1987) failed to establish a strong link between enamel fluoride and caries susceptibility. Attempts to increase enamel resistance to caries through incorporation of fluoride were finally laid to rest when Øgaard *et al.* (1988) showed that carious lesions could be created in shark enamel which has a fluoride content in excess of 30,000 ppm. The lesions were, however, smaller than those created in human enamel under identical conditions. Furthermore, inhibition of demineralisation in human enamel on a similar scale to that found in shark enamel could be achieved using a daily 0.2% NaF rinse.

Recently, it has been suggested that the cariostatic effect of fluoride did not originate solely from fluoride incorporated into the enamel, rather the effect of fluoride in solution has an important role. Laboratory studies (Manly & Harrington, 1959; ten Cate & Duijsters, 1983a, 1983b) had shown that low concentrations of fluoride in solution could reduce the rate of enamel demineralisation. Margolis *et al.* (1986) reported that 0.024 ppm fluoride in an undersaturated solution could inhibit surface demineralisation in enamel, whereas a solution containing 1 ppm fluoride would inhibit all mineral loss. The relative importance of fluoride in solution was demonstrated by Wong *et al.*, (1987). They showed that surface adsorbed fluoride was more effective at reducing apatite dissolution than fluoride incorporated into the apatite lattice. Furthermore, fluoride in solution was more effective at reducing apatite dissolution than either surface adsorbed fluoride or fluoride incorporated into the lattice. This work was continued by Featherstone *et al.* (1990) who reported that inhibition of demineralisation of a synthetic carbonated apatite in acetate buffer was related to the logarithmic concentration of fluoride in solution. The same authors, using a pH cycling model on enamel lesions, showed that mineral loss from the lesions was a negative logarithmic function of the fluoride concentration in solution.

Fluoride in solution may be found in lesion fluid, plaque fluid and saliva, and these are now discussed. Levels of fluoride in each of these media are variable and may be subject to independent regulatory mechanisms.

Lesion fluid has been described by Larsen & Pearce (1992) as the solution in the pores of the body, dark zone and translucent zone of the lesion. It differs from that of fluid in the surface zone which may have characteristics of both lesion fluid and plaque fluid. The authors discussed lesion fluid with respect to movement of hydrogen ions in and out of the lesion and concluded that the hydrogen ion concentration within the lesion fluid reflects longer term changes in hydrogen ion concentration in plaque fluid and saliva. It is reasonable to argue a similar case for fluoride. Baseline levels within lesion fluid represent fluoride levels within enamel and changes are effected by longer term changes in fluoride concentration within plaque fluid and saliva rather than transient intraoral peaks. Fluoride has not been measured in lesion fluid directly, but work by Robinson *et al.* (1982) has demonstrated a 30 - 800% increase in the fluoride content of the body, dark and translucent zones of carious lesions compared with adjacent, sound enamel. Vogel *et al.* (1990) used a micro-drilling procedure on thin enamel sections in conjunction with an adapted fluoride electrode but failed to demonstrate significantly higher levels of fluoride within the lesion.

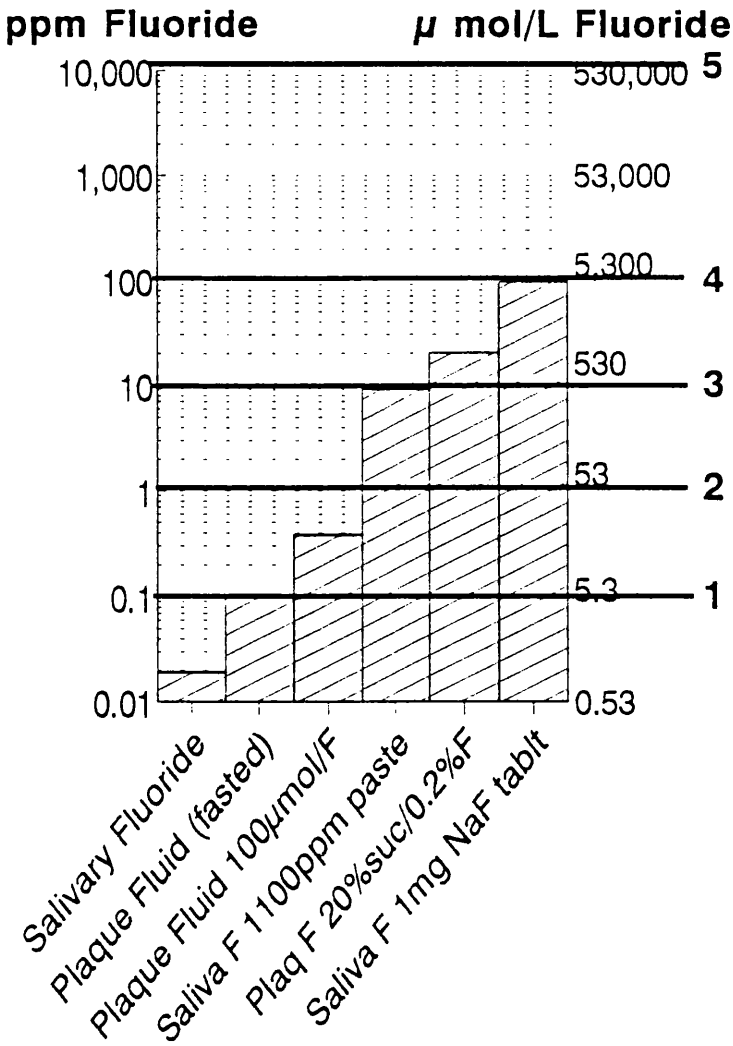
Tatevossian, (1990 and 1991) has reviewed several studies measuring plaque and salivary fluoride levels and makes some pertinent points in relation to mechanisms of fluoride action. Plaque and salivary fluoride levels are indicated in figure 1.1 which has been modified from the text of these papers. Resting plaque fluid fluoride ranges from 2-5 µmol/l. Such levels have been elevated to over 500 µmol/l by Oliveby *et al.* (1990) by rinsing with a 20%

sucrose solution containing 0.2% sodium fluoride. However, these high concentrations persist only for a few minutes. Tatevossian suggested the release of all plaque fluoride bound within the solid phase could theoretically yield a concentration of 1-2 mmol/l, although it is uncertain how much of this fluoride would be released during a fall in pH after fermentation of carbohydrate by plaque bacteria.

Salivary fluoride levels are somewhat lower than plaque. Resting salivary levels are about 1 µmol/l (Shannon, 1977; Gron *et al.*, 1968). Baseline salivary fluoride levels exhibit a dose response to fluoridated dentifrice (Jacobson *et al.*, 1990, 1992). Elevation of salivary fluoride levels by ingestion of a 5mg tablet of NaF did not raise levels above 5 µmol/l (Oliveby *et al.*, 1989). The use of a 1100ppm NaF dentifrice produced peak salivary levels of 0.48mmol/l which fell to 31µmol/l within 30 minutes. (Zero *et al.*, 1988). Duckworth & Gilbert (1992) discussed the complex patterns of fluoride clearance from saliva and concluded that clearance takes the form of a bi-phasic curve with the area under the curve denoting the measure of fluoride clearance in the mouth.

In summary, fluoride incorporated within the apatite lattice will reduce solubility but will have little effect on the inhibition of lesion formation in comparison with similar levels of fluoride in solution (Chow ,1990). The levels of fluoride in lesion fluid are probably representative of an average level of fluoride within the tooth and at the enamel/plaque interface, and are minimally affected by transient intra-oral peaks in fluoride concentration.

Levels of fluoride in plaque fluid are higher than those of saliva and both exhibit transient intra-oral peaks (Tatevossian, 1991). There is also an increased concentration of fluoride in plaque fluid with a fall in pH (Tatevossian, 1991), although, the extent to which this mechanism is of benefit in the inhibition of dental caries remains to be determined.



1 At this level there is reduced enamel solubility and enhanced rates of remineralisation (Margolis *et al.*, 1986; Thirabilok & Fegin, 1978).

2 At this level of fluoride there has been shown to be an effect on fissure shape and inhibition of dental caries. In addition the may be some inhibition of plaque acidogenesis (Aasenden & Peebles, 1974; Jenkins *et al.*, 1969) .

3 Fluoride substitutes for OH^- groups at this level, there is inhibition of plaque metabolism and acidogenesis (Weatherell & Robinson, 1988; Edgar *et al.*, 1970).

4 CaF_2 is formed and albumin is desorbed from enamel surfaces. Plaque formation is reduced (White & Nancollas, 1990; Rolla & Melsen, 1975; Tinanoff & Weeks, 1979).

5 Bacteria desorb from hydroxyapatite (Rolla & Melsen, 1979).

Figure 1.1 Histogram of levels of plaque and salivary fluoride recorded in the oral cavity

1.6.4 Mechanism Of Fluoride Action As A Cariostatic Agent

Having discussed some of the effects of fluoride on caries incidence and the levels of fluoride encountered within the oral cavity, it is relevant to discuss some of the mechanisms of fluoride action. It should be recognised that some mechanisms have only been demonstrated at particular concentrations of fluoride which may only exist as transient peaks rather than baseline levels of fluoride normally encountered in the oral cavity. This information has been succinctly imparted by Tatevossian (1991) and is shown in figure 1.1 which has been adapted from this reference.

Fluoride acts at several sites within the oral cavity. It inhibits demineralisation and promotes remineralisation by various means. Mechanisms of cariostatic action can be seen at the ultrastructural level of the enamel, within the liquid phases in the oral cavity and within dental plaque covering the teeth.

Fluoride can have significant effects on the ultrastructure of enamel at several levels. Nikiforuk (1985) discussed the stability that fluoride brings to the apatite lattice through substitution for hydroxyl groups . This promotes hydrogen bonding between fluoride and other hydroxyl groups as well as electrostatic interactions between fluoride and calcium (Weatherell & Robinson, 1988). Dispersion of small amounts of fluoride throughout the lattice has a large overall effect in terms of lattice stability (Moreno *et al.*, 1974) and crystallinity (Frazier *et al.*, 1967).

Distribution of fluoride within the enamel crystallite is speculative and information is derived indirectly through information on crystallite dissolution characteristics. Demineralisation of enamel crystallites begins at the centre of one end of the crystallite and proceeds **through** the crystallite core parallel to the c-axis (Arends & Jongebloed, 1979a) From this information, one can speculate higher levels of fluoride at the crystallite surface or higher levels of apatite impurities at the crystallite core. Some evidence for increased carbonate levels at the crystallite core is given by Nikiforuk (1985), Daclusi *et al.*, (1979) and Arends & Jongebloed (1979).

The formation of a fluoride rich layer at the surface of the enamel crystallite has been suggested by Brown *et al.* (1977). Arends *et al.* (1984) discussed different interactions of fluoride with the enamel crystallite surface. Those authors described the concept of non-specific adsorption where fluoride is preferentially concentrated in a hydration layer about the crystallite and does not exchange with components of the apatite surface. Such fluoride is attached by hydrogen bonding to hydroxyl groups of HPO_4^{2-} surface ions. Specifically adsorbed fluoride replaces -OH and HPO_4^{2-} groups at the apatite surface. It is thought that both types of surface adsorption will occur at the same time and that adsorbed fluoride can act as a local reservoir for aqueous fluoride in demineralisation and remineralisation cycles. It has been suggested by Arends & Christofferson (1990) that the level of adsorbed fluoride required for strong inhibition of enamel demineralisation *in vitro* correlates to a level of approximately 1ppm in the liquid phase. The authors have shown that areas

of the crystallite not covered with a fluoride rich layer are susceptible to demineralisation. However, such inhibition of demineralisation will be dependent upon the prevailing conditions.

The distribution of fluoride within the enamel prism may be deduced from crystallite sizes within the structure. Johnson (1967) showed increased size of enamel crystallites at the prism junction and suggested that this increase in crystallite size was as a result of remineralisation. This hypothesis has been confirmed by the work of Silverstone (1982).

Recent work has highlighted the role of CaF_2 as a source of ionic fluoride. Arends & Christofferson (1990) have calculated that levels of 10 ppm F in solution should be adequate to form CaF_2 in saliva, plaque, on teeth or within carious lesions. A globular CaF_2 material has been seen on tooth surfaces (Nelson *et al.*, 1983) and in carious lesions (Arends *et al.*, 1988). Such material is probably highly contaminated with phosphates and protein but is very slow to dissolve and may provide a long lasting source of ionic fluoride. However, it is not certain if this would be of sufficient magnitude to inhibit demineralisation.

Fluoride in solution in the oral cavity will promote apatite precipitation from solutions with reduced levels of saturation as well as inhibiting apatite dissolution at low pH and mineral saturation (Margolis *et al.*, 1986).

Two possibilities are proposed for the mechanism of fluoride in solution inhibiting demineralisation and promoting remineralisation:-

i Fluoride interacts with other species such as Ca^{2+} and HPO_4^{2-} ions, thereby buffering the levels of such ions in both lesion and plaque fluid and affecting concentration gradients such that net mineral transfer will be affected.

ii Fluoride acts as a buffer mopping up H^+ ions reducing the cariogenic challenge.

Margolis & Moreno (1990) have discussed the first proposal in relation to a caries model arguing that demineralisation is a function of the degree of saturation of a solution with respect to enamel. Combination of fluoride with Ca and P ions diffusing out of the enamel will increase local saturation with respect to fluoridated hydroxyapatite. This will accelerate mineral redeposition as fluoridated hydroxyapatite upon surfaces of exposed enamel crystallites within a partially demineralised enamel. The net movement of mineral in or out of the tooth is a result of the two simultaneously occurring mechanisms. The large reduction in enamel demineralisation seen with fluoride in acidic solutions could be a result of the ability of fluoride ions to bind hydrogen ions.

Fluoride in dental plaque exerts a cariostatic effect in two main ways. Firstly, by inhibiting plaque metabolism and acid production (Hamilton, 1990) and secondly, by acting as a storage site for fluoride (Tatevossian, 1990) which may inhibit demineralisation and promote remineralisation.

Bowden (1990) quoted plaque fluoride concentrations in the range 0.16 - 0.31 mol/l as having a lethal effect on streptococci *in vitro*. However, such levels of fluoride are rarely, if ever, encountered in the oral environment. Marsh & Bradshaw (1990) have shown that low levels of fluoride (1 mmol/l) will reduce the fall in pH of an *in vitro* mixed culture of bacteria in a chemostat pulsed at regular intervals with glucose. The stabilisation of pH changes results in modification of the composition of the bacterial community and a reduction in levels of acidogenic organisms. Van Loveren (1990) quoted several studies showing a reduction in the pH minimum of plaque exposed to fermentable carbohydrate in the presence of fluoride. Such effects may occur at concentrations as low as 1 ppm fluoride. These findings are supported by the work of Edgar *et al.* (1970) who demonstrated reduced pH minima in plaque with water fluoridation at 1.0 ppm.

Hamilton (1990) discussed the effects of fluoride on bacterial cell biochemistry, notably the inhibition of enolase within the glycolysis pathway and subsequent inhibition of sugar uptake by bacteria via the phosphoenoltransferase system. The maintenance of proton gradients across a bacterial cell membrane is important for cell viability including the uptake of sugar. Marquis (1990) showed fluoride dispersed effectively such proton gradients by inhibition of a bacterial cell membrane bound proton extruding ATP-ase.

Tatevossian (1990 and 1991) has observed that many of the mechanisms by which fluoride affects plaque acidogenicity have been demonstrated using

concentrations of fluoride far in excess of normal plaque levels. Attempts to augment plaque fluoride levels may achieve concentrations at which inhibition of plaque acidogenesis described will occur. Such concentrations are often transitory. Geddes & McNee (1982) argued to the contrary and demonstrated inhibition of plaque acid production for four to ten days after withdrawal of fluoride.

Further effects and mechanisms of fluoride inhibition of dental caries are being determined continually. In the light of our present knowledge, fluoride remains the single most important agent in reducing the prevalence of dental caries. The emergence of physiological mechanisms, whereby host protection from cariogenic factors can be increased is a major area of investigation. Of specific interest, is the field of salivary interactions with the caries process. As stated previously, the importance of saliva in relation to dental caries, has been highlighted. The role of saliva is discussed in greater detail in the next section.

1.7 THE EFFECT OF SALIVA ON ENAMEL CARIES

Saliva has been described as the very antithesis of a pure chemical compound (Nikiforuk, 1985). The ability of saliva to change its composition with increased flow rate, time of day and diet, further increases its complexity. The effects of saliva on enamel caries are profound when it is absent, for example, in radiation induced xerostomia. However, the effects of saliva on enamel caries within a wide range of 'normal' flow rates and chemical compositions are

often subtle and difficult to determine. The mechanisms by which saliva affects enamel caries are often complex and poorly understood. These effects and mechanisms will now be discussed.

1.7.1 Salivary Pellicle

The tooth erupts into an environment where there is a more or less constant flow of saliva. Contact between saliva and tooth surface will result in the rapid formation of salivary pellicle. Hay (1990) described this as a 0.1-1.0 μm thick layer adsorbed onto the dental enamel surface comprising selective hydroxyapatite reactive and other salivary proteins. Eggen *et al.* (1984) quoting Rolla, stated that basic and acidic proteins will adsorb to both exposed tooth surface phosphate groups and calcium ions *in vitro*. However, this remains to be shown for salivary proteins *in vivo*. Eggen *et al.* (1984) showed that pellicle is comprised of immunoglobulins, lysozyme, amylase, albumin and glucosyltransferase. Furthermore, there was a high proportion of phosphoprotein within pellicle which has a high affinity for calcium at the hydroxyapatite surface.

The functions of the salivary pellicle have been suggested by Eggen *et al.* (1984), Marsh & Martin (1984) and Featherstone (1990). They include the ability to affect bacterial colonisation of the tooth surface, protection of the tooth surface against bacterial products, inhibition of mineral loss from the tooth

surface, reduction of friction between the tooth surface and the soft tissue of the oral cavity and, as a possible matrix for remineralisation. Kautsky & Featherstone (1993) have shown an inhibition of carbonated apatite dissolution under an acetic acid challenge after pre-treatment by coating with saliva for seven days. Such inhibition was ascribed to pellicle formation which was analyzed by a combination of thin-layer chromatography and protein quantification. Kautsky and Featherstone (1993) suggested the inhibition observed was due to the limitation of ion diffusion across the pellicle layer and the selective binding of oral bacteria to charged groups on the outermost layer of the pellicle.

Once the pellicle layer is established at the tooth surface, saliva must then minimise the harmful effects of plaque to inhibit demineralisation and encourage remineralisation effectively. Both biochemical and physical characteristics of saliva contribute to this goal. It is often the case that the physical characteristics of saliva modify the chemical effects.

1.7.2 Chemical Characteristics Of Saliva In Relation To Enamel Caries

Saliva is not simply a solution clearing plaque of metabolites, tooth mineral and fluoride. It is a complex mixture of organic and inorganic constituents able to interact and change according to environmental conditions prevailing within the oral cavity. Dawes (1993) identified fifteen different factors which may influence the composition of human whole saliva. Many chemical constituents

of saliva have an effect on enamel caries and some of these will now be discussed.

The buffering capacity of saliva has been defined by Jenkins (1978) as, "the power to resist changes in pH when acid or alkali is added". The buffering capacity of saliva is flow rate dependent (Dawes, 1974), being increased at higher flow rates. The buffering capacity of saliva correlates well with caries incidence (Muracciole 1955; Agus & Schamschula, 1983; Pienikakkinen *et al.*, 1985). Ericsson (1959) stated that, "buffering capacity has the best established connection with caries". This conclusion was drawn from the analysis of many studies of buffering capacity and caries experience. Larmas (1993) suggested that salivary buffering capacity is one of the best indicators of caries susceptibility because it reveals the host response. Patients with a high salivary buffering capacity have a low incidence of caries because of a good host response which can compensate for caries-active habits. A low buffering capacity is indicative of a reduced salivary flow rate and a reduced host response to cariogenic agents. Salivary buffering capacity has been shown to vary throughout the day. Ericsson (1959) showed a progressive increase throughout the day until early evening with a notable increase after meals. This was in agreement with the work of Krasse (1961).

The ability of saliva to neutralise pH is of only limited importance if it is not able to influence the pH at the plaque/enamel interface. Englander *et al.* (1959) measured pH changes and lactic acid concentrations in plaque on the buccal

surfaces of upper molar teeth over a 20 min period following exposure to a sucrose solution. Initial readings were taken with saliva flowing over the plaque. The experiment was repeated and saliva was excluded by cannulating the parotid duct orifices and ensuring good aspiration of saliva from the floor of the mouth. Results showed a steeper and more prolonged pH drop and raised lactate levels when saliva was excluded.

There are two main buffering systems in saliva. These are the bicarbonate system and the phosphate system. The bicarbonate buffering system provides the bulk of salivary buffering capacity (Lilienthal, 1955) and behaves according to the equation below:-

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

The conversion of HCO_3^- to H_2CO_3 has a pK value of approximately 6.1 (Nikiforuk, 1985) and provides maximal buffering at the average ionic strength of saliva (Ericsson, 1959).

Carbon dioxide (CO_2) is lost from saliva as it exits from the salivary duct. This is as a result of a concentration gradient established between the concentration of CO_2 in ductal saliva and a lower concentration of CO_2 in saliva in the oral cavity that has been exposed to air. Ductal saliva has a pCO_2 of approximately 46 mmHg and saliva in the mouth or exposed to air has a pCO_2 of 0.3 mmHg (Nikiforuk, 1985). Such a loss of CO_2 will result in a reduction in the

concentration of hydrogen ions. This phenomenon has been called "phase buffering". The presence of carbonic anhydrase in saliva catalyses the reaction $H_2CO_3 \rightarrow CO_2 + H_2O$ and will reduce further the concentration of hydrogen ions. Dawes (1969 and 1974) showed that bicarbonate concentration increases in parotid and submandibular saliva with an increase in flow rate. Furthermore, levels of bicarbonate are elevated as long as salivary stimulation persists (Dawes & Macpherson, 1992).

Lilienthal (1955) showed that phosphate has reduced ability to buffer saliva in comparison with bicarbonate. This was shown in a series of experiments where bicarbonate was removed leaving phosphate and protein as buffering systems. Removal of the phosphate by dialysis of saliva left only the protein which had no buffering effect. The phosphate buffer system has been suggested as:-

$$PO_4^{3-} + 3H^+ \rightleftharpoons HPO_4^{2-} + 2H^+ \rightleftharpoons H_2PO_4^- \rightleftharpoons H_3PO_4$$

Other methods whereby saliva can counteract a fall in pH include the production of ammonia and decarboxylation reactions. Curtis & Kemp (1983) and Cole & Eastoe (1988) discussed the production of ammonia by bacterial metabolism of salivary urea. Ammonia produced at low pH values will counteract the effect of the acid, raising the plaque pH. In addition the decarboxylation of amino acids, to produce carbon dioxide, will also raise plaque pH by mopping up protons. The effect of simple dilution on plaque pH is difficult to study as saliva has such a complex buffering capacity but as pH

follows a logarithmic scale it is anticipated that large dilutions would be required for relatively small effect. The fact that saliva flows over the oral tissues may have greater effect on chemical concentration gradients between plaque and saliva than a static fluid (Dawes, 1983). This is addressed in greater detail later in this Chapter.

Saliva is generally supersaturated with respect to enamel mineral (Gron, 1973). This also plays a key role in the inhibition of enamel demineralisation and in the promotion of remineralisation. The degree of saturation of saliva with respect to enamel mineral is dependent upon the activity of calcium and inorganic phosphate which are, in part, a function of the ionic strength and pH of the saliva (Vogel *et al.*, 1965). The ionic strength and pH of saliva differs between individuals and with changes in flow rate (Vogel *et al.*, 1965). Lagerlof (1983) assessed the effects of flow rate and pH on calcium phosphate saturation in human parotid saliva. He found an increase in saturation with respect to hydroxyapatite, tricalcium phosphate and octacalcium phosphate with increasing flow rates up to 2.0 ml/min. Parotid saliva remained undersaturated or just saturated with respect to dicalcium phosphate dihydrate at all flow rates assessed. At a constant flow rate, the pH was adjusted and saturation with respect to calcium phosphate compounds re-assessed. Saliva became undersaturated with respect to dicalcium phosphate dihydrate at pH=8.0, octacalcium phosphate at pH=6.75, tricalcium phosphate at pH=6.25 and with respect to hydroxyapatite at pH=5.5. In contrast, ionic calcium levels increased as a result of calcium phosphate dissociation.

The presence of a supersaturated solution would be expected to result in precipitation of calcium phosphate salts within the mouth. This does not occur because of the presence of salivary proteins which inhibit precipitation. Hay & Moreno (1989) discussed the curious nature of salivary supersaturation in terms of its ability to remineralise a carious lesion but to cease mineral deposition at a certain point. Furthermore, mineral precipitation does not normally occur in salivary gland acini or ducts and teeth do not increase in size through mineral acquisition. Speculation was made, therefore, of a mineral precipitation inhibitor.

Analysis of protein fractions of saliva has revealed two types of protein inhibitors of mineral precipitation. These have been identified as statherin and, a family of up to eight proteins with a high degree of charge asymmetry called, the proline rich proteins (Hay, 1990). Statherin is a small tyrosine-rich protein. It has a highly charged amino terminal with its active component located in the first six residues (Hay & Moreno 1989). The actions of these proteins include the ability to inhibit mineral precipitation *de novo* within the salivary gland system; so called primary precipitation, and mineral precipitation inhibition by binding to calcium phosphate crystal surfaces; so called secondary precipitation (Hay, 1990). The ability to bind to the surface of enamel and yet probably not penetrate into the lesion (Hay 1990) would suggest their role as coordinators of mineral deposition within the remineralising carious lesion.

1.7.3 Physical Characteristics Of Saliva In Relation To Enamel Caries

The physical characteristics of saliva such as volume of saliva produced, rate of salivary flow and degree of salivary stimulation will affect the chemical composition of saliva (Dawes, 1969, 1974 and 1975). In addition, salivary flow throughout the oral cavity varies with site (Dawes *et al.*, 1989). Saliva production has been estimated at 500-620 ml per day (Jenkins, 1978). However, this does not represent a constant flow over a 24hr day but rather a circadian rhythm with periods of high flow rate in the early afternoon and evening (Dawes, 1975) and periods of negligible flow rate during sleep (Schneyer *et al.*, 1956). Furthermore, the relative contribution of different salivary glands to whole saliva differs between resting and stimulated conditions (Izutsu, 1987). The relationship between salivary flow rate and caries experience is difficult to determine. Mandel (1974), reporting on the study of Shannon & Terry (1965) , suggested a difference of 0.005 ml/min in resting parotid flow rate between low and high DMF groups. The clinical significance of this is debatable; however, a change in salivary flow rate on an individual level is often related to increased caries experience.

Objective xerostomia occurs as a result of hyposalivation. Such a condition may arise as a result of salivary gland aplasia, removal, or degeneration or as a result of systemic medication or imbalance. The effects of these conditions on dental caries have been described by Newbrun (1989). They include an alteration in the amount and composition of the dental plaque resulting in

increased numbers of *Streptococcus mutans* and*Lactobacilli.* The pattern of attack is somewhat unusual as caries begins at the cervical margin resulting in widespread destruction of tissue and weakening of tooth structure. Dreizen *et al.* (1977) showed a large increase in caries experience following radiotherapy to the head and neck involving the salivary glands and Mason and Chisholm (1975) have described an increase in caries levels amongst patients with Sjogrens Syndrome.

The volume of saliva in the oral cavity is not constant. It varies before and after a swallow and is further affected by the rate of swallowing. Dawes (1983) developed a mathematical model to determine salivary clearance of sucrose from the oral cavity. This model assumes the mouth to be an incomplete syphon such that each time an individual swallows there will be a finite quantity of saliva retained in the mouth. Attempts have been made by Lagerlof & Dawes (1984) to quantify the volume of saliva in the mouth before and after a swallow by determining the change in concentration of potassium or chloride ions in samples of distilled water swished around the mouth. The volume of saliva in the mouth before swallowing was found to be approximately 1.0 ml, whilst the volume after a swallow was approximately 0.7 ml. An increase in flow rate gave rise to an increase in the rate of swallowing and an increase in the volume of a swallow (Lagerlof & Dawes, 1984).

The distribution of saliva in the oral cavity has implications for both the

establishment and progression of enamel caries. Salivary distribution has been addressed by Collins & Dawes (1987). Saliva forms a thin film covering the surfaces of the oral cavity. Measurements of the surface area of the mouth in conjunction with the average volumes of saliva before and after a swallow suggest a film thickness of between 0.07 and 0.1 mm. Furthermore, Dawes *et al.*, (1989b) have shown that such a film moves at different speeds in different parts of the mouth. Rapid film movement may be observed in the lower anterior lingual region, whereas slow film movement is observed in the upper anterior labial region. The direction of salivary flow would appear to be directed to the posterior of the mouth (Weatherell *et al.*, 1984; Lecomte & Dawes, 1987).

The distribution and movement of saliva around the oral cavity has implications for clearance of bacterial substrate, plaque accumulation, local mineral saturation and fluoride levels. A variation of distribution and retention of fluoride in saliva in different areas of the mouth has been demonstrated when NaF tablets and NaF mouth-rinses were used (Weatherell *et al.*, 1984, 1986; Primosch *et al.*, 1986). Similar patterns of retention are seen for glucose and sucrose (Dawes & Weatherell, 1990; Dawes & Macpherson, 1993). The differential patterns of fluoride distribution did not show a tendency to disappear after two hours but it is not known what happens after this period. The upper labial sulcus seemed to be a region of prolonged fluoride retention which correlates with the rate of flow of the salivary film in this area (Dawes *et al.*, 1989). Dawes & Macpherson (1993) have taken this work a stage further

by assessing the distribution of sucrose and saliva around the mouth whilst chewing gum. They found a very uneven pattern of sucrose distribution around the mouth. Low sucrose levels were generally found in regions of high film velocity and *vice versa*. The mathematical model of Dawes (1983) identified the unstimulated salivary flow rate and the volume of saliva immediately before and after a swallow to be the most important parameters in determining the clearance of non-adherent food from the oral cavity. It is unknown if adherent food will clear in the same manner.

Saliva rarely flows over a denuded enamel surface, rather there is a layer of salivary pellicle or plaque. Ions diffuse back and forth between plaque and saliva largely as a result of concentration gradients. Lecomte & Dawes (1987) used an artificial plaque matrix to show that diffusion of potassium chloride from the artificial plaque varied at different sites within the mouth. Rapid loss of KCl was seen in the lower, anterior lingual and upper, posterior buccal regions and much slower loss of KCl from the upper and lower, anterior buccal regions. Stimulation of salivary flow reduced half-times for KCl clearance by 50 per cent. Dawes (1989) discussed the factors that influence the diffusion from dental plaque into a moving film of saliva. The rate of clearance of diffusant from a dental plaque of fixed thickness, area and composition will be dependent upon the velocity of movement of a salivary film over its surface. A reduction in the film velocity results in an exponential reduction in the clearance half time. Dawes (1989) also stated that, given two plaque samples of identical area, depth and composition, the plaque with the slowest

overlying film velocity will be the more cariogenic. However, such a reduction in clearance time has beneficial effects as fluoride retention and calcium and phosphate saturation will be prolonged.

The modifying effect of saliva on the local environment of the tooth makes it very difficult to determine the relative effects of remineralising factors. For example, does the ability of saliva to clear bacterial substrate rapidly from the local environment of an incipient carious lesion, have as much effect on lesion remineralisation as the use of a fluoridated dentifrice? It is one of the aims of this work to determine the ability of stimulated saliva to remineralise artificial caries in a non-fluoridated environment and to compare this to a fluoridated environment without salivary stimulation.

The effects of saliva on enamel caries have so far been described in terms of its ability to form a protective pellicle layer over the tooth, its ability to clear bacterial substrate from dental plaque covering the surface of the tooth, its ability to resist changes in intra-oral pH and its ability to maintain a supersaturated state with respect to enamel mineral. Many other actions of saliva in relation to enamel caries have been determined including specific effects of saliva upon plaque ecology and metabolism. Although, the most profound effects of saliva on enamel caries are seen in its absence, attempts have been made to augment the beneficial role of saliva in the reduction of dental caries.

1.7.4 Augmentation Of The Role Of Saliva In The Inhibition Of Enamel Caries

Increasing the role of saliva in the reduction of caries incidence is not a new concept. It may take the form of either a modified chemical composition of saliva or increasing the amount of saliva in the oral cavity thereby promoting any effect on hard tissue. Attempts to modify the chemical composition of saliva have included an increase in the fluoride content. Fluoride in saliva may be increased above baseline levels with fluoridation of the water supply (Bruun & Thylstrup, 1984; Oliveby *et al.*, 1990a). Jacobsen *et al.* (1990,1992) have demonstrated a dose response increase in baseline fluoride levels in saliva with the use of fluoridated dentifrice. Tatevossian (1991) however, suggested large increases in fluoride levels are often transient and may have only a limited effect. Much work has involved increasing salivary flow rate, and thereby increasing buffering capacity, rather than any artificial augmentation of salivary composition. An effective method of increasing salivary flow and buffering capacity has been through the use of chewing gum (Shannon & Frome, 1973).

The use of gum to stimulate salivary flow rate potentially has both beneficial and detrimental effects (Edgar & Geddes, 1990). Detrimental effects arise largely as a result of the incorporation of sucrose into chewing gum. This has proved difficult to substantiate fully as many studies using sucrose-containing gum are fundamentally flawed in their design due to lack of a negative, (no

gum), control (Finn & Jamison, 1967; Wilson, 1975), or the use of a protocol making comparisons with other studies difficult (Richardson *et al.*, 1972; Makinen *et al.*, 1993). Edgar & Geddes (1990) reviewed five clinical trials which used a sucrose-containing gum. Three trials failed to demonstrate an increased caries incidence with the use of sucrose-containing gum and two trials showed an increase of between 24 and 36 %. Difficulties encountered in comparing these clinical trials included the variable use of a fluoridated background as well as difficulties in consistency of caries diagnosis between trials. The result of such uncertainty as to the effects of sucrose-containing gum chewing on dental caries has prompted investigators to use an intra-oral model in an effort to study such effects in a more controlled environment (Jensen & Wefel, 1989, Creanor *et al.*, 1992; Hall *et al.*, 1993). The results of this work are presented in Chapter 3. Other potential detrimental effects of gum chewing, such as temperomandibular joint pain, have not been reported in studies assessing the effects of gum chewing on dental caries.

Potential beneficial effects of gum chewing include an increase in salivary flow and buffering capacity. Dawes & Macpherson (1992) showed that gum chewing could increase the stimulated flow rate of saliva by a factor of between ten and twelve compared to unstimulated flow rates. Although stimulated flow rate declines with time, it was still approximately three times higher than the unstimulated rate after 20 min. Both sucrose-containing and sucrose-free gums elevated salivary pH to a level significantly greater than that of unstimulated saliva due to increased levels of bicarbonate production.

Jensen & Wefel (1989) assessed the effect of gum-chewing on the pH of dental plaque after a meal. They showed that chewing both sorbitol and sucrose-containing gum for 20 min after a meal would raise the pH of interproximal plaque to a level considered "safe for teeth". Furthermore, the pH remained at this level for over two hours after gum chewing. The effect of non-sucrose containing chewing gum on dental caries is also equivocal and once again difficulties in interpretation of results arise due to variable protocols used to demonstrate this effect. Edgar & Geddes (1990) state that ,"... it would appear that sugar-free gums are (at least) non-cariogenic. However, the suggestion that they inhibit caries requires careful evaluation..."

The difficulty of undertaking a clinical trial to demonstrate the effects of gum chewing on dental caries incidence and the mechanisms of how such effects may arise has persuaded many investigators to use intra-oral models to observe the interaction between stimulated salivary flow, through gum-chewing, and dental caries.

1.8 INTRA-ORAL MODELS FOR THE STUDY OF DENTAL CARIES

Proskin (1992) describes a model as, "A process which simulates, or relates to, some real-world phenomenon of interest; by studying the model, the researcher hopes to derive information about that real world phenomenon." The study of the caries process or actual caries within the oral environment constitutes the basis of the intra-oral model.

1.8.1 Rationale For The Use Of An Intra-Oral Model

The use of a human intra-oral model for the study of dental caries has been advocated by several authors (White, 1992; Stephen *et al.*, 1992; Manning & Edgar, 1992). It is suggested that intra-oral models provide an essential intermediate step between *in vitro* tests, animal studies and clinical trials (Manning & Edgar 1992; Mellberg, 1992). The major advantage of an intra-oral model is the ability to accurately analyze quantitatively mineral changes with the result that the sensitivity of the test is greatly increased. In addition, intra-oral models have the capacity to limit the number of variable conditions which may exist in a clinical trial. For example, strict criteria may be enforced in the choice of specimen for use with an intra-oral model. In addition, dietary control may be more strictly enforced and protocol of test product use may be more rigidly controlled. The ability to repeat and calibrate intra-oral model experiments as well as the use of a crossover trial design are features seldom seen in clinical trials. Mellberg (1992) discussed the value of a natural environment, with its own innate caries inhibiting or promoting effects, superimposed on the effects of any protocol used in a trial with an intra-oral model. Certain ethical problems encountered in a clinical trial may be overcome by the use of an intra-oral model (Manning & Edgar, 1992). Finally, the use of an intra-oral model may have significant advantages over the costing and timing of a clinical trial by prediction of a trial outcome or correlation with previous trial results (White, 1992; Stephen *et al.*, 1992).

1.8.2 Categories Of Intra-oral Model

Ogaard & Rolla (1992) discussed two categories of intra-oral models. These are the *in vivo* intra-oral model and the *in situ* intra-oral model. *In vivo* models involve the study of caries on vital teeth with an intact pulpodentinal complex and a natural covering pellicle. *In situ* models utilise test samples of differing composition, origin and degree of pre-treatment, mounted in a device designed to retain them within the oral cavity.

1.8.3 Evolution Of Intra-Oral Models To Study Dental Caries

The first reported case of an intra-oral model to study dental caries was that of Bunting *et al.* (1926) who attached a gold cup containing a mixture of *B. acidophillus* culture and bread to the surface of a tooth. The culture and bread were replaced at regular intervals for a period of between a week and ten days when the enamel appeared to become white or frank cavitation took place. Subsequent experimental work using intra-oral models was undertaken by Nygard-Ostby *et al.* (1957) and by Von der Fehr (1967). Both of these authors used a gold plate attached to the buccal surface of a premolar tooth scheduled for orthodontic extraction. The resultant gap between the gold plate and the tooth surface permitted the collection of plaque and food debris. Subsequent extraction of the teeth after periods of between four and six weeks permitted analysis of the tooth surface for early signs of decalcification. Hals & Simonsen (1972) modified this model by using an orthodontic band with two pieces of

wire to hold the band away from the tooth, creating a space for the accumulation of plaque and food debris. The advantage of their *in vivo* model over other models was the use of subjects in a caries susceptible age group (Wefel, 1990), whereas, other types of intraoral model have used older subjects. However, the requirement for teeth scheduled for extraction is a major disadvantage. In addition, this model has reduced sensitivity as different teeth are used for test and control. The use of more readily available specimens or specimens which could act as their own control (von der Fehr *et al.*, 1970) would have been a significant advantage.

Kouluorides & Volker (1964) were amongst the first to use specimens of enamel and dentine held in a removable appliance within the oral cavity. Since that time many different types of *in situ* appliance have been developed. *In situ* appliances fall into two main categories, dependent upon the method of specimen preparation (Wefel, 1990): (a) those in which the specimens are in the form of a block, as in the studies of Koulourides (1992) and Dijkman *et al.* (1986), or (b) specimens in the form of a thin section, as used in the studies of Creanor *et al.* (1986b) and Wefel *et al.* (1987) . The different analytical procedures which can be performed in each case are dependent upon the type of specimen used. Differences between the two types of specimen are discussed later.

1.8.4 Components Of An Intra-Oral Model

Intra-oral models all have a common set of components, which in their broadest terms, comprise the specimen, a method by which the specimen can be retained at an appropriate site in the oral cavity, and the presence of environmental factors such as plaque, saliva and other physiological factors in the oral environment. Furthermore, different analytical techniques will contribute to the overall outcome. Each of these components has the capacity to affect the validity and repeatability of any experimental result. These components are discussed in further detail.

Many different types of specimen materials have been used in intra-oral models. These have included human enamel, dentine and cementum, bovine, ovine and primate dental hard tissues and synthetic hydroxyapatite (Featherstone & Melberg, 1981; ten Cate *et al.*, 1992). In addition, specimens may have natural surfaces, abraded surfaces or be pre-treated in some way such as by creation of artificial carious lesions. Finally specimens may be used in the form of blocks of material or thin sections (ten Cate *et al.*, 1992). For the purposes of the following discussion reference will only be made to enamel.

Mellberg (1992) suggested that human enamel should be considered the substrate of choice for the study of human enamel caries because of its near identical composition to the substrate it is intended to mimic. Human teeth may be used *in vivo* where they posses their original surface, intact pellicle and

natural position within the oral cavity, or *in situ* which may compensate for the lack of availability of vital teeth as well as reduced control over lesion reproducibility and, often an inability to repeat *in vivo* experimental work in the same subject. There are, however, potential disadvantages of using human enamel. These are also discussed by Mellerg (1992), and include difficulty in obtaining adequate quantity and quality of material, a variable age and composition of human enamel, which leads to large variations in treatment response, and specimens which seldom have a large flat surface required for ease of analysis. Enamel surfaces used in *in vivo* experiments are usually premolar teeth scheduled for orthodontic extraction (Holmen & Thylstrup, 1986; Artun & Thylstrup, 1986; Holmen *et al.*, 1987).

Bovine enamel has also been suggested as a suitable specimen for use in intra-oral models and a suitable alternative to human enamel (Mellberg, 1992). The advantages of this substrate include a large flat surface for analysis, lower fluoride concentrations compared with human enamel (Mellberg & Loertscher, 1974), no prior caries challenges, which may affect subsequent mineral changes and an increased porosity which results in an increased rate of lesion formation (Featherstone & Mellberg, 1981; Edmunds *et al.*, 1988).

Many *in situ* models use enamel from extracted teeth. The nature of the surface of the enamel may affect the overall result of the experiment (ten Cate *et al.*, 1992). Enamel used in *in situ* experiments may retain its natural surface with, or without, a natural carious lesion. Alternatively an artificial carious

lesion may be created on a natural enamel surface. The enamel surface may also be abraded and subsequently used with, or without, an artificial carious lesion.

The use of a natural carious lesions in *in situ* studies has not been reported. It is speculated that such lesions are more difficult to obtain than samples of sound enamel and that the natural history of the lesion may be almost impossible to elucidate. The creation of artificial lesions in teeth with natural surfaces also presents potential problems. Variation in response to lesion creation, in enamel, has been reported by Creanor *et al.* (1989) who demonstrated that enamel near the cervical margin of the tooth demineralised at a faster rate than enamel towards the cusp of the tooth. In addition the method of artificial lesion creation affects the extent of demineralisation (Strang *et al.*, 1988; ten Cate *et al.*, 1992) and subsequent remineralisation (ten Cate *et al.*, 1992). Lesions which exhibit a large initial loss of mineral have been reported to have a greater capacity for remineralisation (Strang *et al.*, 1987). In order to produce more consistent lesions, the enamel surface may be abraded prior to placement in the oral cavity. This also ensures a flat surface to facilitate subsequent analysis (Arends *et al.*, 1982). Abrasion of the enamel surface results in the loss of any advantage the natural surface might afford (Mellberg, 1992), although Stookey (1992) recommends enamel surfaces be abraded to eliminate the impact of any prior exposure to fluoride.

Enamel specimens for use in *in situ* experiments may take the form of enamel

blocks (Koulourides *et al.*, 1974; Brudevold *et al.*, 1984; Leach *et al.*, 1989) or single sections (Harvey *et al.*, 1982; Creanor *et al.*, 1986a and b). The use of a single section technique was designed to overcome the variability between different sections cut from the same tooth or even the same lesion (Creanor, 1987). Each single section can be analyzed at both the start and end of the experiment and will, therefore, act as its own control (Harvey *et al.*, 1982), providing a technique that is more sensitive to changes in mineral content than the block technique (Strang *et al.*, 1988). The rate at which demineralisation and remineralisation occurs using this technique was the subject of some controversy. Ten Cate *et al.* (1986) showed the rate of demineralisation to be faster in single sections compared with blocks of enamel. Strang *et al.* (1988) showed that demineralisation occurred at the same rate in both blocks and single sections and attributed the difference between the two studies to differences in section preparation. The sections in the earlier study by ten Cate *et al.* (1986) were cut to a thickness of 100 μm resulting in significant crack propagation within the specimen. Those sections in the later study of Strang *et al.*, were cut to a thickness of 250 μm and hand lapped to 120 μm resulting in reduced number of cracks in the specimens.

It has been established that enamel specimens can be used in *in situ* studies in many different forms. However, the method of retention of enamel specimens in the oral cavity is also important. This has become more important as research workers are now aware that the method of specimen retention dictates the site of retention which may exhibit profound differences in local

environmental effects from one site to another (Dawes & Macpherson, 1993). There are several methods by which enamel specimens may be retained in the oral cavity. These may be broadly divided into retention using a removable appliance and retention by means of a fixed prosthesis.

Koulourides & Chien (1992) mounted blocks of enamel in recesses on the buccal aspect of a Kennedy Class I lower partial denture, whereas, Dijkman *et al.* (1986) mounted blocks in recesses of the flanges of complete dentures. Placement of blocks of enamel in areas of buccal surface caries may not be representative of interproximal caries where, it would be anticipated, that plaque formation and salivary clearance would be substantially different. In an effort to place specimens at a more appropriate site, Stookey *et al.* (1985) used a lower partial denture similar to that of Koulourides *et al.* (1974), but mounted the specimen to form a contact point relationship with the distal aspect of the last standing tooth. Creanor *et al.* (1986b) designed a removable appliance to be worn by subjects with a complete natural dentition, retaining multiple enamel specimens as single sections, mounted in a contact point relationship in a recessed trough in the lingual sulcus. However, in the natural situation this is a site of particularly high salivary film velocity (Dawes & Macpherson 1993b) which may have some bearing on the results from this model. Both Brudevold *et al.* (1984) and Ogaard & Rolla (1992) have used a removable palatal appliance similar in design to a Hawley type retainer. Blocks of test specimens are mounted in the appliance, in some cases with an artificial plaque. The appliance is placed in the oral cavity for only a few hours whilst

demineralisation takes place. It is difficult to determine the role of the oral cavity in this model, other than providing a convenient place for bacterial incubation and a container in which to expose the artificial plaque to various substrates.

Fixed prostheses have been used by several investigators to hold enamel sections or blocks. Wefel *et al.* (1987) used a cast fixed prosthesis to retain a specimen at either a buccal or interproximal site. Slight modifications on this basic theme have been proposed by Leach *et al.* (1989), who used a cast band around the tooth, retaining the specimen on the buccal aspect. Slater *et al.* (1986) used temporary acrylic crowns to retain specimens. Recently, Manning & Edgar (1992) bonded an orthodontic bracket with wire loops to retain sections of specimen on the buccal surfaces of molar teeth.

It has been mentioned that the method of retention dictates the site at which the specimen is placed in the oral cavity and therefore, the local environment to which the specimen is exposed. Important factors within the local environment include the surrounding dental plaque, saliva and dietary factors, such as the amount and frequency of sucrose consumption as well as the use of fluoride. In addition, the length of time a specimen is placed in the oral cavity will also determine the amount of de- or remineralisation. The local effects of saliva have been previously described (section 1.6.3) and now discussion will concentrate on plaque, diet and time as environmental factors.

Mellberg (1992) suggested the presence, location and thickness of plaque are

key factors to be considered in intra-oral models for the study of dental caries. The thickness of plaque overlying enamel has been shown to be related to the degree of underlying demineralisation (Mellberg *et al.*, 1990). The location of an enamel specimen will determine the presence and thickness of any covering plaque. It has been suggested that smooth surface and interproximal plaque have different diffusion characteristics and that this would account for the results of a study by Kotsanos (1986) which showed a reduction of approximately 50% in the rate of enamel demineralisation at interproximal sites compared with smooth surfaces. Several methods have been devised to encourage the accumulation of plaque and these are briefly discussed.

Coverage of the specimen with Dacron gauze or steel mesh has been advocated by some investigators (Mellberg *et al.*, 1986; Koulourides & Chien, 1992) to promote plaque accumulation. The use of a recess to house a specimen as proposed by Dijkman *et al.* (1986) has been found to promote a plaque overgrowth of variable thickness resulting in significant differences in demineralisation and remineralisation at central and peripheral parts of the enamel slab (ten Cate *et al.*, 1992). Creanor *et al.* (1986a) have used a trough on the lingual aspect of a lower removable appliance to house single section specimens and to encourage the growth of plaque. Studies by Creanor *et al.* (1986) and Macpherson *et al.* (1990) have shown the plaque within the trough to have quantitatively similar flora to that in the lingual and interproximal areas of the lower molar teeth. Furthermore, acid/anion profiles of plaque from both sites were found to be similar. It is worth noting that those studies

using edentulous subjects will most probably have a different plaque composition to dentate subjects (Melville & Russell, 1981) which will probably affect the rate of de- or remineralisation. Zero *et al.* (1992) used a synthetic plaque made from a culture of *Strep. mutans* Ingbritt-1600 and placed in direct contact with enamel slabs prior to placement in a palatal appliance within the oral cavity. It has been suggested by the authors that such a culture of bacteria placed under a block of enamel would simulate a local environment similar to that found in an interproximal embrasure or deep within a pit or fissure. However a single strain of bacteria would not normally constitute interproximal plaque.

Few investigators have addressed the problem of diet in relation to intra-oral models. Creanor *et al.* (1992) attempted to standardize diet in two related studies by asking subjects to record their food intake for the first week of an experimental test. The dietary record was then returned to the subject and a request made that a similar diet be maintained throughout the trial. Furthermore subjects were asked to consume cariogenic snacks throughout the duration of the experiment to ensure a substantial and on-going cariogenic challenge. Many intra-oral trials are conducted in a fluoridated background with subjects using either a fluoridated toothpaste (Leach *et al.*, 1989; Creanor *et al.*, 1992) or drinking fluoridated water (Koulourides & Chien, 1992). This will obviously have a direct bearing on any experimental outcome.

The duration of experimental protocol has been addressed by ten Cate *et al.*

(1992). They reported that *in situ* studies could vary in length from two weeks to three months. White (1992) suggests this is, "an extraordinarily short interval of what encompasses a 'caries event' in most instances". The rate of remineralisation of artificial enamel lesions has been described by Gelhard & Arends (1984). Initial rates or remineralisation are increased compared with specimens that have been in the mouth for longer periods. It is argued by ten Cate *et al.* (1992) that this represents the deposition of salivary derived mineralisation inhibitors and diffusion-inhibiting compounds. Ten Cate *et al.* (1992) further argued that this is a very different process from that normally occurring in caries.

Ten Cate *et al.* (1992) suggested that participants in intra-oral trials should be representative of the population being studied. It is, however, a very difficult procedure to match up parameters such as age of susceptible population, gender, caries experience and other oral physiological variables. Whilst the matching up of populations is desirable for studies trying to determine the efficacy of a product, it is not necessary for the investigation of mechanisms by which such a change may occur.

In conclusion many intra-oral model systems for the study of dental caries have been described. Each must be taken on its merits with regard to the component parts of the model and the claims made by investigators advocating the model. The evolution of an intra-oral model system at Glasgow University has taken into account many of the factors previously described.

The model and the rationale for its use are discussed in Chapter 3.

1.9 SUMMARY

Dental caries is a multifactorial disease involving the dental hard tissues. Intra-oral factors such as diet, plaque and saliva may influence the rate of progression or remineralisation of early enamel caries. The effects of diet, including fluoride use, and plaque on dental caries have been studied extensively. The relative effect of saliva is less clear. The development of the *in situ* model permits the careful study of factors affecting dental caries. This model may be used to study the effects of stimulated salivary flow on artificial carious lesion in enamel and forms the basis of the work for this thesis. Methods of caries assessment are discussed in Chapter 2 before expanding on the method and materials of the Glasgow *in situ* model in Chapter 3.

CHAPTER 2 THE GLASGOW *IN SITU* MODEL, METHODS OF ANALYSIS OF CARIOUS LESIONS AND STATISTICAL METHODOLOGY

2.1 INTRODUCTION

The use of intra-oral models to study remineralisation and demineralisation has been described in Chapter 1. In addition, the relative merits of various hard tissue substrates in which lesions can be created and the sites at which they are placed in the oral cavity have also been discussed. This chapter will outline the intra-oral model to study caries developed at Glasgow University (Creanor *et al.*, 1986b) and compare and contrast the methods of lesion analysis with those used by other investigators. Finally, the methods of statistical analysis of results will also be presented.

2.2 THE GLASGOW MODEL

The intra-oral model used to study dental caries at Glasgow Dental School was first described by Creanor *et al.* (1986b). It comprises a lower removable appliance (figure 2.1) supporting single sections of human enamel with artificially created carious lesions. The sections are mounted in a contact point relationship within a recessed protective trough on the lingual aspect of the appliance (figure 2.2).

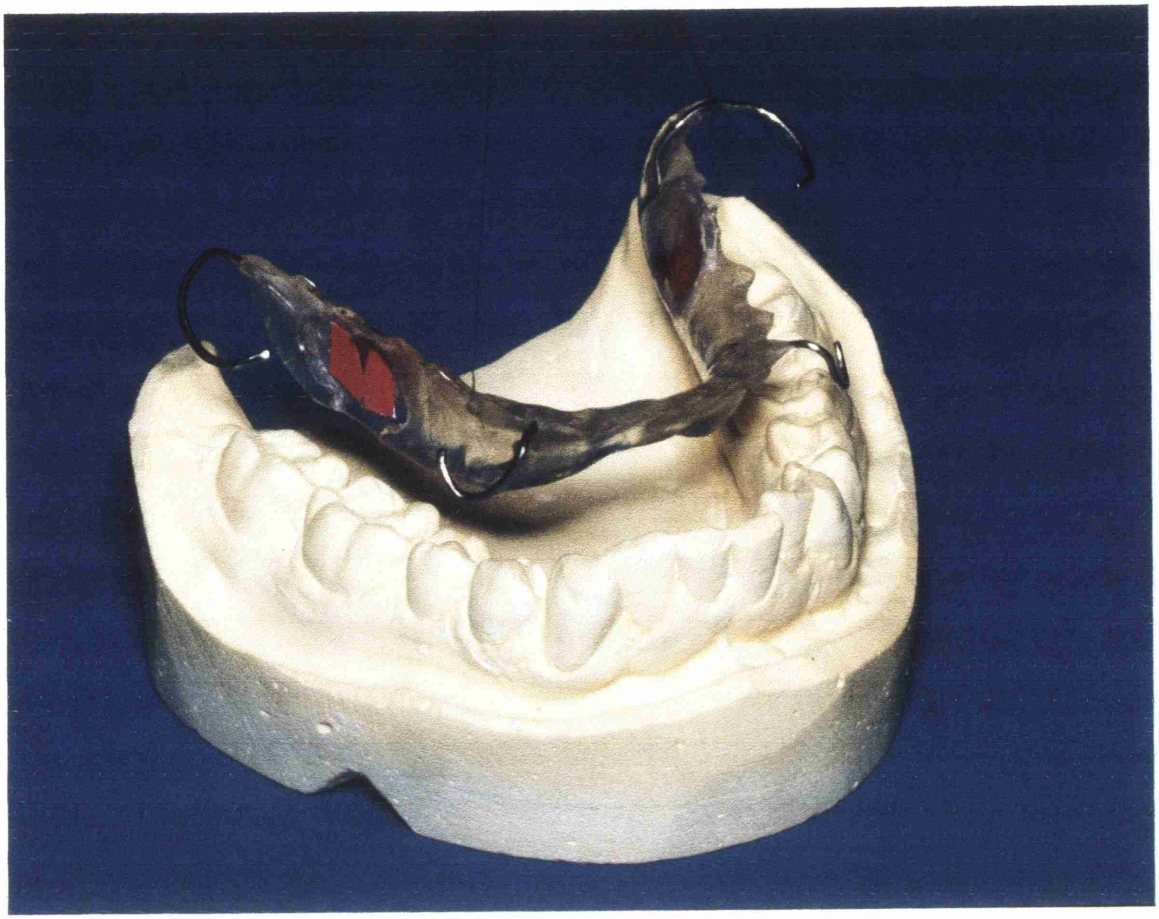


Figure 2.1 Photograph of the lower removeable appliance used for the Glasgow *in situ* model.

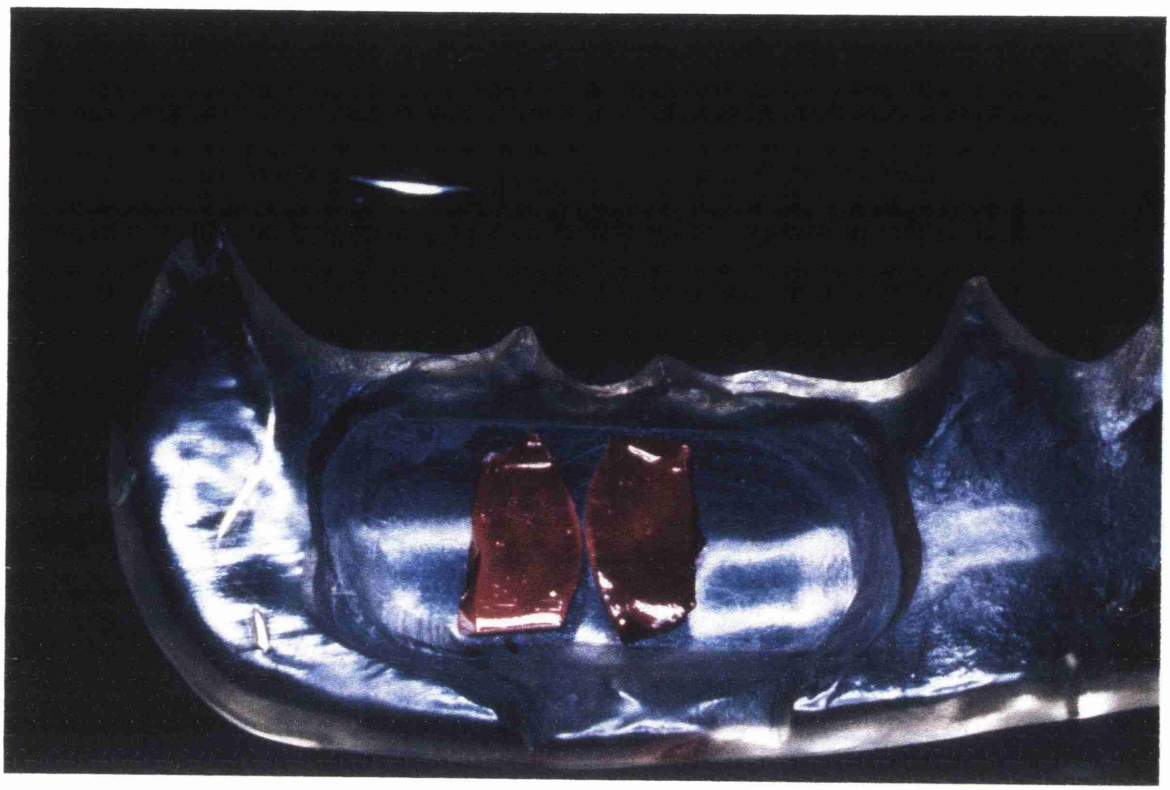


Figure 2.2 Close-up view of the lingual trough on the lower removeable appliance showing the recessed trough with saliva ingress and egress points and varnished sections of teeth mounted in a contact point relationship.

The trough has several functions (figure 2.2). It provides a protective environment against masticatory forces in which the enamel specimens may be housed, it provides a protected environment for the formation of dental plaque and it modifies the specimen exposure to high salivary flow rates experienced in the lingual sulcus (Dawes & Macpherson, 1993) via a salivary ingress and egress. The extent of this modification is presently unknown and was investigated in studies reported in Chapter 6.

Human enamel is used as the medium in which artificial caries is observed. This is the hard tissue of choice (Mellberg, 1992). The use of a single section technique (Harvey *et al.*, 1982) permits each section to act as it own control. The relative merits of single sections and enamel blocks as media for observing changes in artificial caries have been discussed in Chapter 1, Section 1.8.4. Microradiographs are taken at the start and at the end of each experimental period from which quantitative comparisons can be made. Microdensitometry can be used to compare mineral content in the same lesion before and after treatment and has been advocated by Arends & ten Bosch (1992) as the most practical technique for the direct quantitative measurement of mineral content, mineral changes and mineral distributions.

Evaluation of the microbiology and acid anion profiles of plaque covering the sections in the appliance compared with natural interproximal plaque has be undertaken by Creanor *et al.* (1986a) and Macpherson *et al.* (1991). Results of both of these studies suggest no significant differences between the

predominant bacterial species or acid/anion profiles of the plaque from either of these two sources.

Volunteers employed with this particular model are drawn from colleagues working within the Dental School. This does not address the problem of age in relation to dental caries, nor does it represent a group of susceptible people with a high caries activity. However, these factors are compensated by volunteer compliance and accessibility for experimental evaluation.

All trials are designed to be crossover in nature as advocated by Proskin (1992) and enamel specimens are matched to ensure successive sections cut from a tooth are used in the same volunteer in each phase of the trial, thereby reducing experimental variability incurred when comparing sections from different teeth (Creanor, 1987).

The Glasgow intra-oral model has been used to study the effects of various combined fluoride and stimulated saliva protocols on early enamel caries. Most studies have utilised a core protocol which consists of:-

1 The continual wear of the appliance for periods of between five and seven weeks,

2 Appliance removal only for brushing the teeth in the lower arch,

3 The ingestion of sugar containing snacks between breakfast and the midday meal and between the midday meal and the evening meal.

Constant wearing of the appliance ensures the sections are exposed continually to the oral environment and the frequent ingestion of sucrose-containing snacks ensures a regular sucrose challenge. This constitutes a fairly harsh environment in which mineral changes can be observed. Approval was granted by the Local Ethics Committee for use of this model. This is detailed in Appendix I.

2.3 GENERAL METHODOLOGY FOR THE GLASGOW INTRA-ORAL MODEL

This section describes the preparatory stages and general protocol employed in all work using this intra-oral model. The stages are described in the order undertaken by the author and generally follow a logical progression.

2.3.1 Volunteer Selection

Volunteers for this work were generally chosen from colleagues working within the Dental Hospital and School according to the following criteria:-

1 Adequate dentition to retain an intra-oral appliance

2 Controlled caries rate

3 General medical and dental fitness

4 A willingness to participate in the experiment

It has to be accepted that this constituted a biased population sample but it

was considered inappropriate to use volunteers pooled from the general public for reasons of motivation and compliance during fairly prolonged and demanding studies. In addition, it was not one of the aims of this model to address the question of efficacy of a protocol in population terms, rather work has concentrated on a mechanistic evaluation of observed effects.

2.3.2 Appliance Construction

Plaster models were constructed from upper and lower alginate impressions taken of each volunteer. Care was taken to ensure the full functional depth of the lingual sulcus was recorded. The occlusion was indicated to the dental technician by means of a wax inter-occlusal record. All areas of lingual undercut in the region of the lower molar and premolar teeth were blocked out using a soft wax prior to construction of the lower intra-oral appliance.

Appliance construction required the careful adaptation of a wrought lingual bar as a major connector between two clear acrylic lingual flanges. The appliance was retained by four wrought, ball-ended clasps, contoured in such a manner that they did not interfere with the subjects' occlusion (figure 2.1).

The surface of the flange facing the lingual mucosa contained a trough as indicated in figure 2.2. From this photograph it can be seen that the sections of teeth containing artificial caries are mounted on a platform raised from the base of the trough and in a contact-point relationship. The raised platform

permitted exposure of all surfaces of the single section to plaque and saliva thereby ensuring the possibility of ion-exchange over the entire, natural, unvarnished surface of the section. The positioning of the sections in a contact point relationship was suggested in an attempt to simulate conditions occurring with interproximal caries. The shape of the trough encourages plaque collection and the salivary ingress and egress points should permit a continual flow of saliva through the trough.

2.3.3 Artificial Lesion Creation

All of the teeth used for section creation were caries free extracted human teeth. These were collected from Oral Surgery Departments at hospitals in the central Glasgow area. The fluoride level in the domestic water supply for the city of Glasgow is 0.03ppm F (1.6 µmol/l F). All teeth were stored in a saturated solution of thymol until required. Teeth selected for lesion creation were lightly polished with a slurry of pumice and water and then washed in warm, soapy water. Finally all teeth were wiped with an isopropyl alcohol-saturated tissue (Azo-Wipe, Vernon-Carus Limited, Preston, England) to ensure a grease-free surface. The crown of the tooth was inspected for obvious cracks or flaws in either the buccal or lingual/palatal surfaces. Any teeth with obvious flaws were discarded. The selected teeth were allowed to dry before winding adhesive tape (Tape width = 0.7 mm Letraline, Letraset UK) around the anatomical crown of the tooth to form a spiral pattern (figure 2.3). The crown and root of the tooth were covered with two coats of proprietary nail

varnish (Max Factor, Diamond Hard). The nail varnish was permitted to dry for at least 24 hrs before the adhesive tape was removed and the tooth placed into demineralising solution.

It is recognised that there are many different methods in use for the creation of artificial white spot lesions. Such methods have included the production of artificial lesions by natural plaque (Clarkson *et al.*, 1984), the use of acidified gels (Silverstone, 1966) and the use of buffered solutions (Featherstone *et al.*, 1978). Previous experience with both acidified gel and buffered solution techniques has shown the buffered solution method to be faster in terms of lesion creation. Moreover, the lesions created conform to accepted parameters in terms of their size, morphology and are more consistent in their ability to exhibit re- or demineralisation (Strang *et al.*,1988).

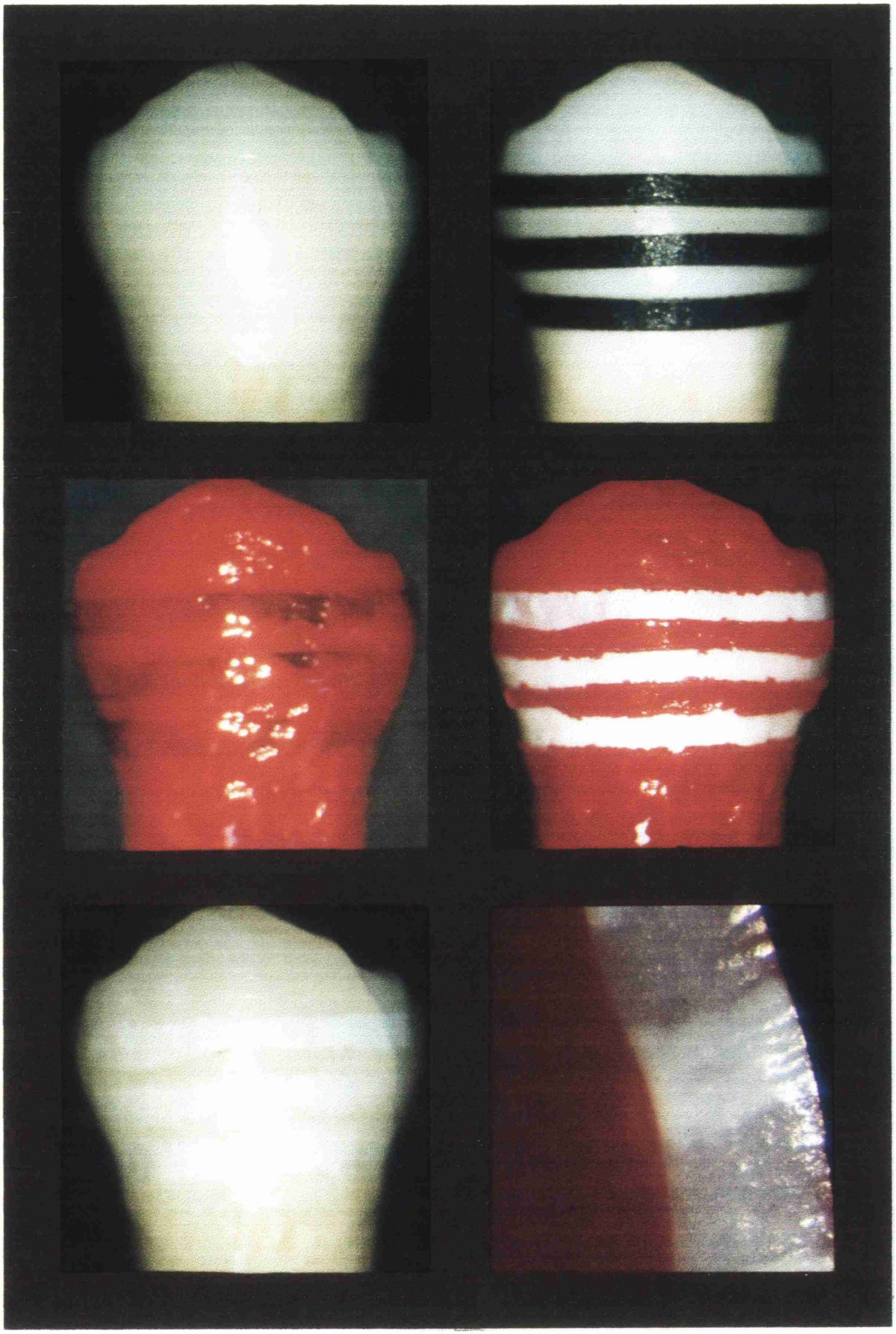


Figure 2.3 Composite photograph of the stages involved in creation of a single section with multiple artificial carious lesions

The buffered solution used contained 2.0 mmol/L calcium as anhydrous calcium chloride, 2.0 mmol/L phosphate as anhydrous sodium dihydrogen orthophosphate and 0.53 μmol/L fluoride as sodium fluoride. The pH was adjusted to 4.55 by the addition of glacial acetic acid and sodium hydroxide. Varnished teeth were suspended in demineralising solution with a volume of approximately 100 ml of solution per tooth. The demineralising solution was changed daily and stirred slowly, using a magnetic laboratory stirrer (Magnetic Stirrer, HI304N, Jencons Scientific Limited, Leighton Buzzard, England), at regular intervals throughout each 24 hr period. Each daily change of demineralising solution was checked to ensure the pH remained constant at 4.55 and, if necessary, was adjusted by the addition of acetic acid or sodium hydroxide. The daily change in demineralising solution also provided an opportunity for the teeth to be inspected regularly for signs of demineralisation.

Teeth were judged to be sufficiently demineralised when the air-dried surface appeared to be a dense, opaque white colour whilst retaining its surface shine and integrity (figure 2.3). Demineralisation usually took between 4 and 7 days. Teeth that had become cavitated or had failed to demineralise during this period were discarded. Those teeth judged to be suitable for inclusion in the experiment had all nail varnish removed by careful swabbing with acetone. The tooth was then washed in absolute ethanol prior to storage in a saturated thymol solution before section creation.

2.3.4 Single Section Creation

The anatomical crown was separated from the bulk of the root of the tooth using a hacksaw to cut approximately 1-2 mm below the cervical margin. The crown portion was then embedded in a hard wax and mounted on a cutting platform in such a manner that sequential sections could be cut in a mesiodistal direction. Each section would, therefore, have a buccal and a lingual, or palatal, aspect. Sections were cut using a Microslice 2 precision slicing machine (Malvern Instruments Ltd., England) (figure 2.4). This machine had an annular, diamond impregnated, blade to cut slices through the embedded tooth with a thickness of approximately 250 μm. The blade was lubricated with Aquagrind (Malvern Instruments, England) and operated at a speed of approximately 350 revolutions per min. Each section had to be supported further, prior to cutting, by a glass slab adhered to the tooth with an ultraviolet light cured acrylic resin (Loctite Glass Bond, Loctite UK, Welwyn Garden City, Hertfordshire) (figure 2.5). This extra support reduced crack propagation within the section as a result of vibration from the cutting blade. The 250 μm thick sections are removed from the wax and glass slab by soaking in acetone. Subsequently, they were passed through ethanol and placed in a saturated solution of thymol prior to hand grinding. The sections at this stage were too thick for microradiography and also had cutting marks on both sides. The section thickness had to be reduced and the cut surface rendered smooth.

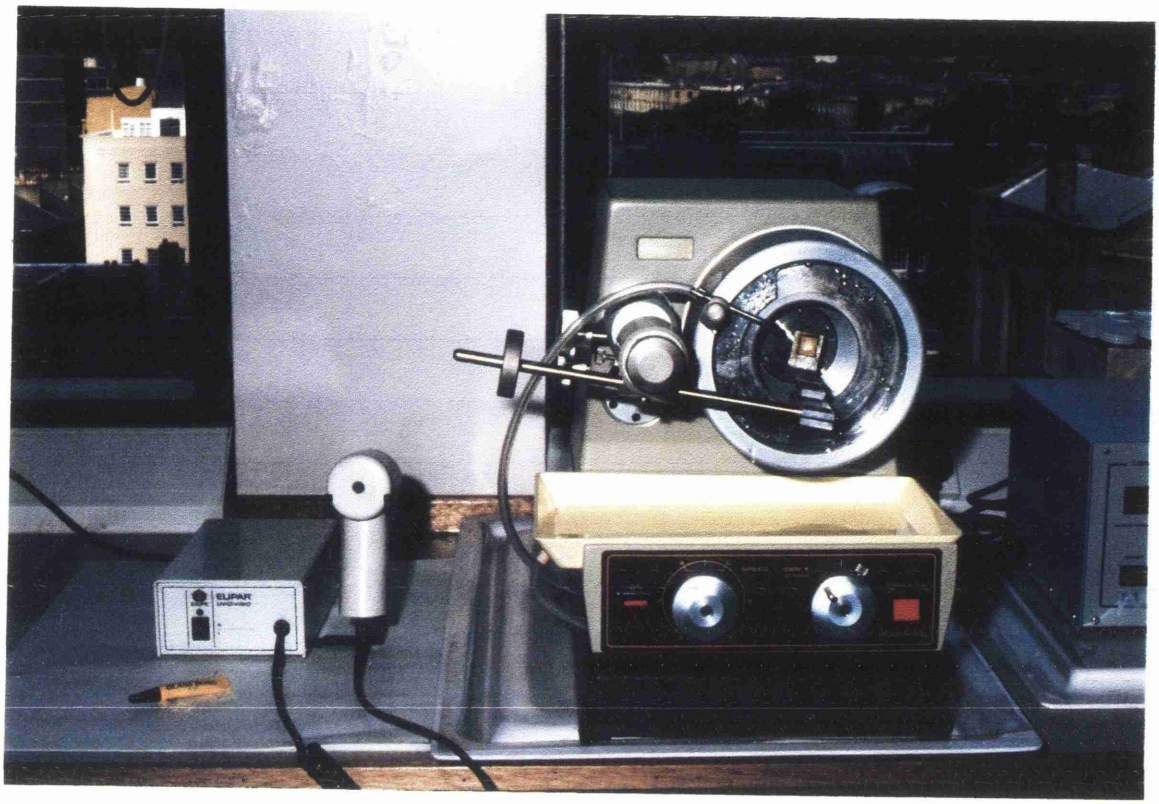


Figure 2.4 Microslice 2 precision slicing machine for creation of 250 µm thick slices of teeth.

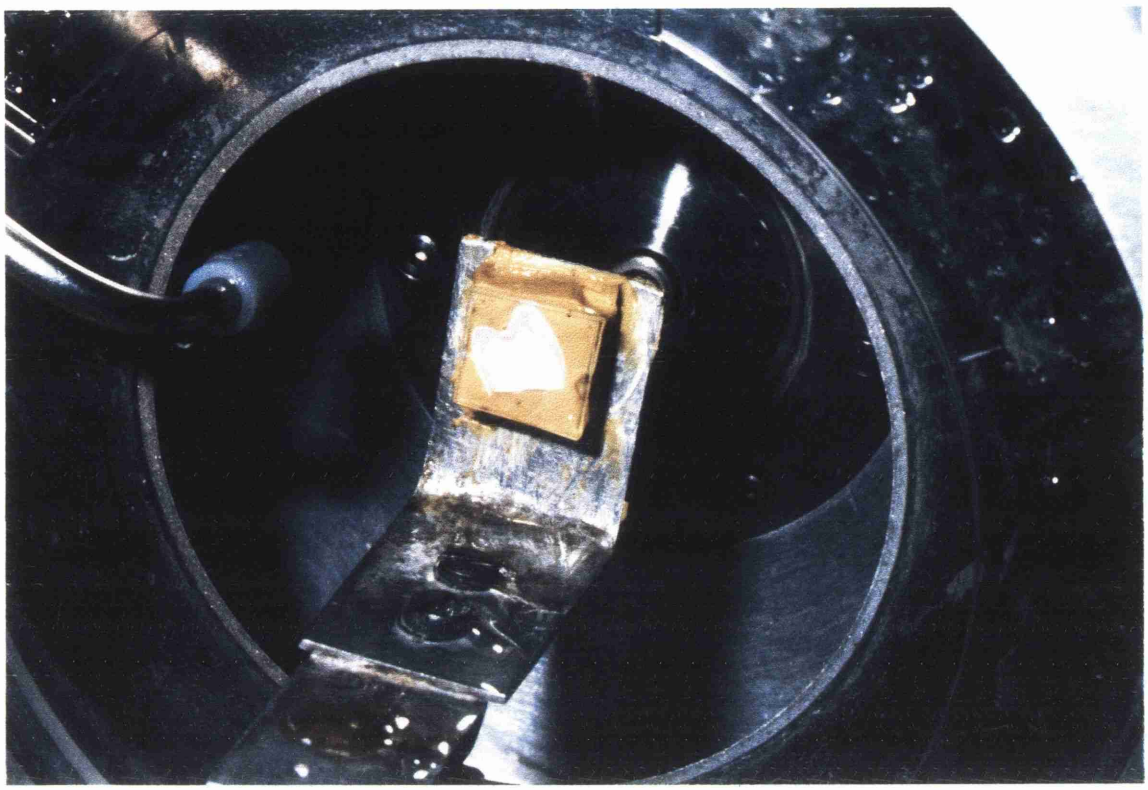


Figure 2.5 Close-up view of Microslice 2 showing tooth section embedded in hard wax with glass slab to support section during cutting.

A heavy brass plate covered with damp gauze (figure 2.6) was used to hold the section in place as each side was carefully ground with a slurry of 1200 grade Bauxilite (Al_2O_3) (Raymond Lamb, London, England) and water on a large glass plate. The section thickness was checked at regular intervals using a digital micrometer (figure 2.7) (Mitutoyo, Tokyo, Japan). Readings were taken along the edge of the section near the enamel surface at approximately 1 mm intervals. These measurements permitted an overall estimation of the section thickness and the degree of planoparallelity of the section. All sections were ground to a thickness of between 120-140 μm. Values within this range of thicknesses ensured an adequate microradiographic grey level range (see later this Chapter). Sections were numbered in such a manner that the tooth from which the section was derived and the order in which the section had been cut was recorded. Sections were then stored in a saturated solution of thymol prior to batch microradiography.

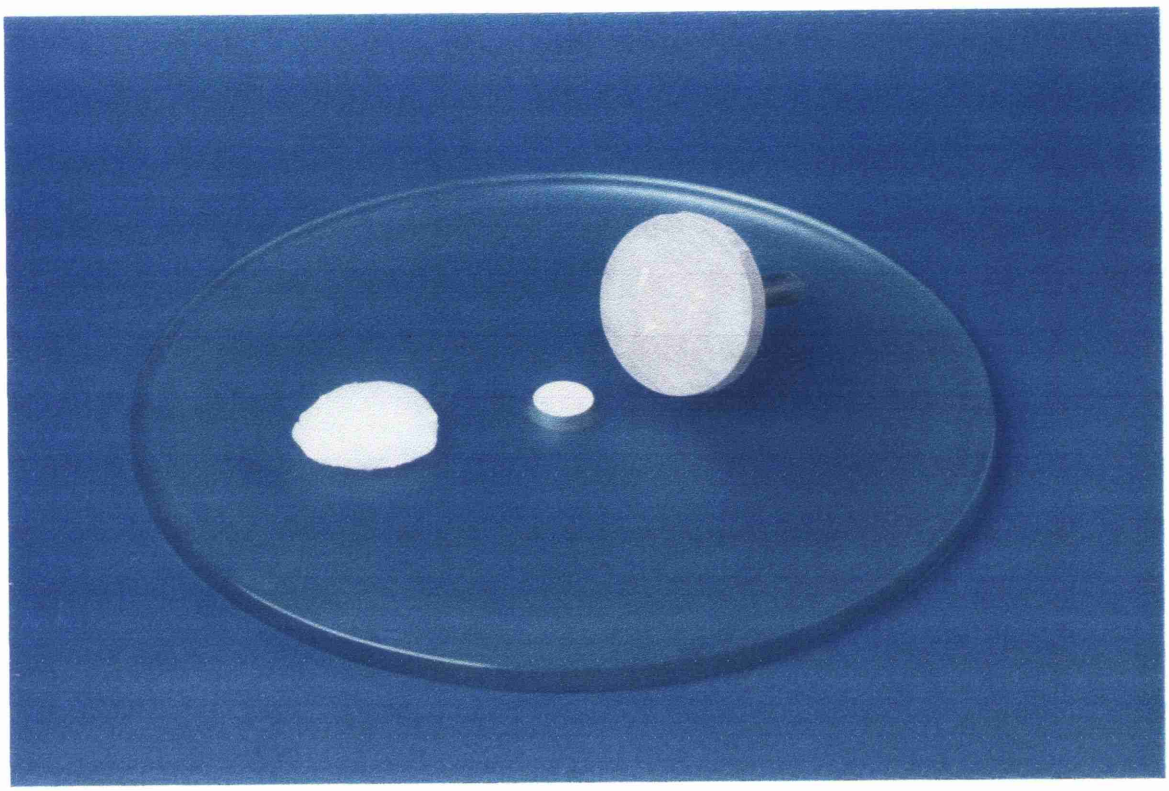


Figure 2.6 Ground glass plate with Bauxilite powder slurry and the gauze covered brass plate. Four enamel specimens can be seen adhering to the dampened cloth.

Figure 2.7 **Mitutoyo digital micrometer for determination of section thickness.**

2.3.5 Batch Microradiography

Assessment of artificial carious lesions in single sections is most accurately undertaken using microradiography (Arends & ten Bosch, 1992). All numbered, ground sections from each tooth were carefully mounted between two sheets of clingfilm (figure 2.8). This permitted the sections to be retained whilst being microradiographed. The clingfilm mounted sections were placed over high resolution microradiographic plates (Kodak high resolution plate type 1A, Eastman Kodak Co., Rochester, New York, USA.). The plates and sections were mounted in light tight plate holders and exposed for 20 min to a Cu(K_α) x-ray source (Diffractis 582, Enraf Nonius, Delf, Holland) (figure 2.9) operating at 20 kV and 30 mA at a source-specimen distance of 300 mm. The plates were developed using standard techniques. Additional discussion of microradiography as a technique for determining lesion characteristics can be found later in section 2.5 of this Chapter.

2.3.6 Section assessment

The most practical method of section assessment was to project the batch plate onto a screen and assess each section individually. This was achieved by placing the plates in a conventional slide carousel in conjunction with a slide projector, thus magnifying each section hundred times. The criteria for the acceptance of a section were:-

-the presence of two or more intact lesions on the same section

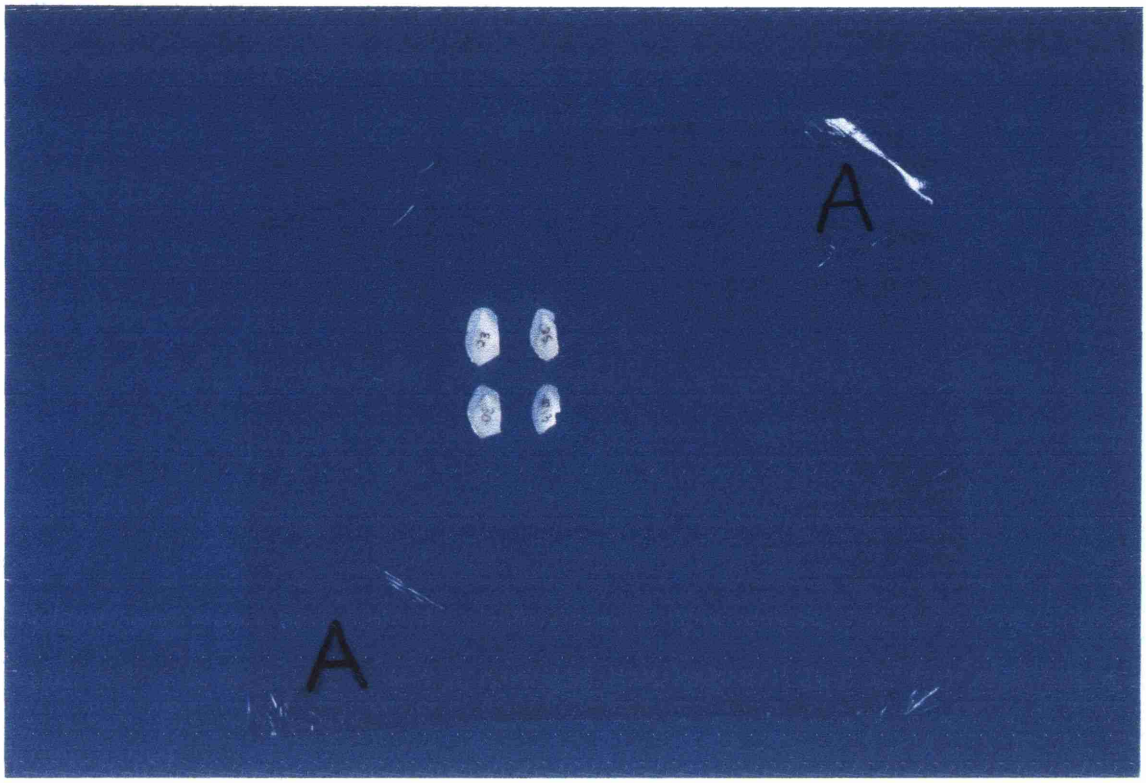


Figure 2.8 Sections of teeth with artificial carious lesion mounted in clingfilm prior to microradiography.

Figure 2.9 Diffractis 582 Cu(K_α) X-ray source

-each lesion should have a well defined and intact surface zone

-each lesion should have a well defined radiolucent lesion body

-there should be no cracks or any other artifacts passing through or near the lesion.

Suitable sections were identified by the tooth from which they had originated and the sequential order in which they were cut from the tooth. Once enough suitable sections had been selected they were compiled into a list prior to allocation to volunteers. Sections were returned to a solution of thymol.

2.3.7 Section Allocation To Volunteers

The crossover nature of the experimental protocol employed required each volunteer to undertake more than one phase to complete the experiment. To allow valid comparisons between phases, the lesions used in each phase required two basic qualities:-

1 The four sections with artificial lesions used for each volunteer during each phase of the experiment came from four different teeth to avoid biasing the results.

2 The lesions used in each phase should be as similar as possible, in terms of size, to those used in other phases to ensure that any re- or de-mineralisation recorded is due to the change in protocol between phases rather than the use of different lesions.

Featherstone & Mellberg (1981) and Mellberg (1992) have shown that

mineralised tissues from different species demineralise and remineralise at different rates. Strang *et al.* (1987) have shown that variation in lesion size, created under identical demineralising conditions, is greatest between different teeth and least in different sections cut from the same artificially produced carious lesion. Finally, ten Cate (1992) stated, on the basis of remineralisation work on lesions of identical size but created by different methods, "It is therefore imperative that comparisons be made between studies or treatments when lesions are comparable." Therefore, the ideal would be to have sequential sections of the same lesion used in each phase of the experiment.

In an attempt to comply with these requirements, the following protocol was employed. Each volunteer took part in all phases of the crossover protocol. In each phase the appliance carried four varnished sections. Each of these sections was from a different tooth. Any overall mineral change in any one particular phase would, therefore, be a composite result based on observations of up to four different teeth. Furthermore, sections from the same four teeth were used in all phases of the experiment. Finally, an attempt was made to ensure that the sections used from any one tooth were cut sequentially so that sections bearing portions of the same lesion were used in the same volunteer in each phase. By using this method of section allocation it was anticipated that similar lesions, which should react in the same manner, would be used from four different teeth, in the same volunteer, in each of phase of an experiment. This would minimise the influence of the lesion characteristics on the protocol and maximise the effect of the protocol on the lesions.

2.3.8 Baseline microradiography

The sections allocated to each phase were mounted in clingfilm and placed over a microradiographic plate. An aluminium step wedge (figure 2.10) was placed alongside the sections for later calibration. The stepwedge consisted of a series of aluminium sheets of known thickness which produced a reproducible series of grey levels on the microradiograph (figure 2.11) which can be subsequently correlated to the amount of mineral present within the lesion according to the equation of Angmar *et al.* (1963) (Appendix II). The step wedge was placed along the *y*-axis of the plate as previous work (Creanor, 1987) had shown an unacceptable variation in X-ray beam homogeneity from the top to the bottom of the plate. This was not the case across the *x*-axis where beam variation was found to be less than 1%. Further information on the principles of microradiography can be found in section 2.5 of this Chapter. Once the microradiographic plates were developed and found to be of a satisfactory standard, the sections were removed from the clingfilm and stored in thymol until required for the experiment.

2.3.9 Section Varnishing

Sections were varnished carefully prior to placing them in the appliance trough. A dissecting microscope (Zeiss Stereo-microscope 4, Zeiss, Wetzlar, Germany) (x 10 magnification) was used to ensure the nail varnish covered all

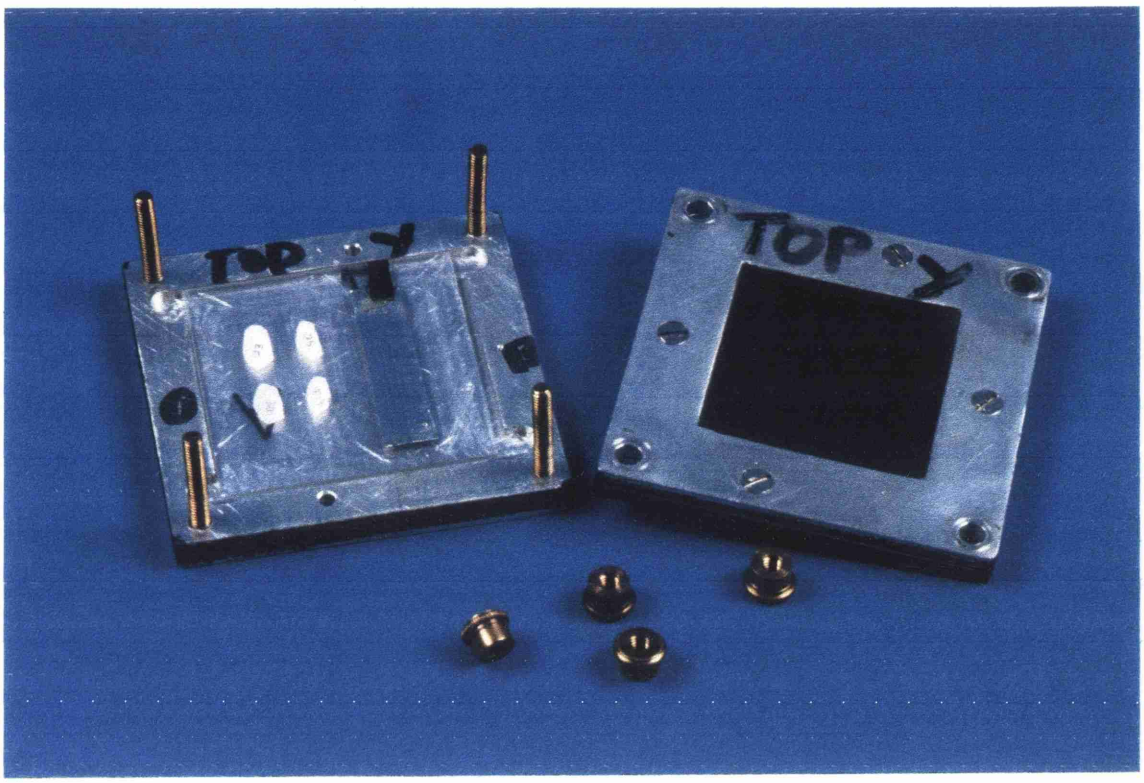


Figure 2.10 Sections mounted in clingfilm covering the glass photographic plate. The aluminium step wedge is placed alongside the sections. The sections, plate and step wedge are placed in a photographic plate holder.

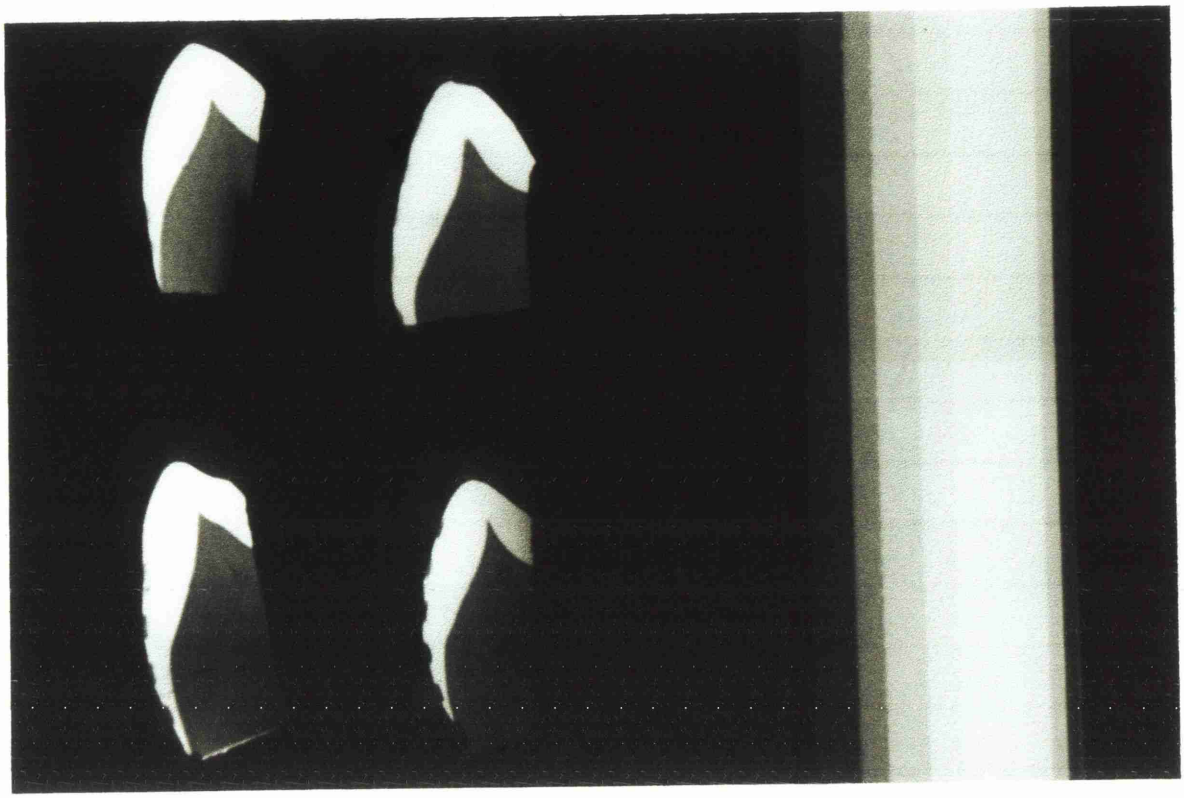


Figure 2.11 Black and white photograph of a developed microradiographic plate showing sections with artificial carious lesions and the aluminium step wedge

the cut surfaces, leaving only the natural tooth surface exposed (figure 2.3). The varnish was left to dry for 24 hrs before the sections were cemented into the appliance trough, in a contact point relationship, with more nail varnish. The sections were left for a further 24 hrs to dry before the appliance was placed in the mouth.

2.3.10 Repeat Microradiography

Once each phase of the experiment had finished, the appliances were returned. After removal from the appliance, sections were suitably cleaned and were mounted in clingfilm prior to re-microradiographing with the aluminium step wedge to produce a second plate. The examination of the second plate in conjunction with the previously developed baseline plate of the same sections permitted a direct comparison of mineral profiles of each lesion by microdensitometry.

2.4 MICRODENSITOMETRY

2.4.1 Introduction

This technique permits a precise assessment of the greyness of a microradiographic image of small volumes of dental hard tissues. The different grey levels can be correlated with the mineral content of the tissue under examination. The technique for analysis of dental hard tissues was first

described by Thewlis in 1940 and was subsequently refined and reported in detail by Angmar *et al.* (1963).

The principle relies on the illumination of a microradiographic image with a constant and uniform light source. Light transmitted by the microradiographic image passes to a detector and a recorder. This information is subsequently correlated with the light passing through a reference image of known radio-opacity. In most cases, the reference image is of an aluminium step wedge. This comprises a series of known thicknesses of aluminium.

2.4.2 Methodology

The microradiograph was placed on the specimen stage of a Leitz Ortholux II microscope in conjunction with a stabilised transmitted illumination system (figure 2.12). The image was recorded by a video camera (ASACA Corporation type 700BE) and transmitted to an analyzer unit (Leitz Image Analyzer). This unit was controlled by a Z8002 microprocessor which digitised the video signal from the camera into 256 grey levels with a resolution of 256 x 256 pixels. Potentiometers on the front panel of the image analyzer enabled the unit to be set up so that the 256 grey levels cover the range of optical, radiographic densities of the material to be analyzed. The digitised image was then transferred to a BBC-Master computer for further analysis and storage of results. The software for both the BBC-Master computer and the Leitz Image Analyzer was written by Dr. R. Strang and Mr I.P.A. MacDonald (formerly of the Department of Clinical Physics, West of Scotland Health Boards).

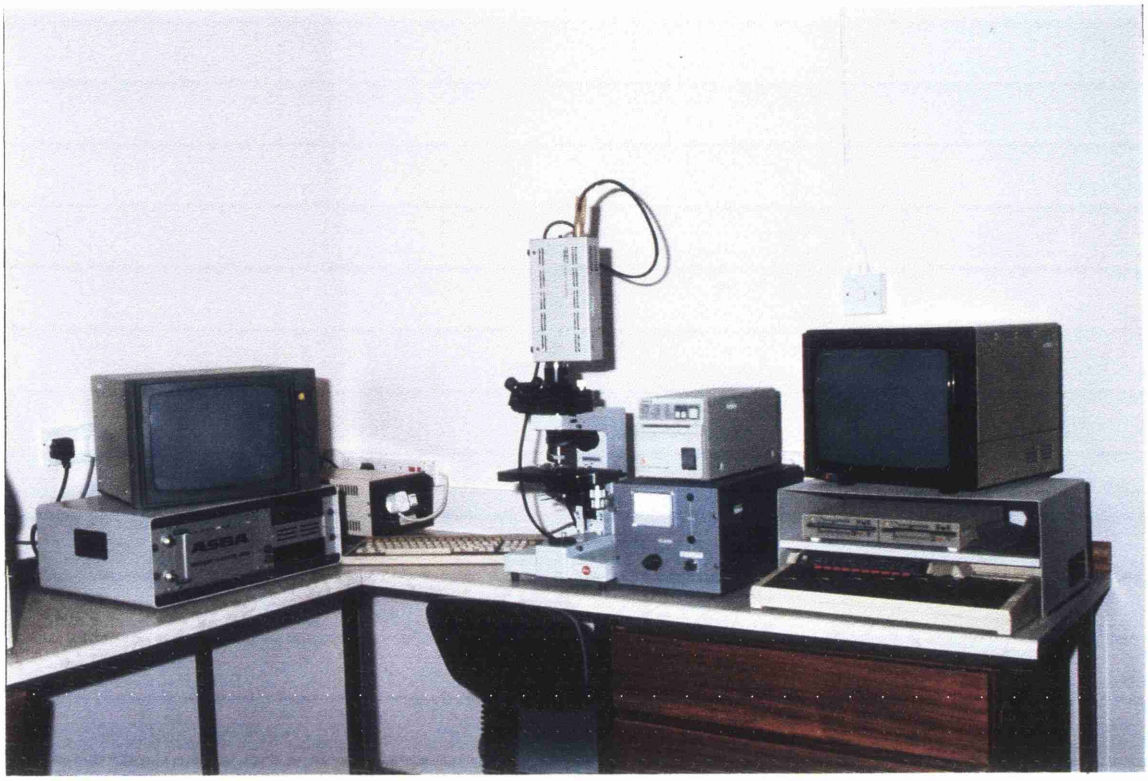


Figure 2.12 System for performing microdensitometry on microradiographs incorporating a Leitz microscope, a video camera, an image analyzer unit (ASBA) and a computer to process and store the information obtained.

The procedure for the analysis of the microradiograph required initial calibration of the system using the aluminium step wedge. The microradiographic plate was initially positioned so that the thickest part of the wedge was in the field of view. The camera was then saturated with light and the potentiometer adjusted to record a value of approximately 250 grey levels. Next, the camera was blanked out so that no light was detected. The potentiometers were once again adjusted to record a reading of between 6 and 9 grey levels. Finally, the light source was adjusted so that the camera recorded the light passing through the thickest part of the wedge at about 241 grey levels. The microscope stage was then adjusted to record the level of light passing through the other portions of the aluminium step wedge. The radiograph was positioned to view the area of the particular lesion of interest on the same horizontal axis as the scan taken from the step wedge. The camera was rotated so the surface zone of the lesion was horizontal on the camera monitor. The image was then digitised into 256 x 256 pixels and displayed on the BBC-Master computer monitor. At the magnification used (x 6.2), 1 pixel corresponded to 3 μm. The BBC-Master computer software re-displayed the image in four different colours corresponding to the grey levels of the image. Using the BBC-Master keyboard, the width of the area of the lesion to be recorded was adjusted by means of two vertical cursors. An average microdensitometric profile could then be calculated of all the data in this region. Horizontal cursors were used to delineate the amount of information stored by the computer (figure 2.13).

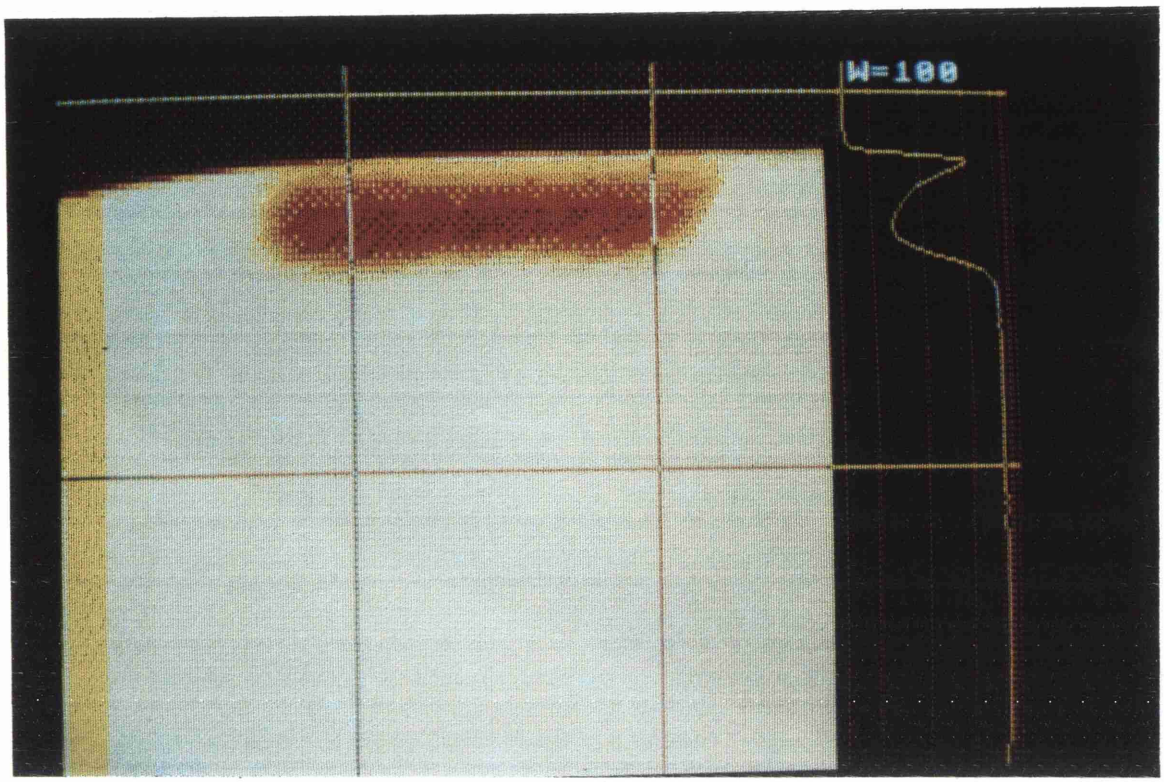


Figure 2.13 An enamel lesion displayed on the BBC monitor. The grey levels of the aluminium step wedge are represented by the red vertical lines on the right of the screen. The scan (indicated by the yellow tracing on the right) has been taken from the area defined by the yellow vertical and horizontal lines superimposed on the lesion.

Microdensitometric scans were performed on lesions from the baseline and later plates. A hard copy of the digitised image from the computer monitor, displaying the lesion on the later plate, was taken to enable the same area of interest in the baseline radiograph to be re-located with an accuracy of one or two pixels.

A fourth order polynomial curve was then fitted to the aluminium wedge grey levels and the grey levels of the profile were subsequently converted to per cent volume mineral using the equation derived by Angmar *et al.* (1963). This equation is given in Appendix II and takes into account the absorbtion coefficients of the mineral and organic components of enamel, as well as the thickness of the section. The profile information and other relevant data, such as section thickness, were stored on floppy disk for later analysis.

2.4.3 Measured parameters

All mineral profiles were stored as per cent volume mineral on the y-axis and depth (μm) on the x-axis. Mineral profiles of all lesions were normalised prior to storage of data. This means that the mineral content of normal enamel in all lesions was adjusted to 80 % by volume. This procedure reduced the influence of second order effects on calculated data such as accidental marks or scratches on the radiographic plate. The measured value for normal enamel varies in the literature from 78% to 87% by volume (Groeneveld, 1974). Therefore, the value of 80% was considered within this accepted range.

Other measured parameters included:-

1 The per cent volume mineral content of the surface zone taken at the maximum mineral content of the surface layer.

2 The minimum lesion mineral content taken as the lowest point on the mineral profile deep to the surface zone.

3 The lesion depth calculated as the distance from the 20% mineral content of the initial slope to an arbitrary cut-off point at approximately 95% of the value of normal enamel. This arbitrary cut-off point was selected because of the unreliability of determining depths in lesions where the mineral content approaches that of sound enamel asymptotically (Mallon & Mellberg, 1985).

4 The integrated mineral loss (Δz) was calculated as the area above the profile from a point at the 20% volume mineral level on the initial slope to a point (S) in sound enamel. Providing the section is planoparallel, the positioning of point S in sound enamel is unimportant. Point S was selected by the operator on the baseline profile. This same distance between the 20% volume mineral point on the initial slope and point S in sound enamel was used by the computer to measure parameters on the second plate of the same lesion. The units for Δz are therefore % vol.mineral x µm.

Other definitions of Δz exist and are described in figure 2.14.

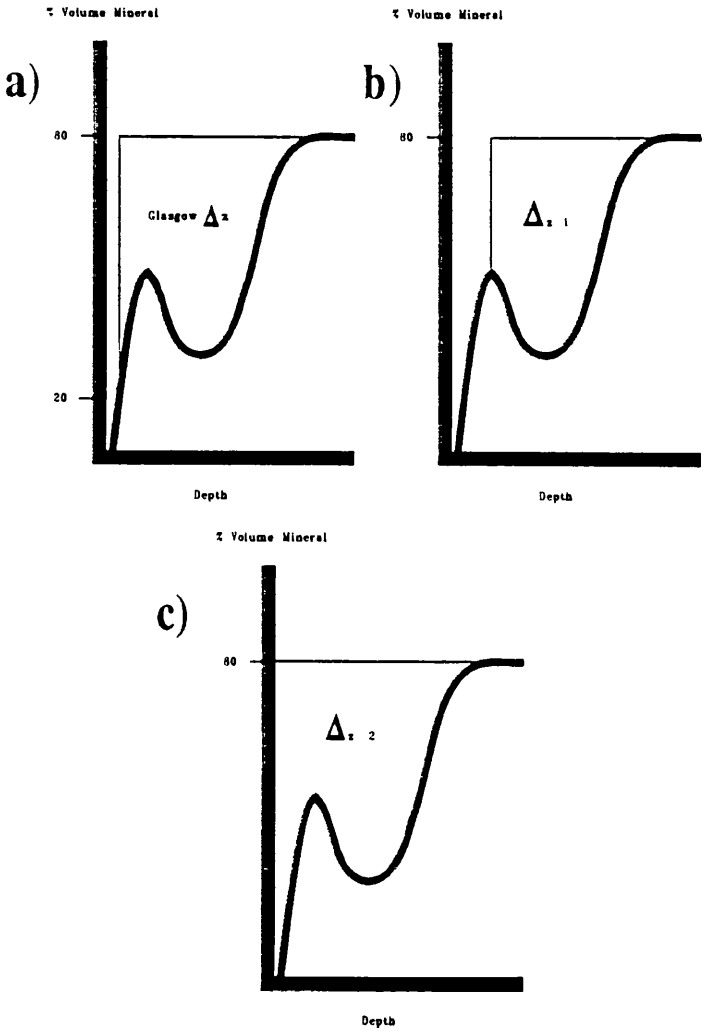


Figure 2.14 Methods of determination of integrated mineral loss (Δz)

a) This method uses the area above the curve from a line perpendicular to the 20% mineralisation point to a point in normal enamel. This method is used in the Glasgow model.

b) This method uses a perpendicular from the point of maximum mineralisation in the surface zone to a line approaching normal enamel asymptotically (mallon & Mellberg, 1985)

c) This method uses a perpendicular from the 0% mineralisation point, at the surface of the lesion, to normal enamel. It is, however often impossible to determine the precise position of the lesion surface (Arends & ten Bosch, 1986; Dijikman *et al.*, 1986)

2.5 CRITIQUE OF LABORATORY METHODS OF MINERAL EVALUATION OF CARIOUS LESIONS IN ENAMEL

2.5.1 Introduction

There are several methods available to determine the mineral content of carious lesions in enamel. These methods are outlined briefly, along with some of their relative merits and drawbacks. Quantitative *in vitro* methods may be destructive or non-destructive and have been used for single or serial determinations of mineral content. Comparisons between techniques are summarised at the end of this Chapter.

Ten Bosch & Angmar-Manson (1991) described three units to express mineral loss from enamel as a result of the caries process. These were:-

1 The mineral loss from a specific point, expressed as a decrease in mineral concentration with units of kg/m^3 or vol%.

2 The integral of the change in mineral content over a known distance from the surface of the tooth. This parameter is expressed in kg/m^2 or vol% x μm.

3 The total mineral loss expressed in kg or g.

These parameters are identified in Appendix III.

2.5.2 Transverse (Contact) Microradiography

This technique involves the assessment of the absorbtion of monochromatic X-rays by a thin plano-parallel tooth section cut perpendicularly to the anatomical surface. The image is compared later with absorbtion by a simultaneously exposed standard, usually an aluminium step wedge (ten Bosch & Angmar-Manson, 1991). X-ray absorbtion is proportional to the resultant optical density of the film which may be calibrated using the step wedge. Mineral content at a point in the sample can be calculated by microdensitometry using Angmar's formula. (Angmar *et al.*, 1963) which calculates mineral loss as vol% or Kg/m^3. The method provides a quantitative measurement of mineral loss or gain at specific points within a lesion. In addition, this method can also be used to provide information about mineral distribution within the lesion if X-ray absorbtion is measured at multiple points from the lesion surface towards the amelodentinal junction.

This technique is destructive as it requires the cutting of a single section, but serial measurements are possible on single sections, thus enabling lesion progression to be monitored (Creanor, 1987). Transverse microradiography of single sections does assume, however, that the section is homogeneous in thickness and White *et al.* (1992) have demonstrated that such homogeneity can be a problem when creating single sections. Most transverse microradiographic techniques are unable to measure the mineral content of the outer 10 μm of enamel, although different systems have different degrees of resolution.

Problems with resolution are due to the use of a point X-ray source producing a non-parallel X-ray beam and the difficulty of producing single sections cut parallel to a curved tooth surface. In addition remineralisation protocols using ions with a very high absorbtion coefficient for X-rays, for example tin ions, may lead to misinterpretation of results (Arends & ten Bosch, 1992).

2.5.3 Longitudinal Microradiography

This technique involves samples of teeth up to 0.5 mm thick which are cut parallel to the anatomical surface and exposed, along with an aluminium step wedge, to Cu-K_α radiation generated by 20keV electrons . The use of a microdensitometer facilitates the determination of the mineral content of a lesion per unit area. Mineral loss is expressed in terms of % vol. x µm or kg/m^2. The method is thought to be subject to errors of up to 20% (ten Bosch & Angmar-Mannson, 1991). This method can now be applied to natural tooth surfaces, although variations in thickness of specimen should not exceed 200 µm (de Josselin de Jong, 1988). The exposure of the anatomical surface of the lesion to the x-ray source, rather than through the cross-section, means that it is not possible to derive information on the variation of mineral content at various depths through the lesion.

2.5.4 Wavelength Independent Microradiography

Polychromatic, high energy x-rays (≥ 60 Kv) are used for non-destructive mineral determination of whole teeth. A reference step wedge is also used with this technique to enable calculation of mineral content. This method can measure, with great accuracy, the amount of mineral per unit area in enamel or dentine samples with a thickness of between 0.3 mm and 6.0 mm, and with either natural or curved surfaces. Accuracy is reduced when mineral determination of whole teeth is attempted rather than blocks. Mineral loss is expressed in units of % vol. x μm or kg/m^2 (Arends & ten Bosch, 1992). Once again, this technique has the disadvantage of being unable to determine the change in mineral content of a lesion with depth.

2.5.5 Microhardness

This technique involves the placing of a Knoop or Vickers Diamond on the sample under a given load for a known time. The size of the resultant indentation is determined microscopically and may be taken as a measure of mineral content of the underlying tissue (ten Bosch & Angmar-Mansson, 1991). This method has been used extensively on enamel slabs which were exposed to the oral environment, (Koulourides *et al.*, 1974; Gelhard *et al.*, 1979) and *in vitro* (Purdell Lewis *et al.*, 1976; Arends *et al.*, 1979, 1980).

Conventional microhardness testing places the diamond over the surface of the

lesion so that any indentation passes through the surface zone towards the lesion body. An alternative technique is to use a cross-sectional slice through a lesion, placing the diamond perpendicular to the original surface at intervals across the surface zone and body of the lesion (Davidson *et al.*, 1974). This technique is known as cross-sectional microhardness testing and, in enamel, correlates to the degree of demineralisation as measured microradiographically (Featherstone *et al.*, 1983). Flat, highly polished surfaces are required for microhardness testing which usually means the destruction of the natural, anatomical surface of the tooth. In addition, the spatial resolution of this technique is less than transverse microradiography. Indentation with the diamond cannot be made every 3 μm which is the resolution of the microradiographic technique. This also means that cross-sectional microhardness testing is less sensitive to mineral changes occurring as a result of experimental protocol.

2.5.6 Polarised Light Microscopy

The splitting of an unpolarised beam of light into two polarised beams when it enters a crystal is known as intrinsic birefringence (ten Bosch & Angmar-Manson, 1991). Enamel is a crystalline structure although the alignment of the crystallites and the existence of pores within the crystalline structure contributes to a second type of birefringence, called form-birefringence. The pores can be imbibed with various media with different refractive indices, thus changing the form-birefringence. The difference in the two types of

birefreingence can be used to determine the mineral porosity (Shellis & Poole, 1985) and mineral changes within a carious lesion. The technique is not thought to be quantitative but does demonstrate the change in mineral distribution in various regions of the lesion. Polarised light microscopy will only provide data on lesion mineral content if the same section is imbibed in several different imbibing liquids, such as water, naphthalene and Thoulet's solution. When only a single imbibition is undertaken, certain assumptions have to be made which ensure that the only factor that can be determined accurately is lesion depth (ten Bosch & Angmar-Mansson, 1991). In addition, the assumption is made that the imbibing medium reaches even the smallest pores of the lesion, it assumes that the crystallite optical axes are in the same plane as that of the tooth section and that there are no pores so large that they do not contribute to form-birefringence. (Shellis & Poole, 1985). Finally, it has been suggested that the use of imbibition media may affect the subsequent ability of the lesion to remineralise (White *et al.*, 1992).

2.5.7 Light Scattering

Scattering of light within a lesion makes incipient lesions look whiter than surrounding sound enamel. This is probably due to the mineral within a lesion being surrounded by lesion fluid rather than sound enamel (ten Bosch & Angmar-Mansson, 1991). Methods involving optical fibre technology enable such light scattering to be quantified and correlated with mineral loss (ten Bosch *et al.*, 1984). This technique is now being applied to *in vivo* caries

assessment.

2.5.8 Chemical analysis

This method of analysis involves the dissolution of samples of hard tissue in acid and analysis for a range of inorganic ions by atomic absorbtion spectrophotometry or other colorimetric techniques (ten Bosch & Angmar Mansson, 1991). Samples may be obtained by microdrilling (Hallsworth *et al.*, 1973; Sakkab *et al.*, 1984), sequential acid etching (Weatherell *et al.*, 1973), abrasion (Weatherell *et al.*, 1985). The technique is a very sensitive method for detection of mineral changes. However, it is time consuming, destructive and, depending upon the method of sampling, does not provide information regarding change in mineral content with depth.

2.5.9 Iodine Absorbtiometry

A collimated beam of Sn-filtered radiation from an ^{125}I source passes through a section of tooth. Transmitted radiation is detected with a scintillation crystal and photomultiplier unit and can be used subsequently to calculate the mass per unit area of mineral in the section. The beam area at the surface of the tooth is approximately 0.7 mm^2 (ten Bosch & Angmar-Manson, 1991). The technique is similar in many ways to microradiography and microdensitometry.

2.5.10 Iodide Permeability/Penetration

This technique was developed by Bakhos *et al.* (1977) and involves the penetration of iodide into the porous carious lesion. Iodide is then recovered by back diffusion into a known volume of water and quantitatively determined using an iodide-specific electrode. Pilot studies demonstrated that the iodine did not bind to enamel. Zero *et al.* (1990) showed a moderate correlation between a modified form of the iodine permeability test, surface microhardness and mineral dissolution following an acid challenge. The iodine permeability test has recently been used by Zero *et al.* (1992) as a rapid intra-oral model to assess plaque/hard tissue interactions. Such a test may be affected by surface zone pore blockage, especially if used *in vivo* or *in situ* (Arends & ten Bosch, 1992).

2.5.11 Summary

The comparative abilities and applications of laboratory based techniques for mineral evaluation of white spot lesions have been succinctly reviewed by Arends & ten Bosch (1992) and are presented in a modified form in Table 2.1

Table 2.1
Evaluation techniques to assess de and remineralisation

Technique	Determines mineral content in Vol%, Wt% or kg/m³ ?	Determines mineral change in vol%, wt% or kg/m³ ?	Determines mineral distribution?	Sample destruction?	Sample preparation	Estimated mineral loss threshold (after Arends & ten Bosch, 1992)	Repeat	Used with dentine
Microradiography TMR	yes (direct)	yes (integration)	yes	yes (no if single section technique)	sections	0.03×10^3 kg/m³	yes	yes
Microradiography LMR	no	yes (direct)	no	no	longitudinal slabs	0.01 kg/m²	yes	yes
Microradiography WIM	no	yes (direct)	no	no	whole tooth	0.01-0.05 kg/m²	yes	yes
Microhardness (on surface)	no	?	no	minor	flat surface	0.005 kg/m²	yes	maybe
Microhardness (cross-sectional)	yes (direct)	yes (indirect)	yes	yes	sections	0.15×10^3 kg/m³	yes	not known
Polarized light	no	only qualitative	no	yes (no if single section technique)	sections	0.3×10^3 kg/m³	yes	no
Light Scattering	no	yes (indirect)	no	no	whole tooth	0.02 kg/m²	yes	no
Chemical analysis	yes (direct)	yes (direct)	no	yes	whole tooth	0.02×10^{-6} kg	no	yes
Iodine absorption	no	yes (direct)	no	no	whole tooth	0.01 kg/m²	yes	yes
Iodide permeability	no	only qualitative	no	no	flat surface/ whole tooth	0.005 kg/m²	yes	not known

2.6 STATISTICAL ANALYSIS OF MEASURED PARAMETERS

The following method of statistical analysis of data obtained from the intra-oral model was suggested by Mr W.H. Gilmour, Department of Public Health, Glasgow University.

Microradiography and quantitative microdensitometry yield the following data for each phase of experiment.

Week 0 data	Week "*x*" data
Δz (%vol.mineral x μm)	Δz (%vol.mineral x μm)
Surface zone (SZ) (%vol. mineral)	Surface zone (SZ) (%vol. mineral)
Lesion Body (LB) (%vol. mineral)	Lesion Body (LB) (%vol. mineral)

Each of these three measurements (Δz, SZ, LB) were analyzed independently and compared to data from the crossover phase(s) of the experiment.

For each phase the *week "x" - week 0* values were calculated for Δz, surface zone (SZ) and lesion body (LB) for each lesion for each subject in the trial. Initial analysis of this type of data suggested that the baseline lesion size (*week 0* values) would be a suitable covariate. A standard method of analysis would be to use the arithmetical mean *week "x" - week 0* value for each subject and the mean *week 0* lesion size as a covariate in an analysis of variance to

determine significance between treatments. However, this method makes two assumptions:-

1 the relationship between the *week "x" - week 0* change and the *week 0* lesion size is the same for each subject.

2 the relationship between the *week "x" - week 0* change and the *week 0* lesion size is the same for each treatment.

Formal statistical assessment of these hypotheses suggested that the relationship between *week "x" - week 0* change and *week 0* lesion size is not the same for all subjects or all treatments and that the size of the difference between treatments will change for lesions of different baseline sizes. Therefore, another method of analysis was devised using a predicted change model with separate regression lines for each subject and each parameter.

2.6.1 The Predicted Change Model

Plots were constructed for *week "x" - week 0* changes against *week 0* values for each subject for Δz, surface zone and lesion body data for each phase of the experiment. Separate linear regressions were calculated for all groups of data. Using these regression lines predicted values for changes in mean *week 0* Δz, surface zone and lesion body data could be determined for each phase of the experiment (figure 2.15). The mean week 0 values were calculated by adding up all *week 0* values for each parameter in the trial and dividing by the total number of lesions. This was, therefore, a trial mean *week 0* value as opposed

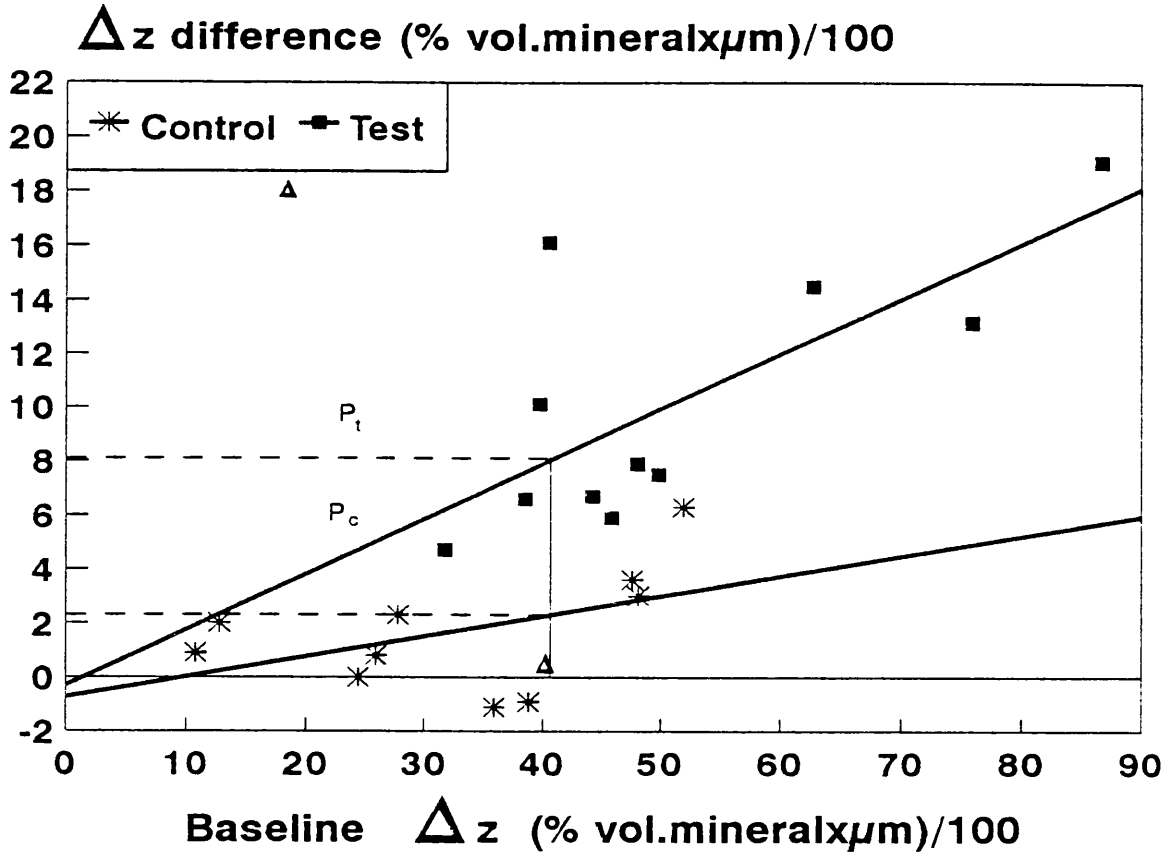


Figure 2.15 Plot of Δz difference [(*week "x" - week 0*) x -1] against baseline Δz for each lesion for test and control protocols for one subject. P_t and P_c represent the predicted changes for test and control protocols respectively for that subject.

to a phase or subject mean. Predicted changes for each of Δz, surface zone and lesion body for each subject in each phase of the experiment are, therefore, determined for a constructed regression line made from observations of lesion behaviour for each subject in each phase. The number of observations of lesion behaviour will be one factor in determining the accuracy of the fit of the regression line to the data. It became apparent that each predicted value for change of a lesion of mean *week 0* size should be weighted.

2.6.2 Weighting Of Predicted Values For A Lesion Of Mean Week 0 Size

The square root of the number of lesions on which the regression line was based was used as a method of weighting for predicted changes prior to analysis. This would ensure that the standard error of the estimate of predicted change was inversely proportional to the square root of the number of lesions. It would have been possible to use other methods of weighting, such as the inverse of the standard deviation of the predicted change but it was suggested that the square root of the number of lesions was most appropriate.

2.6.3 The Mean Change Model

An additional method of analyzing this data used the arithmetical mean change between *week 0* and *week "x"* for all lesions analyzed for each subject in each phase. However, this method was unable to compare the change for a lesion of mean trial baseline size for each subject. Comparison of subject

performance based on lesions of different sizes is complicated, hence the use of the predicted change model.

2.6.4 Incorporating A Period Effect

All studies using this intra-oral model should ideally be of a random crossover design and, therefore, subjects were randomly assigned to undertake either a test or control protocol during the first phase of the experiment. Significant differences between all test and all control results for phase one and all test and all control results for phase two represent a period effect (Armitage & Berry, 1987). A period effect, when present, was used to adjust the predicted values for one phase prior to comparison with another. Whilst this is not a method of weighting results, it does permit their adjustment prior to analysis.

2.6.5 Analysis of data

Weighted predicted changes for each of Δz, surface zone and lesion body for each subject are analyzed for differences between protocols using analysis of variance (ANOVA) to determine two sided significance tests.

CHAPTER 3 AN *IN SITU* TRIAL TO DETERMINE THE EFFECT OF SUCROSE-CONTAINING GUM ON REMINERALISATION OF ARTIFICIAL ENAMEL LESIONS

3.1 INTRODUCTION

Several clinical studies have been undertaken to determine the effect of sucrose-containing chewing gum on both caries incidence and plaque pH response. The studies relating to caries incidence are summarized in tabulated form in Table 3.1. In general, they are parallel studies with matched baseline groups. The gum chewing protocols are variable, as are methods for determination of caries incidence. In some instances results are presented as a change in DMF from baseline levels, in other instances they are presented as a change in caries incidence relative to other protocols.

Additional work by Jensen & Wefel (1989) has provided information concerning the effects of sucrose-containing gum on plaque pH responses. Using interproximal telemetric appliances with glass pH electrodes, Jensen & Wefel (1989) were able to demonstrate the ability of sucrose-containing gum, when chewed for 20 min after a meal, to restore the plaque pH to baseline levels. Furthermore, the pH remained constant for up to 100 min after cessation of chewing. The results obtained with sucrose-containing gum were no different from those obtained with sorbitol-containing gum.

Table 3.1

Summary of clinical trial data to determine the effect of sucrose-containing chewing gum use on dental caries

Reference	Duration	Protocol	n	Result	Comments
Finn & Jamison 1967 Effect of a dicalcium phosphate chewing gum on caries incidence in humans. JADA 74 Pg 987	30 month results	Deaf/blind children, 6-18 yrs old. Divided into 3 matched groups at baseline with respect to age, sex and DMFS. One group on each of sugar-free gum, sucrose-containing gum and sucrose gum with 1% dicalcium phosphate added	416	33% increase in caries increment with sucrose-containing gum compared to sugar-free gum	No negative control Results quoted in relation to sucrose-containing gum ?ethics of a study on deaf/blind children
Slack *et al.*, 1972 The effect of chewing gum on the incidence of dental diseases in Greek children BDJ 133 Pg 371	3 year study	Two groups of children aged 11-13 yrs. No significant difference in baseline levels of disease. Test group chewed 5 sticks of sucrose-containing gum per day for 3 yrs. Control group chewed no gum. Clinical and standardised radiographic examination every 6 months	228 test group 236 control group	No evidence of an increase in caries incidence in the test group	Very detailed results Some difficulty with the reproducibility of the clinical scoring
Wilson, 1975 The effect of calcium sucrose phosphate chewing gum on caries incidence in children. J.Wisconsin Dent. Assoc. 51 Pg 521-525	2 year study	Double blind trial with children in mixed dentition. Compare a sucrose-containing gum with a test gum with 5% of sucrose replaced by calcium sucrose phosphate. Each protocol required 3 sticks to be chewed after meals and 2 sticks between meals for at least 10 minutes	247 subjects	Significant decrease in caries increment in calcium sucrose phosphate group compared to sucrose group	No data on examiner reproducibility/reliability Examinations performed with reference to previous data Clinical examination only, no radiographs

Table 3.1 (Contd.)

Summary Of Clinical Trial Data To Determine The Effect Of Sucrose-Containing Chewing Gum Use On Dental Caries

Reference	Duration	Protocol	n	Result	Comments
Glass, 1981 Effects on dental caries incidence of frequent ingestion of small amounts of sugars and stannous EDTA in chewing gum Caries Res 15 Pg 256-262	2 year study	7-11 yrs schoolchildren with non-fluoridated water. Divided into 3 matched groups. Group 1 chewed sucrose-containing gum twice per day for two years. Group 2 chewed no gum. Group 3 chewed sucrose-containing gum with 0.05% SnEDTA added.	503 children 167 - group 1 157 - group 2 182 - group 3	36% increase in caries incidence with sucrose-containing gum compared to no gum No significant difference between no gum and gum with 0.05% SnEDTA	Large standard deviations in caries increments exceeding the mean observation in many cases
Makinen *et al.*, 1993 Caries preventative effects of polyol-containing chewing gums JDR 1993 Abstract 1945	28 month data	On-going trial with Belieze schoolchildren to assess differences between sucrose-, sorbitol- and xylitol-containing chewing gum and no gum. Gum chewed *ad libitum*	?	Substantial increase in caries incidence with sucrose-containing gum	Data quoted as WHO classified DMFS data. Concern expressed by scientific community (Fejerskov 1993 IADR meeting) re. validity of examination procedures.

Clinical studies, to date (see Table 3.1), indicate a wide variation in observed response to chewing sucrose-containing gum. Difficulty exists in relating the results of one clinical trial to another as standardized experimental methods have not been used. The use of an intra-oral model provided an opportunity to study the effects of sucrose-containing gum on dental caries without the problems involved with a further clinical trial. In addition, the intra-oral model allowed mineral changes within lesions to be studied.

An intra-oral study to determine the effects of chewing sucrose-containing gum on remineralisation of artificial caries has been undertaken previously by Creanor *et al.* (1992). The results of this work showed a strong trend towards increased remineralisation with the use of sucrose-containing chewing gum in conjunction with a fluoridated toothpaste, compared to the use of a fluoridated toothpaste alone. Of the three parameters measured (Δz, surface zone and lesion body mineral contents), only lesion body showed a significant difference in favour of the chewing gum protocol ($p = 0.02$). The size of the data set in this trial was felt to be too small to easily observe any differences between test and control protocol and this additional study was planned with the specific aim of increasing the size of the data set. The aim of this work, therefore, was to determine further the effect of chewing sucrose-containing gum on remineralisation of artificial caries using additional *in situ* model data from a different group of volunteers in conjunction with a previous data set (Creanor *et al.* 1992). In addition, this data could be analyzed further to determine the presence of any lesion size effects as described by Strang *et al.* (1987).

This experiment was conducted whilst volunteers used a fluoridated toothpaste. The rationale behind this was:-

1 To maintain the protocol of the original experiment.

2 Repeated exposure to sucrose may promote caries in the natural teeth of volunteers. A fluoridated toothpaste would, hopefully, prevent this.

3 The common use of fluoridated toothpaste in the UK suggests that any effect observed would need to be in addition to that of normal toothpaste use to be of benefit to the majority of the UK population.

3.2 METHOD AND MATERIALS

Following approval by the local Ethics Committee (See Appendix I), sixteen additional volunteers were recruited for a further study to determine the effect of sucrose-containing gum chewing on artificial lesion remineralisation using an *in situ* model. The size of this group was suggested by Mr W.H. Gilmour, Department of Public Health, Glasgow University, to be sufficient to obtain enough data to permit a valid statistical analysis of the results, although only thirteen volunteers completed both phases of this two phase experiment. The choice of subjects was made according to the criteria discussed in Chapter 2.

The general methodology for this study has been discussed in Chapter 2. The study was a randomised two-way crossover design with the following protocols:-

1 TEST PROTOCOL: NaF paste and 5 sticks of sucrose-containing chewing gum per day. The chewing gum was to be chewed for a timed 20 minutes after each of breakfast, mid-morning snack, lunch, mid-afternoon snack and evening meal.

2 CONTROL PROTOCOL: NaF paste, no gum but maintain the same dietary pattern as above.

Volunteers were asked to wear appliances for seven weeks for each different protocol. The appliances were worn continuously, including eating and sleeping, and only removed whilst subjects cleaned the lingual aspect of the lower teeth. Volunteers were asked to brush their teeth twice daily using a pea-sized amount of 0.32% NaF dentifrice (Crest Decay Prevention Formula, Proctor and Gamble, Eggham, Surrey) (figure 3.1). In addition, all volunteers were asked to snack mid-morning and mid-afternoon with a piece of chocolate, a biscuit or some other cariogenic food.

FIGURE 3.1 Photograph of 0.32% sodium fluoride dentifrice used in *in situ* study to determine the effect of sucrose-containing gum on remineralisation of artificial carious lesions

This additional study was identical to the previous study (Creanor *et al.*, 1992) in all aspects except for:-

1 This study used 0.32% NaF dentifrice, the previous study used 0.24% NaF dentifrice. There was only one NaF formulation dentifrice on the UK market at this time and the manufacturers changed the concentration of fluoride between the two studies.

2 This study employed an additional, increased set of volunteers. The original study (study 1) had results from 12 volunteers, this study (study 2) had results from 13 volunteers. No subjects were common to both studies.

3 The criteria for enamel section allocation to volunteers in this study was more rigid than the previous study in an effort to match up baseline lesion size for each subject in each phase (see Chapter 2 section 2.3.7).

Appliance construction, baseline microradiography, section allocation to volunteers, microradiography, microdentsitometry and subsequent statistical analysis were all performed as described in Chapter 2.

Additional statistical analysis was performed to provide a combined result from the two trials. Since the two trials have slightly different protocols, the data were <u>not</u> pooled to give a single sample size of twenty five. Instead, a separate estimate of the difference between test and control was obtained for

each trial and these estimates were combined, using meta analysis (Fleiss, 1993), by taking their weighted average using the sample size for each trial as the weight. The standard error of their weighted average was also calculated, and the ratio of the weighted average to its standard error was treated as a Standard Normal Deviate. Independence of the studies is assured as there were no volunteers common to both studies.

3.3 RESULTS

3.3.1 Predicted Changes For Δz, Surface Zone (SZ) And Lesion Body (LB) Mineral Contents

The full set of predicted changes for the second sucrose-containing gum study are presented in Table 3.2. The test data represent the predicted changes for the fluoride dentifrice and sucrose-containing gum protocol and the control data represent the predicted changes for the fluoridated dentifrice alone protocol.

Table 3.2a shows the data for the predicted Δz values which were determined according to the method described in Chapter 2 (Section 2.6). A negative value for the predicted change indicates remineralisation as the Δz value has decreased over a seven week appliance wearing period. The difference between predicted changes (test - control) is also shown in Table 3.2a. A negative difference indicates more remineralisation with the control protocol

compared to the test protocol and *vice versa*. The 95% confidence intervals indicate the possible spread of the difference between test and control predicted changes.

The data in Tables 3.2b and 3.2c show data for surface zone and lesion body predicted values respectively. In both cases these measurements represent mineral content at a point within a lesion and were calculated in the same way as Δz data. However, a positive predicted change indicates increased mineralisation, whereas, a negative value indicates decreased mineralisation. A negative value for the difference between predicted test and control indicates greater mineral deposition for the control phase.

In addition, the overall results of the first sucrose-containing gum study (study 1) (Creanor *et al.*, 1992) are presented for comparison (Table 3.3).

Table 3.2

Predicted changes, for each volunteer in study 2: (a) Integrated mineral loss (Δz), (b) Surface-Zone (SZ) mineral content and (c) Lesion-Body (LB) mineral content for Test and Control. (n=number of lesions)

Table 3.2a Integrated mineral loss

Subject	Test n	Test change	Control n	Control change	Difference	95% C.I. for difference
Δz (%vol.min. x μm)/100						
A	7	-3.85	11	-7.09	3.24	-0.34 to 6.81
B	8	-2.98	10	-6.25	3.27	-4.78 to 11.32
C	9	-11.32	8	-6.32	-5.00	-10.70 to 0.71
D	8	-4.27	9	-6.10	1.82	-2.03 to 5.67
E	8	-5.28	9	-0.94	-4.34	-11.48 to 2.81
F	9	-3.85	10	-4.39	0.54	-10.08 to11.16
G	9	-12.24	9	-14.05	1.80	-3.32 to 6.92
H	10	-5.92	10	-0.18	-5.74	-9.91 to -1.58
I	8	-5.43	8	3.03	-8.47	-19.01 to 2.08
J	8	-7.32	8	-6.31	-1.02	-7.42 to 5.39
K	8	-14.16	8	-10.20	-3.96	-10.63 to 2.71
L	6	-2.87	6	0.51	-3.39	-9.43 to 2.66
M	8	-10.43	8	-11.81	1.47	-2.68 to 5.62

Note: A -ve value for the Δz predicted change indicates remineralisation

Table 3.2b Surface zone

Subject	Test n	Test change	Control n	Control change	Difference	95% C.I. for difference
Surface Zone mineral content (%vol.min.)						
A	7	-1.3	11	2.08	-3.37	-7.48 to 0.74
B	8	0.69	10	0.36	0.33	-6.12 to 6.79
C	9	0.06	8	3.14	-3.08	-9.38 to 3.23
D	8	-3.71	9	3.73	-7.45	-12.12 to -2.78
E	8	2.53	9	2.87	-0.34	-2.69 to 2.02
F	9	1.17	10	5.65	-4.48	-10.02 to 1.07
G	9	3.99	9	6.45	-2.55	-7.11 to 2.19
H	10	1.31	10	-1.54	2.84	-1.67 to 7.36
I	8	3.08	8	-2.10	5.18	-1.02 to 11.38
J	8	4.94	8	5.07	-0.13	-7.01 to 6.76
K	8	3.97	8	4.36	-0.39	-5.62 to 4.84
L	6	-0.53	6	0.57	-1.09	-8.64 to 6.45
M	8	7.65	8	10.16	-2.52	-5.84 to 0.81

Note: A -ve value for SZ predicted change indicates demineralisation

Table 3.2c Lesion body

Subject	Test n	Test change	Control n	Control change	Difference	95% C.I. for difference
Lesion Body mineral content (%vol.min.)						
A	7	4.78	11	7.92	-3.14	-6.9 to 0.62
B	8	7.98	10	4.60	3.38	-2.14 to 8.89
C	9	10.77	8	9.80	0.97	-4.41 to 6.34
D	8	4.35	9	3.45	0.81	-3.52 to 5.14
E	8	6.4	9	-0.64	7.03	3.21 to 10.85
F	9	5.24	10	4.74	0.50	-7.11 to 8.11
G	9	13.25	9	14.93	-1.68	-7.26 to 32.89
H	10	5.67	10	-0.29	5.96	2.11 to 9.81
I	8	4.42	8	2.66	1.76	-3.03 to 6.55
J	8	9.81	8	8.91	0.90	-4.58 to 6.38
K	8	8.95	8	9.46	-0.51	-5.36 to 4.35
L	6	6.57	6	2.26	4.31	-12.13 to20.76
M	8	15.36	8	12.54	2.82	-1.71 to 6.81

Note: A -ve value for LB predicted change indicates demineralisation

Table 3.3

Summary of results for both sucrose-containing gum studies

	Weighted Mean Predicted Change			95 % C.I. for the Difference in Weighted Mean Predicted Changes	p (2-sided)
	Gum	Control	Diff		
Study 1					
Δz	7.43	4.38	3.05	-1.45 to 7.55	0.16
SZ	-0.76	-0.57	-0.19	-3.60 to 3.22	0.90
LB	6.11	2.81	3.30	0.69 to 5.91	0.02
Follow up study					
Δz	6.93	5.45	1.47	-0.85 to 3.79	0.19
SZ	1.84	3.17	-1.33	-3.27 to 0.61	0.16
LB	7.97	6.21	1.76	0.01 to 3.51	0.05

Δz - (% vol. mineral x μm) / 100
SZ - surface-zone mineral content, % vol. mineral
LB - lesion-body mineral content, % vol. mineral

3.3.2 Analysis Of Predicted Changes

Analysis of variance of test and control predicted changes for each study demonstrated independently, significantly increased lesion body remineralisation for the sucrose-containing gum chewing protocol compared to the use of a fluoridated dentifrice alone ($p = 0.02$ - study 1 and $p = 0.05$ - study 2; Table 3.3). However, the statistical analysis of predicted changes for the Δz and surface zone measurements failed to demonstrate any significantly increased remineralisation for the test protocol compared with the control protocol.

No evidence of a period effect was observed in the second study. The p-values for the probability of a period effect in the analysis of variance were 0.45 for Δz, 0.56 for surface zone, and 0.58 for lesion body predicted changes.

The results of meta analysis on the data from the two independent studies are shown in Table 3.4. The use of sucrose-containing chewing gum in conjunction with a fluoridated dentifrice produced statistically significantly increased remineralisation for Δz ($p = 0.046$) and lesion body ($p = 0.0004$) compared to the use of a fluoridated dentifrice alone, in this group of volunteers. The results for the surface zone predicted changes indicated a trend for more remineralisation with the control protocol compared to the test protocol although this result was not significant ($p = 0.32$).

Table 3.4

Pooled results from the two sugared gum studies.

	Study 1				Follow up study				Combined data		
	Treat. Effect	SE	n	p 2-sided	Treat. Effect	SE	n	p 2-sided	Treat. Effect	SE	p 2-sided
Δz	3.05	2.02	12	0.16	1.47	1.07	13	0.19	223	111	0.046
SZ	-0.19	1.53	12	0.90	-1.33	0.89	13	0.16	-0.78	0.87	0.32
LB	3.30	1.17	12	0.02	1.76	0.80	13	0.05	2.50	0.87	0.0004

Δz - (% vol.mineral x μm) / 100
SZ - surface zone mineral content, % vol. mineral
LB - lesion body mineral content, % vol. mineral

3.3.3 Further Analysis Of Predicted Changes

Table 3.5 shows the data for mean baseline Δz, SZ and LB and their predicted changes for both test and control protocols in each phase of both sugar-gum studies. These data are also presented as graphs in figure 3.2. Data for predicted changes were calculated by determining the arithmetical mean of the weighted predicted change for each volunteer for each protocol. The weighting used was the square root of the number of lesions for each volunteer.

3.3.4 Analysis Of Results To Determine A Lesion Size Effect

Simplicity of analysis and an increased data set results from analysis of actual lesion changes rather than predicted changes. In addition, actual lesion changes were used in previous work to determine the relationship between baseline lesion size and the change in mineralisation over a period of intra-oral wear (Strang *et al.*, 1987). Data were available for Δz, surface zone and lesion body measurements for 207 lesion in the first study and 270 lesions in the second study. Graphs plotted of baseline lesion size against the actual change over the seven week intra-oral period are plotted in figures 3.3 and 3.4. Linear regression analysis of this data is presented in Table 3.6 in the form of regression equations. These equations take the form of $y = mx + c$ where "m" represents the gradient of the slope and "c" represents the intercept with the y-axis.

Correlation is best for the Δz measurement ($r = -0.53$ to -0.71) and less for surface zone and lesion body measurements. In each trial, lesions with a larger Δz measurement tended to undergo greater amounts of remineralisation. Lesions with a higher mineral content in the surface zone tended to loose mineral over the period of intra-oral wear. In general lesions with a lesion body greater than 60 %vol.min. tended to demineralise, whereas lesions with a lesion body less than this tended to remineralise.

The slopes of the regression lines indicated in Table 3.6 are similar for the Δz parameter in both sugar gum studies with a gradient of approximately -0.3. Data for surface zone measurements tended to be similar for test and control protocols within a study, but differences were apparent between studies. The largest differences between the slopes of test and control regression lines were observed with the lesion body measurements and may, indeed, reflect the significant differences between test and control predicted changes observed for this data in both sugar gum trials.

Table 3.5

Comparison of mean baseline Δz, SZ and LB values and predicted changes for the two sugar gum trials

		1st Sugar-Gum Trial				2nd Sugar- Gum Trial			
		n	mean baseline Δz	SD	Predicted Change	n	mean baseline Δz	SD	Predicted Change
Phase 1	Test	45	50.1	16.8	-11.15	64	45.05	15.00	-7.19
	Control	45	49.6	17.8	-8.79	68	45.01	11.16	-6.26
Phase 2	Test	60	34.4	18.5	-4.71	68	46.57	11.87	-8.28
	Control	57	32.7	18.5	0.60	70	42.08	13.23	-6.08
Totals and means		207	40.66	17.98		270	44.65	12.90	

		1st Sugar-Gum Trial				2nd Sugar- Gum Trial			
		n	mean baseline SZ	SD	Predicted Change	n	mean baseline SZ	SD	Predicted Change
Phase 1	Test	45	63.63	8.63	-2.62	64	52.46	8.47	2.85
	Control	45	63.51	8.19	-2.07	68	52.31	7.13	3.83
Phase 2	Test	60	63.30	7.24	1.04	68	53.20	7.50	2.97
	Control	57	61.88	6.72	1.69	70	53.57	8.13	3.97
Totals and means		207	63.03			270	59.92		

		1st Sugar-Gum Trial				2nd Sugar- Gum Trial			
		n	mean baseline LB	SD	Predicted Change	n	mean baseline LB	SD	Predicted Change
Phase 1	Test	45	38.72	8.17	7.90	64	38.77	8.51	7.55
	Control	45	38.38	8.62	2.68	68	38.25	6.85	5.95
Phase 2	Test	60	45.15	11.39	5.06	68	35.75	5.52	8.41
	Control	57	46.42	10.15	3.67	70	38.03	7.02	6.95
Totals and means		207	42.69			270	37.69		

Δz (%vol.mineral x μm) / 100
SZ %vol.mineral
LB %vol.mineral

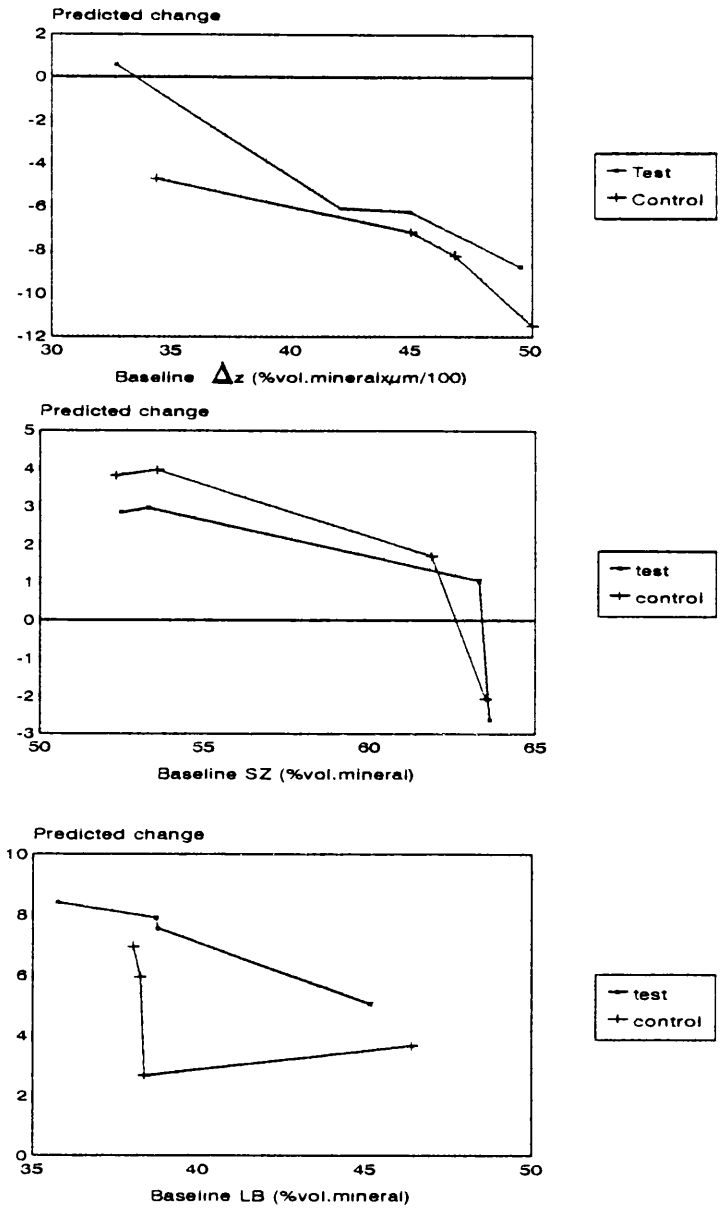


FIGURE 3.2 **Graphs of predicted lesion change against mean baseline lesion size for Δz, surface zone and lesion body for both sugar gum trials. Each trial was a randomised two phase crossover design hence two test and control results for both trials** (Test data = fluoride and sucrose-containing gum protocol, control data = the use of fluoride dentifrice alone)

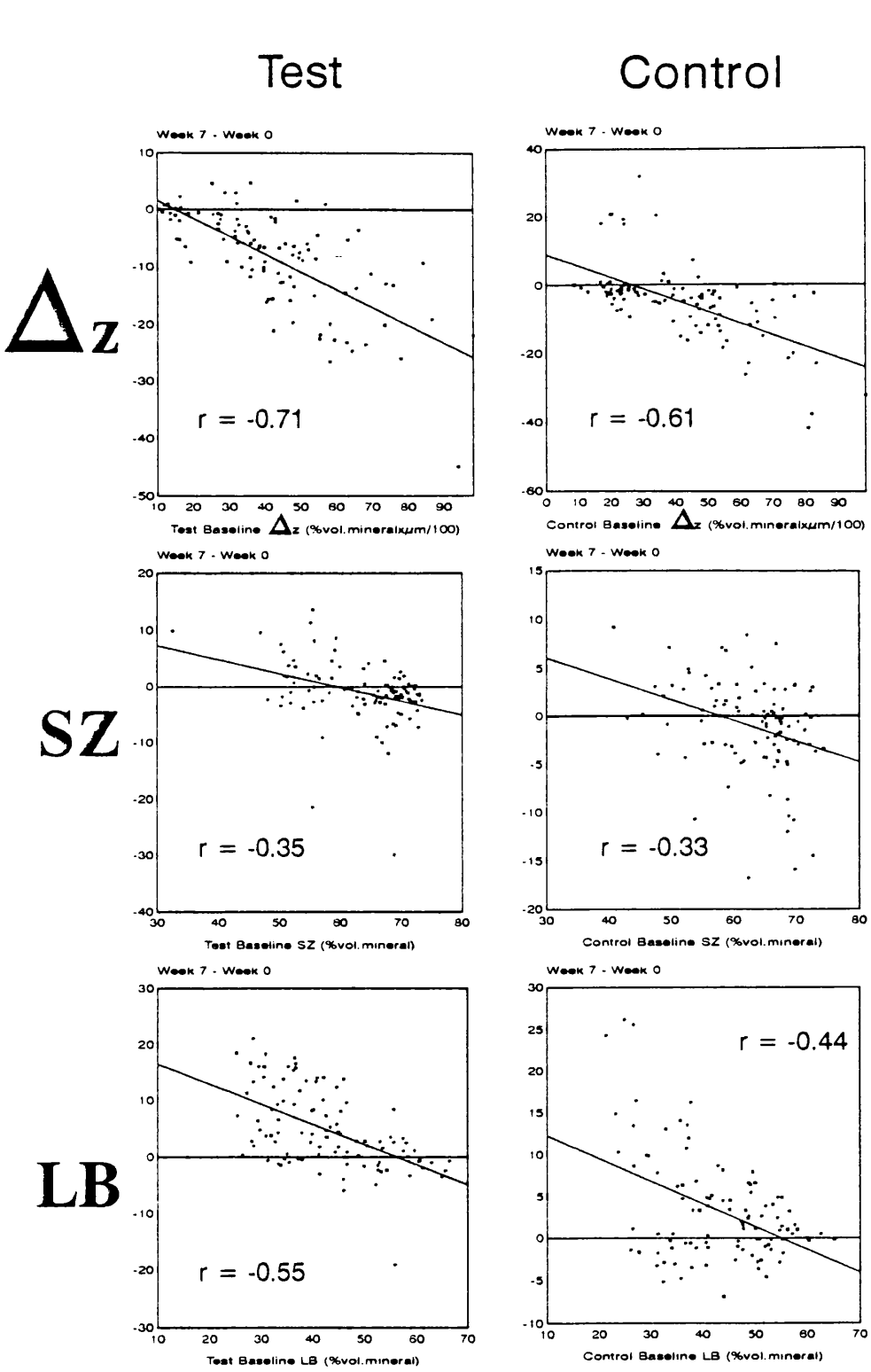


FIGURE 3.3 **Graph to show the relationship of baseline lesion size to actual mineral change over a seven week period of intra-oral wear for the first sugar gum trial.** Week 7 - Week 0 data units are the same as those units for corresponding *x*-axis values.

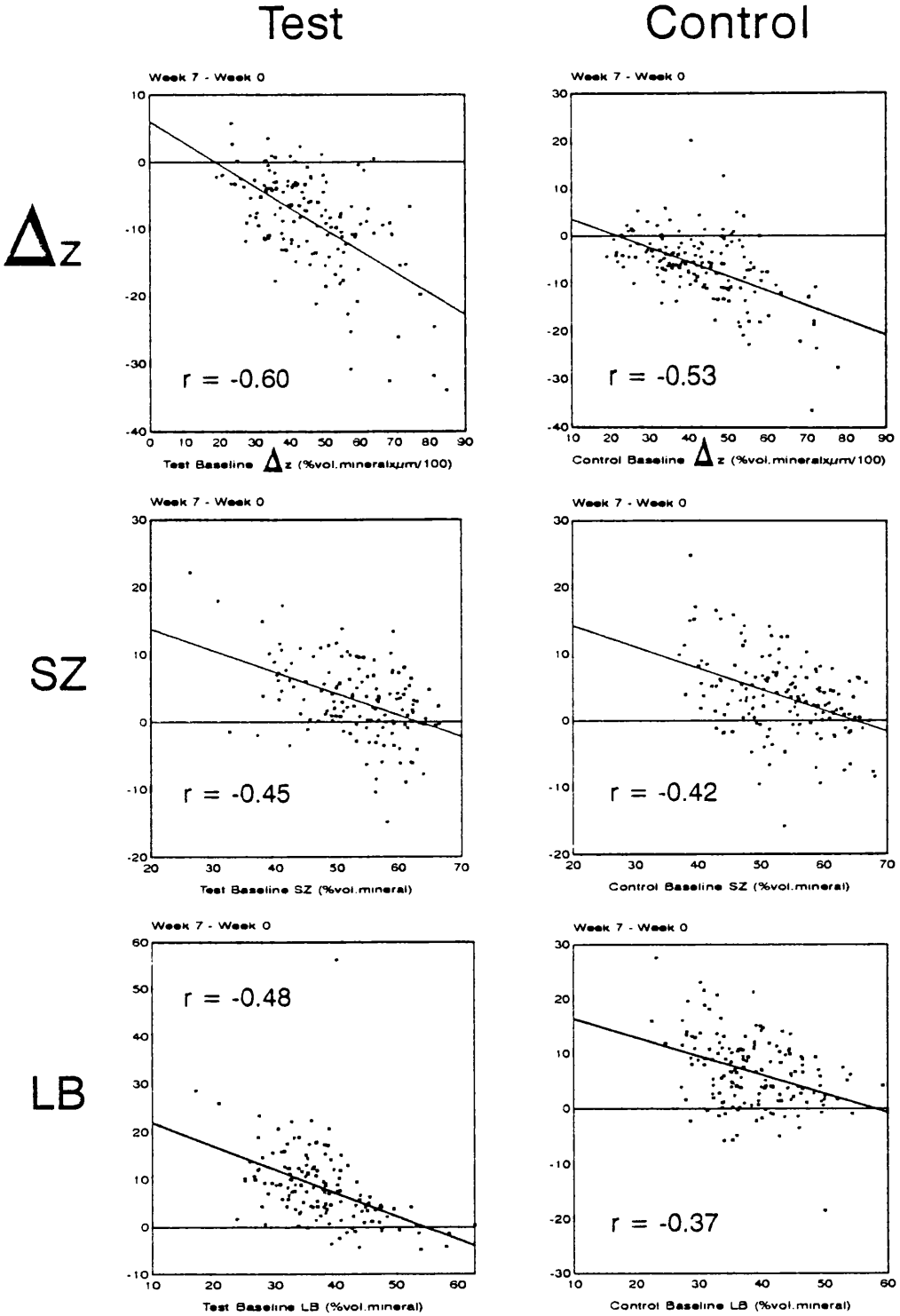


FIGURE 3.4 Graph to show the relationship of baseline lesion size to actual mineral content over a seven week period of intra-oral wear for the second sugar gum trial. Week 7 - week 0 data units are the same as those units for the corresponding *x*-axis values.

Table 3.6

Regression equations for linear regression analysis of the relationship of baseline lesion size to mineral change over a seven week period of intra-oral wear for two sugar gum *in situ* studies.

1st sugar gum study			P-value for linear regression analysis
Δz	Test	Δz difference = 4.638 - 0.310 baseline Δz	0.000
	Control	Δz difference = 8.85 - 0.333 baseline Δz	0.000
Surface Zone	Test	SZ difference = 14.7 - 0.248 baseline SZ	0.000
	Control	SZ difference = 12.6 - 0.218 baseline SZ	0.001
Lesion Body	Test	LB difference = 20.1 - 0.357 baseline LB	0.000
	Control	LB difference = 14.9 - 0.271 baseline LB	0.000
2nd sugar gum study			
Δz	Test	Δz difference = 6.00 - 0.319 baseline Δz	0.000
	Control	Δz difference = 6.57 - 0.302 baseline Δz	0.000
Surface Zone	Test	SZ difference = 20.1 - 0.318 baseline SZ	0.000
	Control	SZ difference = 20.5 - 0.317 baseline SZ	0.000
Lesion Body	Test	LB difference = 31.5 - 0.620 baseline LB	0.000
	Control	LB difference = 19.8 - 0.341 baseline LB	0.000

3.4 DISCUSSION

3.4.1 The Second Sucrose-Containing Gum *In Situ* Trial

The results of the second sucrose-containing gum study have shown a significant difference between test and control protocols for the lesion body measurement ($p = 0.05$). No significant difference between the two protocols was observed for either Δz or surface zone measurements. This result was identical to that observed in the previous study. The aim of this work was to generate additional *in situ* model data in conjunction with previous data (Creanor *et al.* 1992).

3.4.2 Combination Of Data From Two Sucrose-Containing Gum *In Situ* Trials

The results from the two studies can be combined for the purposes of statistical analysis. This method of analysis is known as meta-analysis (Boissel *et al.*, 1989; Fleiss, 1993) and is commonly used in combining results from separate clinical trials in the medical literature. A recent publication by Proskin (1992) discussed this method in relation to dental research, and more specifically intra-oral models. He suggested that meta-analysis is a powerful tool but care should be taken to determine the "combinability" of studies and presents three points for guidance:-

1 The acceptance of this method of analysis, by the professional

community, is difficult to gauge at present.

2 Any advantage to be gained by the use of meta-analytical techniques will be diminished if the data in the combined studies is correlated across the studies. That is, if the studies involve identical or overlapping subjects.

3 The amount of work involved in determining an overall conclusion will not be reduced. If a large sample size is required to draw an appropriate conclusion, then the use of several smaller, inconclusive studies not equal in size to a single large study will not provide a superior result compared to the use of the large study in the first instance.

The results of the combined data set support the trends observed in the first study and show significant differences for lesion body in favour of the test protocol ($p = 0.0004$). In addition, statistical significance has also been achieved for Δz ($p = 0.046$). These results indicate that chewing sucrose-containing gum using this protocol and with this panel of subjects, produced remineralisation beyond that which could be expected from a fluoridated dentifrice alone.

3.4.3 The Effect Of A Change In Dentifrice Concentration

The overall result from both studies was determined assuming the differences in dentifrice fluoride concentration between the studies had no effect. Meta-analysis does allow for a concentration difference to some extent. Fluoride

dose response curves for this particular model are not available for these concentrations of NaF dentifrice and so the confidence with which these values can be quoted is difficult to assess accurately. Dose response curves for NaF dentifrice have been produced for a single section model by Mellberg *et al.* (1994) but only evaluate the range 0 - 1100 ppm F (0 - 0.24% NaF). The authors quote a value of 24.8% (+/- 29.2%) remineralisation for lesions exposed to a 0.24% NaF dentifrice for a two week period. Comparable data from study 1 (Creanor *et al.*, 1992) demonstrated 10.8% remineralisation for lesions exposed to a 0.24% NaF dentifrice for seven weeks. Data from the second sucrose gum study indicate a figure of 12.2% remineralisation with the use of a 0.32% NaF dentifrice. In addition, data from a sorbitol gum study (Creanor *et al.* 1992) demonstrate 12.1% remineralisation with a 0.24% NaF dentifrice.

3.4.5 Significant Change In Δz And Lesion Body Measurements

The significant change in Δz and lesion body should be addressed in greater detail. It may be that the ultimate aim of early enamel remineralisation should be to direct mineral deposition to the deeper portions of the lesion (Creanor *et al.*, 1992). This would permit complete lesion remineralisation and a return to normal appearance. The combined results from these studies would indicate that mineral deposition has been directed to the deeper parts of the lesion as evidenced by the lesion body results.

Twice daily fluoride dentifrice exposure may result in the formation of

phosphate and protein contaminated calcium fluoride at the enamel surface (Fejerskov *et al.*, 1981; Featherstone & ten Cate, 1988; Bruun & Givskov, 1991). Access to deeper portions of the lesion can only be achieved if the surface layer is cleared of precipitated debris. This may be achieved by a decreased pH at the enamel/plaque fluid interface as a result of exposure to fermentable carbohydrate. A decreased pH may result in unsaturation of fluid in the local environment with respect to tooth mineral, thereby opening the surface pores of the lesion.

The effects of sucrose in the gum may be transitory due to increased salivary flow and elevated salivary bicarbonate levels (Dawes & Macpherson, 1992). These combined effects will increase the rate of sucrose clearance (Dawes & Macpherson, 1993) and elevate the local pH. If the local environment is undersaturated with respect to tooth mineral, elevation of the pH will help to restore saturation levels. In addition, increased salivary flow results in increased salivary saturation with respect to calcium and phosphate salts (Lagerlof *et al.*, 1983). Concentration gradients will be established between the fluid at the lesion surface and that within the deeper layers of the lesion. The lesion body has the largest pore volume (Kidd & Joyston-Bechal, 1987) and, therefore, the greatest internal surface area. Subsequent ion transfer down a concentration gradient would make this the probable site for greatest mineral deposition as evidenced by these results.

3.4.6 Comparison Of Baseline Lesion Size And Other Data Between The Two Studies

Analysis of the data for baseline lesion size is of help in comparing the two studies. There was a greater variability in baseline lesion size between the phases of the first sugar-gum study compared to the second sugar-gum study. This was observed for Δz and lesion body baseline lesion sizes but not for surface zone baseline lesion sizes. The use of a section pairing method in the second study, whereby sequential sections from the same tooth were used in each phase would appear to have reduced the variability in baseline lesion sizes between the two phases of the second study.

The comparison of baseline lesion size with predicted lesion change was demonstrated in figure 3.2. For the Δz predicted change it would appear that a larger baseline lesion size was associated with increased mineral deposition over the seven week intra-oral period. This is in agreement with work by Strang *et al.* (1987). Additional data for surface zone and lesion body measurements are less clear in their behaviour.

Data for the actual change in mineral content of lesions over a seven week period of intra-oral wear, when compared to the baseline lesion size, demonstrated a significant lesion size effect for the Δz measurement which was also in agreement with Strang *et al.* (1987). The magnitude of this effect can be judged by the gradient of the slope of the regression line. For the Δz

measurement this was approximately - 0.3 for both test and control protocols in both sugar gum studies. No obvious differences were observed in the slopes of the regression lines between test and control protocols for surface zone measurements in either sugar gum trial. This may reflect the much larger impact on lesion behaviour of fluoride in comparison to salivary stimulation. The larger difference in test and control regression line gradients for lesion body does mimic the statistical results obtained using the predicted change model. However, the low correlation coefficients for this data introduce a note of caution when interpreting this data.

The repeatability of studies with this model has not been investigated in detail. Results from these sugar-gum studies are not suited to address this question as no volunteers were common to both studies. The small numbers of volunteers in each trial will only serve to increase the effect any volunteer may have on the overall result. However, both studies have demonstrated individually remineralisation with a fluoridated dentifrice and the additional use of sucrose-containing gum. The analysis of both studies suggested that stimulating salivary flow on a regular basis, within a fluoridated environment, significantly increased remineralisation of artificial enamel lesions beyond that observed with a fluoridated dentifrice alone.

3.4.7 Summary

The observation that fluoride can remineralise artificial carious lesions in an intra-oral model in conjunction with the clinical observation that salivary insufficiency increases caries levels suggests that both factors are important in the prevention and remineralisation of early carious lesions. The relative effects of fluoride and saliva on the dynamics of the incipient carious lesion are presently unknown. This intra-oral model may provide an opportunity to attempt to answer this question. However, further work on the effect of fluoride on lesion remineralisation is required prior to determining the relative effects of fluoride and saliva. This forms the basis of the work presented in Chapter 4.

CHAPTER FOUR A PILOT TRIAL TO DETERMINE THE EFFECT OF A NON-FLUORIDATED TOOTHPASTE ON ARTIFICIAL ENAMEL LESION REMINERALISATION USING AN *IN SITU* MODEL

4.1 INTRODUCTION

The aim of this work was to determine the relative effects of fluoride on artificial enamel lesion remineralisation using an intra-oral model. As part of this work, it was important to ascertain whether the model could distinguish between fluoridated and non-fluoridated dentifrices prior to studying the effect of stimulated salivary flow on lesion remineralisation. This work has been previously undertaken by Creanor (1987) using two subjects to compare the effect of two concentrations of NaF dentifrice with a non-fluoridated dentifrice on remineralisation of artificial enamel lesions using a single section model. Analysis of Δz data demonstrated significantly increased remineralisation for both concentrations of NaF dentifrice (1000 and 1500 ppmF) compared to the non-fluoridated paste. Similar data were also available for 1000, 1500 and 2500 ppmF dentifrices as sodium monofluorophosphate dentifrice compared to a non-fluoridated placebo. On this occasion, two out of three volunteers demonstrated a significant difference between fluoridated and non-fluoridated dentifrice.

This work was repeated by Damato (1990) using the same *in situ* model with

a group of 7 volunteers to determine the relative effects of a non-fluoridated dentifrice and sodium monofluorophosphate dentifrice with fluoride concentrations of 1000 and 2500 ppmF. However, one potentially significant difference between the two studies was that no programmed cariogenic snacks were consumed between meals in the study by Damato (1990). The intra-oral period lasted for 4 weeks and the results indicated significantly increased remineralisation with the 2500 ppmF dentifrice compared to the non-fluoride dentifrice. No difference was observed between the 1000 ppmF dentifrice and the non-fluoride placebo. In the light of these results, it was decided to repeat the original work by Creanor (1987) to compare a non-fluoridated dentifrice with a sodium fluoride dentifrice using an expanded group of volunteers.

4.2 METHOD AND MATERIALS

For the purposes of this study, a parallel study design was employed to enable the use of previous seven week data from subjects using a fluoridated dentifrice alone. Following approval by the local Ethics Committee (Appendix I), six subjects wore intra-oral appliances supporting varnished sections of human enamel with artificially created carious lesions as described in Chapter 2. In keeping with the core protocol, all volunteers wore appliances continually, except for cleaning the lingual aspect of the lower teeth. Volunteers used a non-fluoridated dentifrice (Boots Non-fluoride Toothpaste, Boots the Chemist, Nottingham, England)(figure 4.1) for a 4 week period before the trial and for the duration of the trial. In addition cariogenic snacks

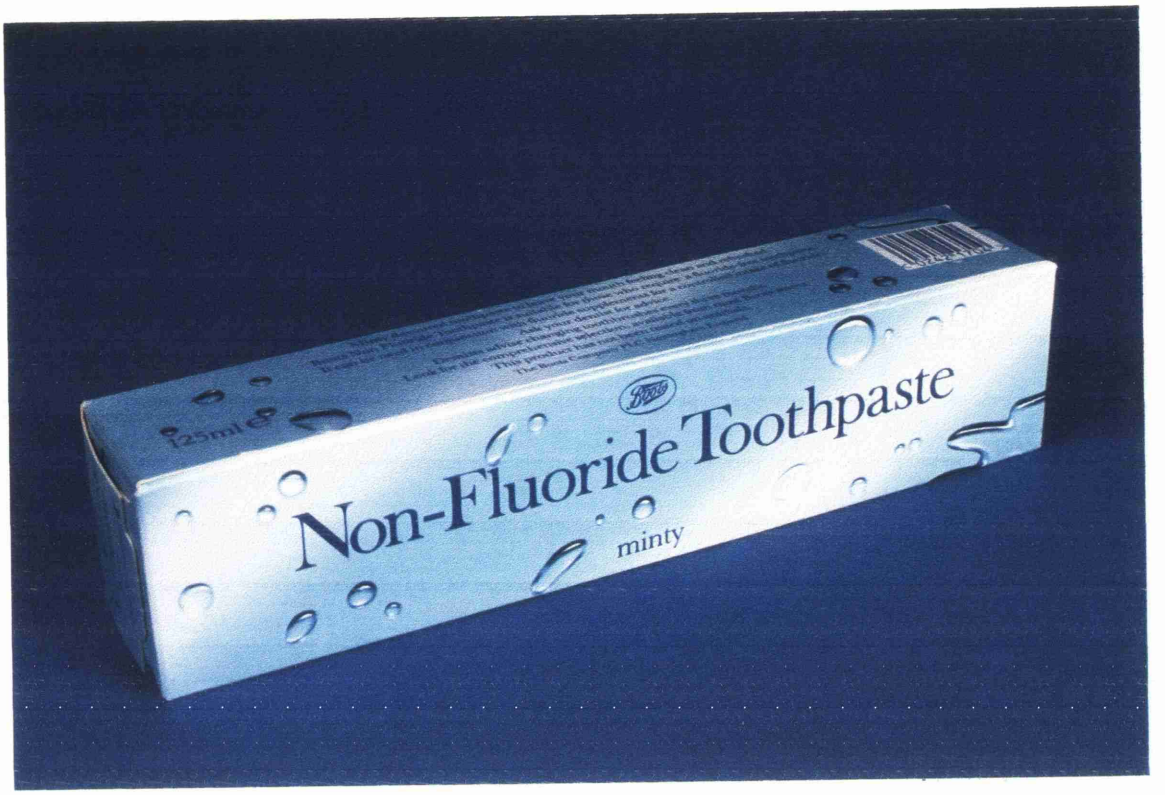


Figure 4.1 Non-fluoride dentifrice used in *in situ* trial

were consumed twice daily between breakfast and lunch and between lunch and evening meal. All volunteers wore the appliances for 5 weeks. The previous sugar-gum trial used a seven week period of appliance wear. However, the final two weeks of each phase of this trial appeared to be testing the limits of volunteer cooperation. In addition, analysis of previous data (Creanor, 1987; Damato, 1990) indicated that 5 weeks should be adequate to observe mineral changes. As additional studies were planned, it was considered appropriate to shorten the period of appliance wear for the sake of continued cooperation.

Microradiography and microdensitometry were undertaken as described in Chapter 2. Statistical analysis of these results requires further comment.

The time-discrepancy between the seven week fluoridated dentifrice results and the five week non-fluoridated dentifrice results was overcome by multiplying the five week results by a factor of 7/5. Thus, it was assumed that remineralisation or demineralisation occurred linearly over time. Creanor (1987) and Damato (1990) both showed linear demineralisation of single sections using an *in vitro* model. Damato (1994) also demonstrated linear remineralisation *in situ*. No period effect can be estimated as this was not a crossover study. In addition baseline lesions in each phase could not be matched as in previous studies. They were however, of similar average size.

The predicted changes for Δz, lesion body and surface zone, for a lesion of

average baseline size, were calculated for each subject in each phase. Paired t-tests or Wilcoxon Signed Ranks Tests were used as appropriate to determine any significant difference between the two protocols. The Wilcoxon Signed Ranks Test was used with non-normally distributed data or with small numbers of data and the paired t-tests were used when data were normally distributed. Weighted ANOVA was not used as no measurement, and subsequent adjustment, could be made for a period effect and the numbers of lesions analyzed per subject was relatively constant (between 8 and 11 lesions).

4.3 RESULTS

This relatively small data set was, on occasion, difficult to demonstrate a normal distribution. In addition, the Δz data from some individuals behaved differently to other volunteers. All these factors suggested a non-parametric analysis of data would be appropriate, hence the use of the Wilcoxon Signed Ranks test.

The mean baseline lesion size data from the two appliance wearing periods of this trial are presented in Table 4.1. Initial observations indicated the data for the two periods were well matched. The unequal size of the data sets indicated a two sample t-test would be appropriate to detect any differences between the baseline lesion sizes. No significant differences were observed between baseline Δz and SZ data but there was a significant difference between the two LB data sets ($p = 0.0001$). The results for each parameter are now described.

Table 4.1

Summary of mean baseline lesion size data for the two periods of appliance wear

Protocol	N	Baseline Δz (%vol.mineralxμm)/100		Baseline SZ (%vol. mineral)		Baseline LB (%vol.mineral)	
		Mean	SD	Mean	SD	Mean	SD
Fluoride	59	41.81	13.41	54.59	8.05	39.34	8.59
Non-fluoride	48	43.09	13.91	54.94	10.28	32.14	8.34
Total	107	42.38	13.59	54.75	9.08	36.11	9.17
p values for two sample t-test		0.68		0.89		0.0001	

4.3.1 Δz Data

The predicted changes for Δz data are shown in Table 4.2. Five subjects showed a greater remineralisation with the fluoride, compared to the non-fluoride, dentifrice. This increased remineralisation was significant for two subjects. The mean predicted change in Δz for all subjects using the fluoridated toothpaste was -6.23 (%vol.mineralxμm)/100 (14.7% remineralisation, p = 0.004). The mean predicted change in Δz for all subjects using the non-fluoridated dentifrice was 0.57 (%vol.mineralxμm)/100 (p = 0.91) The mean demineralisation observed with the non-fluoridated dentifrice was distorted by one subject with a very high demineralisation. If this subject were excluded, the mean predicted change in Δz for subjects using the non-fluoridated dentifrice became 10% remineralisation. The difference between fluoridated and non-fluoridated dentifrice was non-significant using a paired t-test (95% Confidence Interval for Fluoride vs Non-fluoride = -19.4 to +5.8, p = 0.22, 2,sided). The use of a Wilcoxon Signed Ranks Test also demonstrated no significant difference between the two data sets (p = 0.098, 2,sided).

The assessment of a lesion size effect (Strang *et al.*, 1987) was made by plotting actual lesion changes over a period of intra-oral wear against the baseline lesion size. This data is shown in Figure 4.2. Linear regression lines through a plot of the Δz change over the appliance wearing period against the baseline Δz value produced the following regression equations:-

Table 4.2

Results from non-fluoride pilot study

Subject	Predicted Change: Fluoride	Predicted Change: Non-fluoride	Difference	95% CI for difference in predicted change	Sig. Diff. in favour of:
Δz					
A	- 2.45	- 4.04	1.59	- 1.39 to 4.56	-
B	- 7.24	- 0.71	- 6.53	- 8.86 to -4.13	Fluoride
C	- 3.91	- 2.09	- 1.82	- 1.50 to 6.87	-
D	- 5.87	24.75	-30.62	-46.54 to -14.7	Fluoride
E	- 6.60	- 4.45	- 2.15	- 6.57 to 2.26	-
F	-11.32	-10.05	- 1.27	- 6.08 to 3.55	-
Lesion Body					
A	5.11	3.64	1.47	- 3.91 to 6.85	-
B	7.10	- 0.67	7.77	4.12 to 11.42	Fluoride
C	5.04	2.62	2.42	- 3.42 to 8.25	-
D	5.29	- 2.15	7.44	1.12 to 13.76	Fluoride
E	8.48	- 3.31	11.80	5.04 to 18.55	Fluoride
F	12.90	9.55	3.35	- 3.08 to 9.79	-
Surface Zone					
A	- 3.43	1.60	- 5.03	-12.48 to 2.43	-
B	3.24	1.43	1.81	- 0.59 to 4.21	-
C	3.67	- 0.57	4.23	- 0.94 to 9.41	-
D	- 0.24	- 8.09	7.85	- 4.01 to 19.71	-
E	1.27	0.88	0.39	- 4.02 to 4.80	-
F	9.37	2.05	7.32	3.43 to 11.21	Fluoride

Units for Predicted Change and Difference
Δz (%vol.mineralxμm)/100
LB %vol.mineral
SZ %vol.mineral

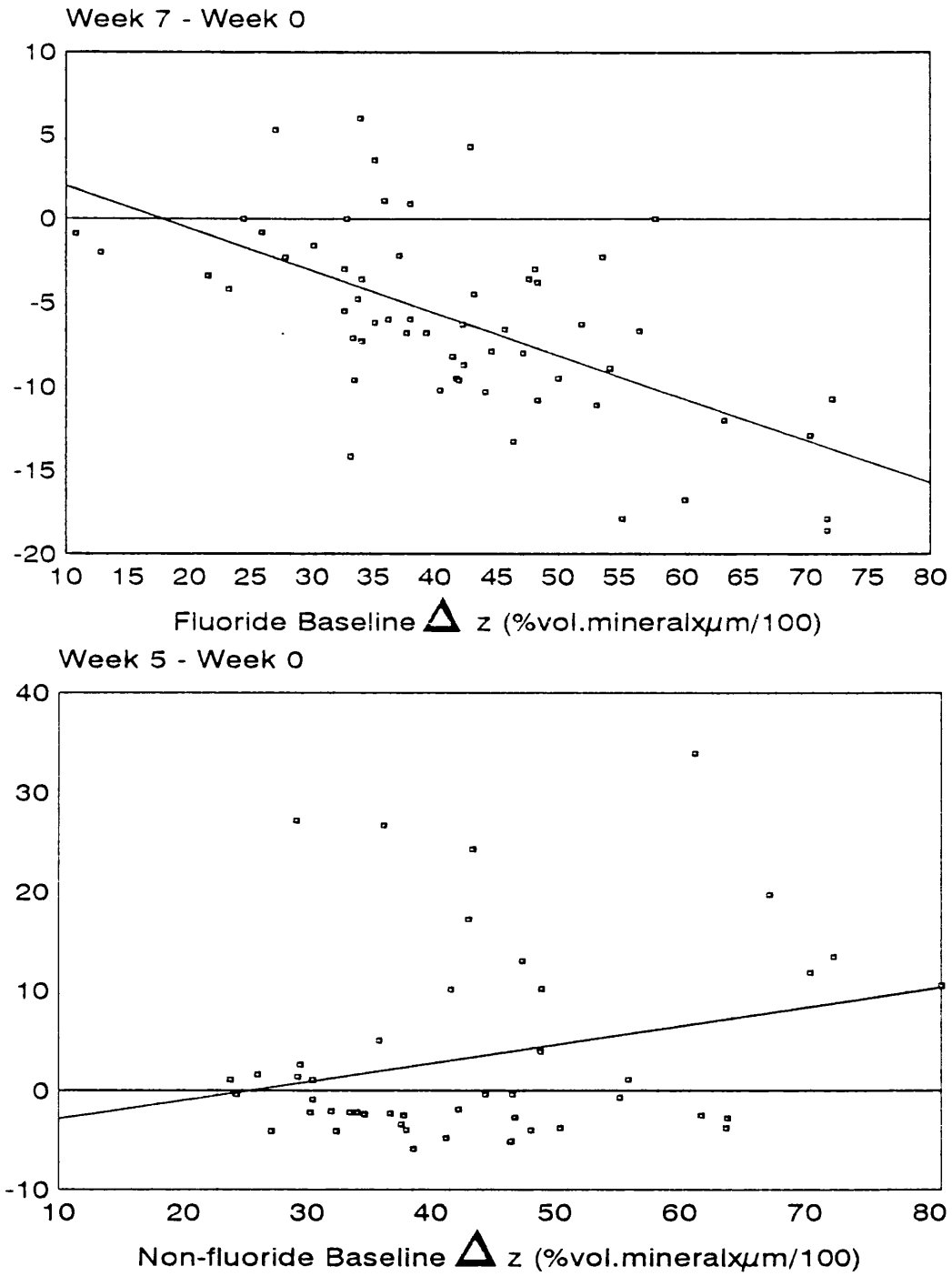


Figure 4.2 Graphs of baseline Δz data plotted against change in Δz over a period of intra-oral wear for fluoride and non fluoride protocols.

Δz Difference (Fluoride) = 4.46 - 0.252 baseline Δz ($p = 0.000$)

Δz Difference (Non-fluoride) = -0.17 + 0.007 baseline Δz ($p = 0.946$)

The significant lesion size effect for the fluoridated dentifrice indicated that a lesion with large baseline Δz underwent significantly increased remineralisation compared to a lesion with a smaller baseline Δz. The data for the non-fluoride dentifrice showed no significant relationship between mineral change and baseline lesion size.

4.3.2 Lesion Body Data

The predicted changes for each volunteer for lesion body data are shown in Table 4.2. All subjects showed greater remineralisation with the fluoridated toothpaste compared with the non-fluoridated toothpaste. This was significant in three subjects. The mean predicted change was +7.32 %vol.mineral for the fluoridated paste (20.3% remineralisation, $p = 0.002$) and +1.61 %vol.mineral for the non-fluoridated dentifrice ($p = 0.44$). The difference between fluoridated and non-fluoridated dentifrice was significant using a paired t-test (95% Confidence Interval for fluoride vs. non-fluoride = 1.5 to 9.9; $p = 0.02$, 2-sided).

4.3.3 Surface Zone Data

The mean predicted changes for surface zone data are shown in Table 4.2. Five

subjects showed greater remineralisation with the fluoridated dentifrice compared with the non-fluoride dentifrice. In one case this difference was significant. The overall mean predicted changes were +2.31 %vol.mineral for the fluoridated dentifrice ($p = 0.25$) and -0.45 %vol.mineral with the non-fluoridated dentifrice ($p = 0.79$). The difference between fluoridated and non-fluoridated dentifrice was non-significant using a paired t-test (95% Confidence Interval for fluoridated vs non-fluoridated dentifrice = -2.3 to +7.8; $p = 0.22$, 2 sided).

Comparison of these results with previous data for NaF dentifrice use is difficult as the study by Creanor (1987) used only two subjects. Data from the study by Damato (1990) are available for 6 out of a total of 7 subjects comparing non-fluoride dentifrice use with sodium monofluorophosphate dentifrice use. Data for the non-fluoride protocol were calculated as a remineralisation rate per week over a four week study. Similar data for Δz can be calculated for the present study and the two data sets compared. Data from both trials are presented in Table 4.3. Two sample t-tests were used to determine differences between the data sets and produced a p-value bordering on significance ($p = 0.073$). However, subject 4 from the present pilot study behaved in a markedly different manner form the other volunteers. Exclusion of this data in a comparative analysis between the two studies increased the p-value for the two sample t-test to 0.89 and the overall trial mean remineralisation rates became more comparable. These data are displayed in Table 4.4.

Table 4.3

Comparative subject mean Δz data for the present, pilot, study and that of Damato (1990).

Subject	Present pilot trial		Damato (1990)	
	n	Subject mean Δz remin. rate per week (%vol.mineralxμm)/100	n	Subject mean Δz remin. rate per week (%vol.mineralxμm)/100
1	8	-0.45	9	-1.82
2	8	-0.09	8	-0.95
3	8	-0.3	6	-0.34
4	8	3.55	9	-1.10
5	8	-0.76	11	0.96
6	8	-1.78	8	-0.85
Total/ mean	48	0.03	51	-0.64

Note: A -ve value for Δz indicates remineralisation

Table 4.4

Comparison of data between present non-fluoride pilot trial and that of Damato (1990) with subject 4 from present pilot trial removed

Experiment	Number of lesions analyzed	Trial mean remineralisation rate per week (%vol.mineralxμm)/100	SD of trial remineralisation rate
Present pilot trial (Subject D removed)	40	-0.674	0.927
Damato (1990)	51	-0.640	1.54

4.4 DISCUSSION

The results of this parallel design study indicate significantly increased lesion body remineralisation with the use of a 0.24% NaF dentifrice compared to a non-fluoridated dentifrice. The failure to demonstrate a significant difference for Δz data was surprising although analysis of the lesion size effect regression equations does indicate some difference in lesion behaviour between the two protocols.

This was a parallel design study and ideally should have been a single blind crossover design to maximise the statistical power with this small number of subjects. However, given the shortfall in trial design, the results do indicate the ability of this intra-oral model to demonstrate demineralisation in the absence of fluoride. This would indicate that changes in experimental protocol will affect conditions within the trough. This was a relatively simple method of gauging an effect prior to a more complex *in situ* trial.

Once again, it has been demonstrated that significant remineralisation with fluoridated dentifrice was observed in the lesion body in preference to other parameters. This may be due to the relatively stable nature of lesion fluid in the body of the lesion. Larsen & Pearce (1992) have discussed the relatively stable nature of the fluid in the lesion body compared with fluid at the enamel/plaque interface. The composition of lesion body fluid represents the average, or long-term effects of an external environment, as opposed to the

rather more changeable composition of the fluid at the surface of the lesion representing the day-to-day or even hour-to-hour changes in the local environment. In addition, the composite nature of the Δz measurement (See Appendix III) suggests an influence by changes in both lesion body and surface zone measurements. If these component parts of the lesion behave in different ways then this will affect the overall Δz result. In such circumstances it may be better to adopt a different definition of Δz such as that suggested by Mallon & Mellberg (1985) (See figure 2.15 in Chapter 2) where proportionately more emphasis is placed on the lesion body component.

The stability of lesion body fluid is in apparent contradiction to the argument for increased mineral deposition with the use of fluoridated dentifrice and sugar-gum chewing presented in Chapter 3. In this present pilot study there was no demineralisation period followed shortly by a influx of saliva supersaturated with calcium and phosphate salts induced by gum chewing. The concept of opening up surface pores to permit greater access to the deeper portions of the lesion, therefore, does not apply.

The regression equations for the Δz data requires further comment. The magnitude of the lesion size effect indicated by the gradient of the regression line suggests a difference in the mineral deposition between the two protocols. The fluoride data is essentially a sub-set of the data from Chapter 3 and it is not surprising that the lesion size effect is similar to this data. The lesion size effect for the non-fluoride data is much smaller and, although non-significant,

perhaps indicates a different type of lesion behaviour under these circumstances.

The results of a recent *in situ* trial by Damato and Stephen (1994) demonstrated a linear artificial lesion remineralisation in response to 0, 250 and 1000 ppm sodium fluoride dentifrice. This study was a double blind crossover design with 12 subjects. There was a five week intra-oral period and a 4 week washout period between period of appliance wear. The more ideal trial design and increased number of subjects probably accounts for the discrepancy between the results of Damato & Stephen (1994) and the pilot study reported in this Chapter.

The possibility of demonstrating differences between chewing and non-chewing protocols, as evidenced by the results in Chapter 3, and between fluoride and non-fluoride protocols, as evidenced by the results of this pilot study and the work of Damato & Stephen (1994), suggested that it may also be possible to demonstrate differences between the effect of stimulated salivary flow and fluoride on the remineralisation of artificial carious lesion in enamel. This formed the basis of a third *in situ* experiment described in Chapter 5.

CHAPTER FIVE AN *IN SITU* TRIAL TO DETERMINE THE RELATIVE EFFECTS OF STIMULATED SALIVARY FLOW AND FLUORIDE ON ARTIFICIAL LESION REMINERALISATION

5.1 INTRODUCTION

The potential effects of saliva on enamel caries have been discussed in Chapter 1. They are poorly understood, probably due to their complexity. Saliva is a necessity for good oral health and the absence of saliva, or states which result in objective hyposalivation, produce rampant caries (Mason & Chisholm, 1975; Dreizen, 1977; Newbrun, 1989). Normal resting salivary flow rates are difficult to correlate with caries experience at the population level (Mandel, 1974) and it may be the changes in salivary flow rate for individual subjects which provide a better correlation with caries experience (Newbrun, 1989).

The combination of mechanical and gustatory stimuli is the most potent stimulus for saliva production (Dawes & Macpherson, 1992). Flavoured chewing gum, therefore, provides an ideal salivary stimulus for observing the effects of stimulated salivary flow on artificial caries using intra-oral models. Several *in situ* studies have been undertaken using chewing gum to stimulate salivary flow (Leach *et al.*, 1989; Creanor *et al.*, 1992; Manning *et al.*, 1992; Manning & Edgar, 1992). These papers are summarized in Table 5.1. All have shown increased remineralisation of artificial enamel lesions with gum chewing. Different fluoride and gum chewing protocols make comparisons

Table 5.1

Comparison of different *in situ* trials to demonstrate remineralisation with gum chewing

	Model type	Specimen	N	Time	Protocol	Method of Analysis	Results	Discussion
Leach *et al.*(1989)	Cast silver band with buccally placed specimen on lower molar	Enamel block with artificial carious lesion created by acid buffered solution and divided into three parts - original lesion, control lesion & test lesion	10	3 weeks	Two way crossover trial. Both phases had between meal snacks and some F dentifrice (?ppmF). Test protocol had 5 sticks of sorbitol gum per day chewed for 20 min. after each ingestion of food	Create sections (80 μm thick) from each of three blocks. Imbibe each with water and use polarised light microscopy (PLM) to determine the area of the lesion with pore size >5%. Compare areas of lesions for original, control and test lesions. Microradiograph each section and compare Δz, I.B and lesion depth. All comparisons by ANOVA or paired t-tests.	PLM - sig. remin for both test and control compare to original lesion Microradiography - sig. remin for Δz, I.B and lesion depth for test and control compared to original. Effect of gum approx. double that of no gum	Each section does not act as its own control. No information on variation in lesion sizes
Manning *et al.* (1992)	Modified orthodontic bracket bonded to buccal surface lower molar	As above	9	3 weeks	Double blind randomized crossover trial with between meal snacks and 1500 ppm F dentifrice. Gum chewing after each meal and snack with either 100% sorbitol gum or 25%/75% xylitol/sorbitol gum	Create sections and analyze as above using PLM and microradiography	PLM - xylitol gum showed sig. remin. compared to original lesion. Not so for sorbitol. No difference between xylitol and sorbitol. Microradiography - sig remin for Δz and I.B measurements for xylitol and sorbitol. No difference between xylitol and sorbitol.	Model restricted to analysis of two lesions per subject.

NB. PLM = polarised light microscopy

Table 5.1 (Contd)

Comparison of different *in situ* trials to demonstrate remineralisation with gum chewing

	Model type	Specimen type	N	Time	Protocol	Method of analysis	Results	Discussion
Manning & Edgar (1992)	Orthodontic pad with two circles of ortho wire holding lesion in dacron gauze on buccal surface lower molar	As above	19	3 weeks	Two parallel studies each of a crossover design. All subjects have between meal snacks and use 100 ppm F dentifrice. 9 subjects chew sugar-gum for 30 min five times per day. 10 subjects chew sugar-gum for 20 min. five times per day	Create sections and analyze using PLM and microradiography	PLM - sig remin with sugar-gum for 30 min chew but not for 20 min chew. Microradiography - sig remin for Δz for 30 min protocol but not for 20 min protocol.	Authors suggest crossover between protocols would have been ideal. Have large variation in lesion size. Results based on 9 or 10 observations only.
Creanor *et al.* (1992)	Removable appliance with lingual trough	Varnished single sections placed 2 each side of the appliance created by buffered acid solutions.	12	7 weeks	Two independent crossover studies, each study 1100ppmF dentifrice with snacks and gum five times per day. Each study involved test and control protocols. One study with sorbitol gum and the other with sucrose gum	Baseline and 7 week microradiography. Analysis based on predicted change model.	Sorbitol gum 264 lesions. Sig remin from baseline for test and control protocols. No sig differences between test and control protocols. Sucrose gum 207 lesions. Sig remin. from baseline. No sig difference between test and control for Δz and SZ parameters. Sig remin on test for LB measurement.	Single section acts as its own control. Possible that initial drop in pH clears surface pores permitting mineral deposition to deeper parts of the lesion.

NB. PLM = Polarised light microscopy

between studies difficult. However, it is clear that salivary stimulation is not detrimental to artificial lesion remineralisation. Difficulties arise in separating the individual effects of salivary stimulation, fluoride-use and diet.

Each of these trials has been conducted in a fluoridated environment. The effects of salivary stimulation were observed in addition to the remineralising ability of fluoride. There is a relative paucity of information concerning the effects of salivary stimulation alone on artificial lesion remineralisation. To the author's knowledge this has only been addressed by one study (Leach *et al.*, 1986) which showed that stimulated salivary flow could remineralise artificial carious lesions in the absence of fluoride. This study is discussed in detail later in this Chapter.

Further difficulties with these intra-oral studies included the relatively small numbers of volunteers, presumably recruited from staff within the different research institutions. In addition, only one observation of mineral change was made for each individual. The study by Creanor *et al.* (1992) is an exception as individual results were based on the behaviour of up to 10 lesions per subject. The results of intra-oral studies may be influenced by the biased sample population, who are probably quite "dentally aware", or by outstanding individual results, affecting the overall mean. This is not an easy problem to resolve. Larger sample sizes may exclude dentally aware subjects and possibly increase the model validity or sensitivity to detect more subtle changes between protocols. Inevitably, such trials become more complex,

demanding more input from the volunteers taking part. The danger with the use of volunteers who do not retain some interest in this type of research, is non-compliance with the protocol as a result of volunteer fatigue.

The aim of this study was, therefore, to determine the separate effects of stimulated salivary flow and fluoride on the remineralisation of artificial enamel lesions using an *in situ* model. This study would also provide additional data to compare remineralisation in fluoridated and non-fluoridated environments. It was intended to compare the results of this trial with those of previous studies (Creanor *et al.*, 1992) which determined the effect of salivary stimulation in a fluoridated environment. To undertake this additional comparison within the confines of a single study was not thought to be practicable.

5.2 MATERIALS AND METHODS

Following approval by the local Ethics Committee (Appendix I), eighteen subjects were recruited for this study. Many of the subjects had taken part in the previous sucrose gum study and the non-fluoride pilot study. The study was a randomised three-way crossover design with the following protocols:-

1 0.32% NaF dentifrice (Crest Decay Prevention Formula, Proctor and Gamble, Eggham, Surrey) twice daily. Mid-morning and mid-afternoon snacks with no gum chewing.

2 Non-fluoride dentifrice (Non-Fluoride Toothpaste, Boots the

Chemist, Nottingham) twice daily. Mid-morning and mid-afternoon snacks and 5 sticks daily of sorbitol-containing chewing gum ("Orbit" peppermint flavoured chewing gum, Wm. J.Wrigley Jr. Company, Chicago, USA), one stick to be chewed for 20 min immediately after each of breakfast, mid-morning snack, lunch, mid-afternoon snack and evening meal.

3 Non-fluoride (Non-Fluoride Toothpaste, Boots the Chemist, Nottingham) toothpaste use twice daily. Mid-morning and mid-afternoon snacks with no gum chewing.

Each phase lasted five weeks with a period of four weeks between phases. Prior to wearing the appliances, all subjects were asked to use either a fluoridated or non-fluoridated dentifrice, for the duration of a four week "washout period". The choice of dentifrice was dependent upon the protocol to which each volunteer had been randomly assigned. All subjects continued to use the same dentifrice throughout the following five week period of appliance wear. The four week periods between phases were also utilised as "washout periods" so that any change in oral fluoride levels as a result of the change in dentifrice fluoride concentration would have a chance to become established. The use, and length of, "washout periods" was in accordance with recommendations by Damato (1990, 1994) and Stephen (1992) and was felt to be especially important when subjects changed from a fluoridated dentifrice to a non-fluoridated dentifrice. This particular study ran over the Christmas and New Year holidays and all subjects were returned to on a fluoridated

dentifrice over this period prior to commencing a further four week "washout period" before phase 3 in January.

The use of eighteen subjects for this study was based on statistical advice from Mr W.H.Gilmour, Department of Public Health, Glasgow University. The randomised three-way crossover was a more powerful design of trial, thereby reducing the need for large numbers of volunteers. In addition, a sample size divisible by three permitted easier analysis of the results. However, it was still necessary to reach a compromise in the numbers of volunteers and eighteen subjects was potentially insufficient. The logistics of undertaking a three-way crossover trial with more than this number were felt not to be feasible.

Artificial lesion and single section creation were undertaken as described in Chapter 2. Section allocation to volunteers followed the same basic principles outlined in Chapter 2, but required three sequential sections from each different tooth mounted in the appliance for each volunteer. A total of 216 sections were required for this experiment which necessitated batch microradiographing in excess of 750 sections. Baseline and week five microradiography and microdentsitometry as well as section placement in, and removal from, the appliance have been described previously in Chapter 2.

5.3 RESULTS

5.3.1 Introduction

All eighteen volunteers completed all three, five-week phases of this experiment. Section loss from the appliances was minimal, accounting for only three sections from all three phases. One subject broke an appliance in the first week of the first phase but this was quickly re-made with minimal loss of time. Occasional incidences of minor soft-tissue trauma occurred during the first few weeks of phase 1 but these were resolved quickly.

The mean baseline sizes for lesions measurements for the whole trial were:-

Δz = 43.0 (%vol.mineral x μm) / 100 (SD 16.0)

SZ = 52.7 %vol.mineral (SD 9.2)

LB = 35.7 %vol.mineral (SD 8.1)

5.3.2 Comparison Of Protocols By Component Lesion Measurements

Integrated Mineral Loss (Δz)

One subject (no. 14) in the trial showed dramatic demineralisation in all phases of the trial by a factor of between two and three times that of any other subject. This subject was excluded from the analysis of Δz. A further two subjects (15 and 18) produced regression lines based on lesions which were

considerably different from the experimental mean baseline Δz. In addition, the slopes of these regression lines were so steep that extrapolation to determine the effect on a lesion of mean baseline size produced results which had a profound effect on the analysis. Therefore, the analysis for Δz was performed on fifteen subjects. The mean predicted changes for each volunteer are shown in Table 5.2. Subjects 14, 15 and 18 were removed prior to analysis of variance.

Analysis of variance of mean predicted change in Δz values for fifteen volunteers revealed a non-significant difference ($p = 0.225$) between the three protocols, which, when ranked in order of ability to remineralise artificial carious lesions show the following, non-significant trend:-

Protocol	**Mean predicted mineral change**
Fluoridated toothpaste alone	2.28 (%vol. mineral x μm) / 100
Non-fluoridated toothpaste alone	0.06 (%vol. mineral x μm) / 100
Non-fluoridated toothpaste and sorbitol gum	0.30 (%vol. mineral x μm) / 100

Table 5.2

Mean predicted changes in Δz for individual subjects

Subject	Protocol		
	Fluoride dentifrice only	Non-fluoride dentifrice and sorbitol gum	Non-fluoride dentifrice only
1	-0.857	-0.804	2.987
2	0.398	-0.173	3.754
3	-5.245	0.061	-7.334
4	-1.534	-0.562	-6.979
5	-3.653	-6.957	-2.750
6	2.009	2.148	5.247
7	-1.038	1.799	1.679
8	-5.353	-2.659	1.728
9	4.007	1.272	-2.612
10	-7.118	-0.776	-8.335
11	-7.699	14.668	10.360
12	2.051	-7.515	-0.465
13	-0.299	0.762	-1.347
14	44.118	23.081	31.162
15	28.261	4.226	-1.065
16	-5.308	2.956	5.146
17	-4.540	0.345	-1.977
18	-2.862	-9.100	-26.691

Note: A negative result, in this table, for Δz indicates remineralisation.
All units are (%vol.mineral x μm) / 100

Lesion Body (LB) Mineral Content

Analysis of variance for lesion body mineral content data was performed for all eighteen subjects. No subjects demonstrated patterns of lesion behaviour which would have made a profound impact on the overall result as with the Δz data. No period effect was observed for lesion body in any phase. Mean predicted changes for each subject are shown in Table 5.3. Analysis of variance demonstrated no significant difference between any of the protocols ($p = 0.094$) but, when ranked in their order to remineralise, the following, non-significant trend could be observed:-

Protocol	**Mean predicted mineral change**
Fluoridated toothpaste alone	2.91 %vol. mineral
Non-fluoridated toothpaste alone	2.42 %vol. mineral
Non fluoridated toothpaste and sorbitol gum	0.72 %vol. mineral

Table 5.3

Mean predicted changes in lesion body mineral content for individual subjects

Subject	Protocol		
	Fluoride dentifrice only	Non-fluoride dentifrice and sorbitol gum	Non-fluoride dentifrice only
1	5.65	1.206	-6.090
2	0.395	2.317	4.715
3	6.638	-0.490	9.009
4	4.764	0.804	7.244
5	4.934	4.034	3.560
6	0.753	-1.844	0.481
7	0.424	-2.624	-1.140
8	5.448	0.439	3.253
9	-6.801	-3.779	-0.630
10	7.961	1.607	12.088
11	6.282	2.513	-0.866
12	1.030	5.594	2.739
13	-1.036	-2.090	1.769
14	3.208	0.897	0.908
15	0.309	-4.979	0.908
16	1.594	1.284	-3.523
17	2.634	-1.512	2.520
18	8.312	9.634	4.740

Note: a positive result on this table indicates remineralisation

All units are %vol.mineral

Surface Zone (SZ) Mineral Content

All subjects had data which were suitable for analysis of the surface zone mineral content. Mean predicted changes for each subject are shown in Table 5.4.

Analysis of variance demonstrated no significant difference between any of the protocols ($p = 0.448$) but, when ranked in their order to remineralise, the following, non-significant trend could be observed:-

Protocol	**Mean predicted mineral change**
Fluoridated toothpaste	1.08 %vol. mineral
Non-fluoridated toothpaste	0.63 %vol. mineral
Non-fluoridated toothpaste and sorbitol gum	0.14 %vol. mineral

Table 5.5 displays the p-values for comparison of lesion measurements in all three phases of the trial. In addition, p-values for the mean change model, previously described in Chapter 2, are also included. The data would suggest that in no case was the mean change model superior to the predicted change model in the determination of statistical significance.

Table 5.4

Mean predicted changes in surface zone mineral content for individual subjects

Subject	Protocol		
	Fluoride dentifrice only	Non-fluoride dentifrice and sorbitol gum	Non-fluoride dentifrice only
1	-1.342	-0.452	-6.766
2	4.832	-0.092	2.251
3	3.890	0.303	3.550
4	2.892	0.495	5.988
5	3.537	4.823	2.778
6	-3.167	2.724	3.199
7	0.593	-3.845	0.089
8	4.031	2.996	4.960
9	-7.172	-1.009	0.546
10	3.812	-2.159	3.709
11	1.488	0.168	-3.984
12	0.3345	4.529	1.765
13	-3.106	-5.821	-9.101
14	0.807	2.585	-1.116
15	-0.598	-7.769	0.513
16	2.797	-1.944	-2.801
17	0.185	-5.120	1.375
18	5.591	7.233	4.338

Note: A positive result in this table indicates remineralisation

All units are %vol.mineral

Table 5.5

Comparative results of the three-way crossover trial

Measured parameter	ΔZ (%vol.mineral x μm) / 100		Surface Zone Mineral Content (%vol.mineral)		Lesion Body Mineral Content (%vol.mineral)	
Number of Subjects in analysis	15		18		18	
Method of analysis	Mean change ANOVA	Predicted change ANOVA	Mean change ANOVA	Predicted change ANOVA	Mean change ANOVA	Predicted change ANOVA
p value of period effect	0.462 (n=18)	0.379	0.924	0.912	0.497	0.679
p value of difference between protocol without period effect	0.286 (n=18)	0.225	0.260	0.448	0.200	0.094
Ranking of means according to protocol		Fluoride=-2.2786 Non-Fluoride+Gum=0.304 Non-Fluoride=-0.0599		Fluoride=1.0782 Non-Fluoride+Gum=-0.14 Non-Fluoride=0.6274		Fluoride=2.914 Non-Fluoride+Gum=0.72 Non-Fluoride=2.4212

5.4 DISCUSSION

The results form this trial showed no significant differences between any of the protocols when using either a predicted or a mean change model. This was not as expected considering the results from previous work, the changes in experimental protocol, which had been designed enable a better comparison between phases, and the randomised crossover design of the trial.

The ideal protocol would have included a fourth phase using a fluoridated dentifrice with sorbitol gum. However, this was felt to be beyond the limits of cooperation of the volunteers. In addition, data was already available for the fluoride dentifrice and sorbitol gum protocol from a previous trial (Creanor *et al.*, 1992) and it was possible this could have been used for comparative analysis with data from this study.

The present experiment started in September 1993 and finished in March 1994. The total length of time involved was 27 weeks which included three, 4-week "washout" periods and three 5-week appliance wearing periods with a 2 week break over Christmas and New Year. Most subjects were pleased when the trial had ended which suggested they were becoming somewhat fatigued. Compliance with experimental protocol was taken on trust. Whilst there was no way of determining accurately subject compliance, the results from the third phase of the experiment suggested no significant change in volunteer behaviour compared with other phases.

It is interesting to note that, once again, despite no statistically significant differences between protocols, the parameter closest to achieving significance was lesion body. Comparison with data in Chapters 3 and 4 has shown lesion body measurements to consistently show significant differences between protocols whereas Δz and surface zone measurements have less frequently shown significant differences between protocols. Previous discussion in Chapter 3 of the relative stability of the intercrystalline fluid in this part of the lesion reflecting long-term changes in the oral environment, as opposed to the more rapid changes in intercrystalline fluid at the surface zone, may account for this result. In addition, the composite nature of the Δz parameter, also discussed in Chapter 3, may again explain the results observed by measurement of lesion body.

It is possible that the changes in salivary conditions, in the absence of a fluoridated dentifrice, were too subtle to be detected by this model. Problems with the model may have included the length of the intra-oral period, the nature of the lesions used or the intensity of the cariogenic challenge.

This experiment was based on the premise that if some saliva is good for remineralisation, then more is better. Perhaps the word "more" should be expanded upon. "More" could mean a greater rate of salivary flow than other subjects, a greater increase in bicarbonate content compared to other subjects or higher levels of salivary calcium and phosphate. The changes in salivary composition upon stimulation are complex and "more" could mean many

things.

If "more" saliva is better, then could it be that "less" is worse? Although data from xerostomics have demonstrated an increased caries rate with a reduction in salivary flow rate, this has not been demonstrated using an intra-oral model and is perhaps just as important as demonstrating the effects of hypersalivation to validate the model. Hyposalivation in a group of volunteers with normal salivary flow rates would have to be induced pharmacologically. This may have significant effects on salivary biochemistry. Another possibility would be to study the effects of hyposalivation using a group of volunteers with pathologically induced hyposalivation such as Sjogren's syndrome. Obviously this raises problems of a significantly biased population sample but such data has not been collected as far as the author is aware.

Variations in salivary flow rates and salivary biochemistry occur at different sites in the oral cavity (Dawes *et al.*, 1989). This may provide a possible solution to the problem of pathologically or pharmacologically induced hyposalivation. Volunteers with normal salivary flow rates could be used to determine the relative effect of salivary flow rate on remineralisation of artificial carious lesions at different sites within the oral cavity. Dawes & Macpherson (1993) have demonstrated significant changes in salivary film velocity between different areas of the mouth. For example, the unstimulated salivary film velocity in the upper labial sulcus was observed to be approximately eight times less than the film velocity in the lower lingual

sulcus (Dawes & Macpherson, 1993). Such differences in salivary film velocity may provide a source of regions in the oral cavity of relative hyposalivation.

The use of a sorbitol-containing gum for the stimulation of salivary flow requires further comment. The intention was to find a method of providing good salivary stimulation without inducing plaque acid production. This was particularly appropriate considering volunteers were already using a non-fluoridated dentifrice and ingesting mid-morning and mid-afternoon cariogenic snacks. It is known that stimulation of salivary flow is high when both mechanical and gustatory stimuli are encountered at the same time (Dawes & Macpherson, 1992). Furthermore, plaque bacteria should, initially, be unable to metabolise sorbitol as a period of induction is required for the development of a specific sorbitol phosphotransferase system (Hogg & Rugg-Gunn, 1991). Studies by Birkhead *et al.* (1978) showed that plaque takes approximately six weeks to adapt to sorbitol and, once adaptation has occurred, the pH drop experienced is only 30% of that seen with glucose. It would, therefore, appear that a sorbitol-containing chewing gum would meet with the required provision of good salivary stimulation and minimal plaque acid production.

The effect of stimulated salivary flow on artificial lesion remineralisation in the absence of fluoride has seldom been demonstrated. However, one study worthy of discussion is that by Leach *et al.* (1986) who demonstrated the ability of stimulated saliva to remineralise artificial enamel lesions *in situ*. This study used an orthodontic band cemented to a lower molar tooth. An enamel block

with an artificially created enamel lesion was attached to the orthodontic band. Five volunteers took part in a two phase non-randomised crossover study. Each phase of the study lasted for 14 days. All volunteers used a non-fluoridated dentifrice throughout the study. During the first phase all volunteers consumed 15 Lycasin candies per day. Lycasin is a hydrogenated glucose syrup and, when exposed to dental plaque, results in minimal acid production. During the second phase of the study, volunteers consumed no candies and refrained from eating between meals. The artificial lesions used for each phase were in the form of enamel blocks cut from a single, larger, uniform lesion. Sections were created from the enamel blocks and examined using polarised light microscopy and microradiography. Each subject was, therefore, allocated three specimens from the same lesion. One specimen was an original sample from the lesion, a second specimen was used for the phase requiring the consumption of Lycasin candies and the third specimen was used for the phase where no candies were consumed.

Comparisons were made between all three samples from each lesion for all volunteers using paired t-tests to determine differences between the protocols. Analysis using polarised light microscopy determined the area of the lesion with a pore size >5%. Microradiography and subsequent microdensitometry were used to determine the mineral content of the surface zone, lesion body and area under the mineral profile which the authors named "Z".

Results indicated statistically significant remineralisation, for all polarised light

and microradiographic measurements, in the specimens used for the Lycasin candy chewing phase, compared to either of the other two specimens. Furthermore, the authors suggested that up to one third more mineral was deposited in the Lycasin group compared to the other samples.

Comparison of data from this study with the work by Leach *et al.* (1986) is difficult. That study was based on only 5 observations in 5 subjects whereas the present study was based on 140 observations in 19 subjects yet still failed to demonstrate any significant difference between unstimulated and stimulated salivary flow.

Leach *et al.* (1986) created their lesions using an acid buffered solution containing 0.5 ppm F^- as NaF. This is a higher concentration of fluoride than was used to create lesions for the present study (0.01 ppm F^- as NaF) and is evidenced by the higher mineral content of the surface zone (Arends & Christofferson, 1986) of the lesions in the study by Leach *et al.* (81.8 % vol mineral compared to 52.6 % vol. mineral in the present study). The protocol for the study by Leach *et al.* used a single lesion divided into three parts to act as baseline, test and control lesions respectively. It is possible that variation in the mineral content within a lesion, as demonstrated by Creanor (1987), may account for some of the differences with such a small sample size. No data is given regarding individual lesion sizes although the mean lesion size was approximately 14.0 %vol.mineral x μm / 100 (where Δ is taken as the area above the curve). Such lesions are very small compared to the ones used in the

present study (mean Δz = 43.0 %vol.mineral x μm / 100) and, according to the current data on the behaviour of lesions according to baseline lesion size (Strang *et al.*, 1987), would not be expected to remineralise to a large extent. The use of 15 Lycasin candies per day at 30 - 60 min. intervals probably constituted a more prolonged stimulation of salivary flow than the present study and this may also have contributed to the result obtained. Finally, it is assumed that all subjects were using a fluoridated dentifrice prior to commencing the trial. No mention was made of any "washout" period when a non-fluoridated dentifrice was used prior to the placement of enamel blocks in the mouth. The short intra-oral period (14 days) may have had carry over effects from previous fluoridated dentifrice use (Damato, 1990; Stephen, 1992, Damato & Stephen, 1994)) with the Lycasin candy phase but not for the later control phase.

The main problems with the present study were an inability to detect a significant difference between the three protocols and especially with regard to the difference between fluoridated and non-fluoridated dentifrice use. This second observation was of particular concern in light of previous work presented in Chapter 4. Possible reasons for these results may have included problems with volunteer compliance or the innate reactivity of the lesions used. It is difficult to argue these points, as previous trials have progressed quite satisfactorily under similar conditions. However, these problems are now discussed in further detail.

Volunteer compliance is of crucial importance in this type of study. All volunteers were chosen for their willingness to comply with experimental protocol. The ingestion of cariogenic snacks between meals generally conformed with most volunteers' daily routines. The change of dentifrice for twice daily tooth brushing was of minor inconvenience to most subjects. No subjects reported discomfort with the appliances after the second week of wear and gum chewing after ingestion of food quickly became a habit during the relevant parts of the experiment. However, occasionally, volunteers would forget to replace the appliance after cleaning their teeth. This usually occurred before setting out for work. An appliance at the side of the bathroom sink all day would probably kill most of the plaque bacteria. There would then be a period of re-growth and re-colonisation prior to re-establishment of a plaque capable of previous patterns of acid production, permeability and fluoride uptake. It is unknown how this would affect the mineral transfer in and out of lesions. There is no evidence, however, to suggest that this happened any more frequently in this study than with any other study carried out.

The innate reactivity of the lesions used may also be a factor accounting for these results. All lesions were stored in 0.12% thymol prior to cementing into appliance troughs. The establishment of a bacterial colony would have to overcome such an antibacterial agent. In addition, the range of sizes of such lesions or indeed the relative mineral distribution within such lesions may also account for the inability to distinguish between these protocols.

5.5 FURTHER ANALYSIS OF THREE-PHASE TRIAL DATA

5.5.1 Introduction

The failure to observe differences between fluoride and non-fluoride, as well as chewing and non-chewing protocols, suggested further analysis of the data was required to determine why this result had been obtained. The methods of statistical analysis used in the following examination of the data may not be the most appropriate, but endeavour to demonstrate how the protocol may have influenced the outcome of the experiment. Furthermore, suggestions for changes in protocol for future work are made in light of these results.

5.5.2 Baseline Lesion Size

The methodology outlined in Chapter 2 went to considerable length to ensure each phase of the trial starts with artificial lesions of similar size. The need for lesion sizes to be coincident between phases of a trial is reduced using a single section model where each specimen acts as its own control. However, the presence of a lesion-size-effect may still influence the overall result.

No guarantee can be made that all experimental phases which commence with baseline lesions of similar sizes will finish with baseline lesions of similar sizes. Each subject started with approximately 15 lesions on 4 sections. Sections may be lost or lesions may cavitate. The mean baseline lesion size is, therefore,

calculated retrospectively on lesions which survived intact the five week period of intra-oral appliance wear. Mean baseline lesion size can be calculated phase by phase or protocol by protocol. For convenience, the initial allocation of the sections was on a phase by phase basis. However, the results of this experiment were based on differences between protocols. Therefore, the mean baseline lesion measurements by protocol were determined and are presented in Table 5.6.

The mean baseline values and standard deviations for Δz, SZ and LB appeared to be quite similar for each protocol. Formal statistical analysis suggested no difference between baseline Δz values for each of the three protocols. However, differences did exist between some SZ and LB measurements.

Table 5.6

Comparison of baseline lesion size by protocol

	Fluoride only (F)	Non-fluoride and sorbitol gum (NF+G)	Non-fluoride only (NF)	Two sample t-tests		
				F vs NF+G	F vs NF	NF+G vs NF
Δz	43.0 (SD 16.3)	41.6 (SD 15.7)	44.7 (SD 15.6)	0.54	0.31	0.096
SZ	53.6 (SD 9.1)	53.2 (SD 9.0)	51.1 (SD 9.3)	0.72	0.026	0.059
LB	36.1 (SD 8.4)	36.8 (SD 8.3)	34.2 (SD 7.4)	0.49	0.05	0.007

Δz - (%vol.mineral x μm) / 100
SZ - %vol.mineral
LB - %vol.mineral

Regression plots of baseline lesion measurements against five week changes were made, for all lesions, for Δz, SZ and LB data (figures 5.1 - 5.9). The regression line was indicative of mean lesion behaviour for each protocol. The confidence in the regression line for each protocol was indicated by the p - values of the regression equation. Using mean trial baseline lesion data and mean protocol baseline lesion size data, predicted changes could be determined using these regression lines. Figures 5.10 - 5.12 show how differences between mean trial baseline lesion size and mean protocol baseline lesion size could have affected predicted changes.

Predicted changes were calculated for data of mean protocol lesion size as opposed to mean trial lesion size. The predicted changes and their differences are shown in Table 5.7. In those cases where a statistically significant difference in baseline lesion sizes was detected, the use of a lesion of trial mean baseline lesion size tended to spread out the results between the protocols. However, these changes in results were minor and probably would not have affected the overall outcome of the trial.

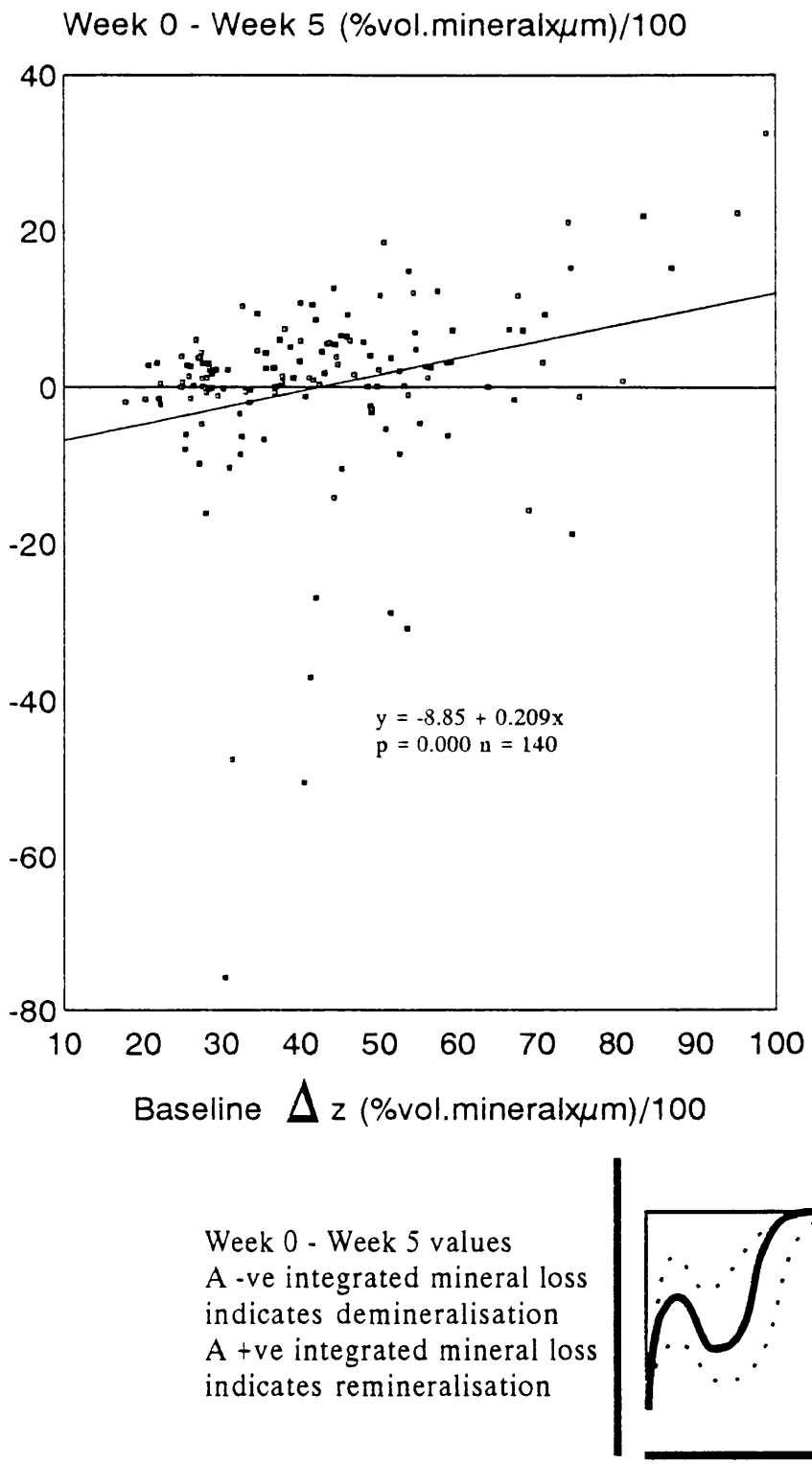


Figure 5.1 **Regression plot of baseline Δz against Δz mineral change over 5 weeks for fluoride dentifrice only.**

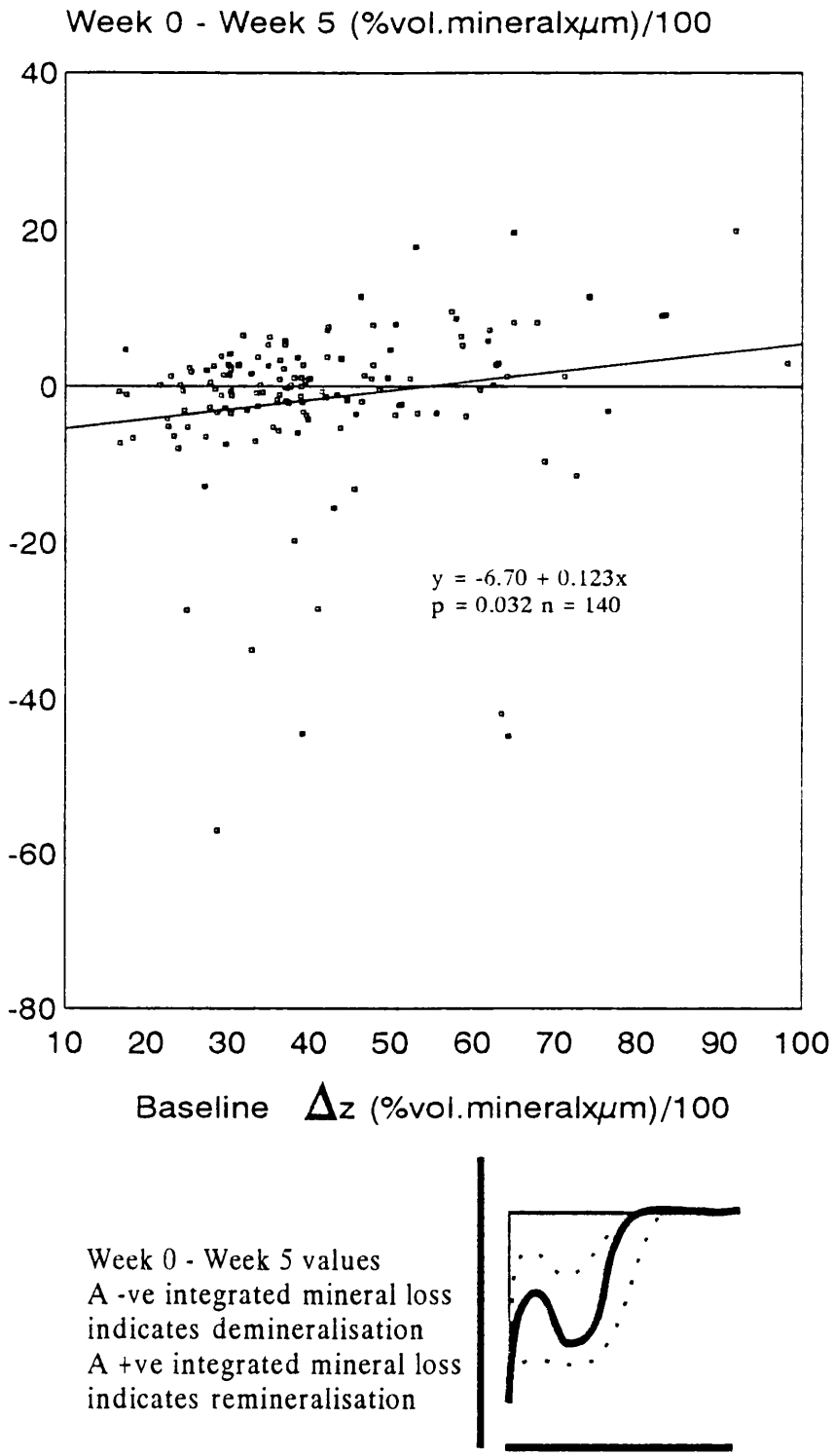


Figure 5.2 **Regression plot of baseline Δz against Δz mineral change over 5 weeks for non-fluoride dentifrice and sorbitol gum.**

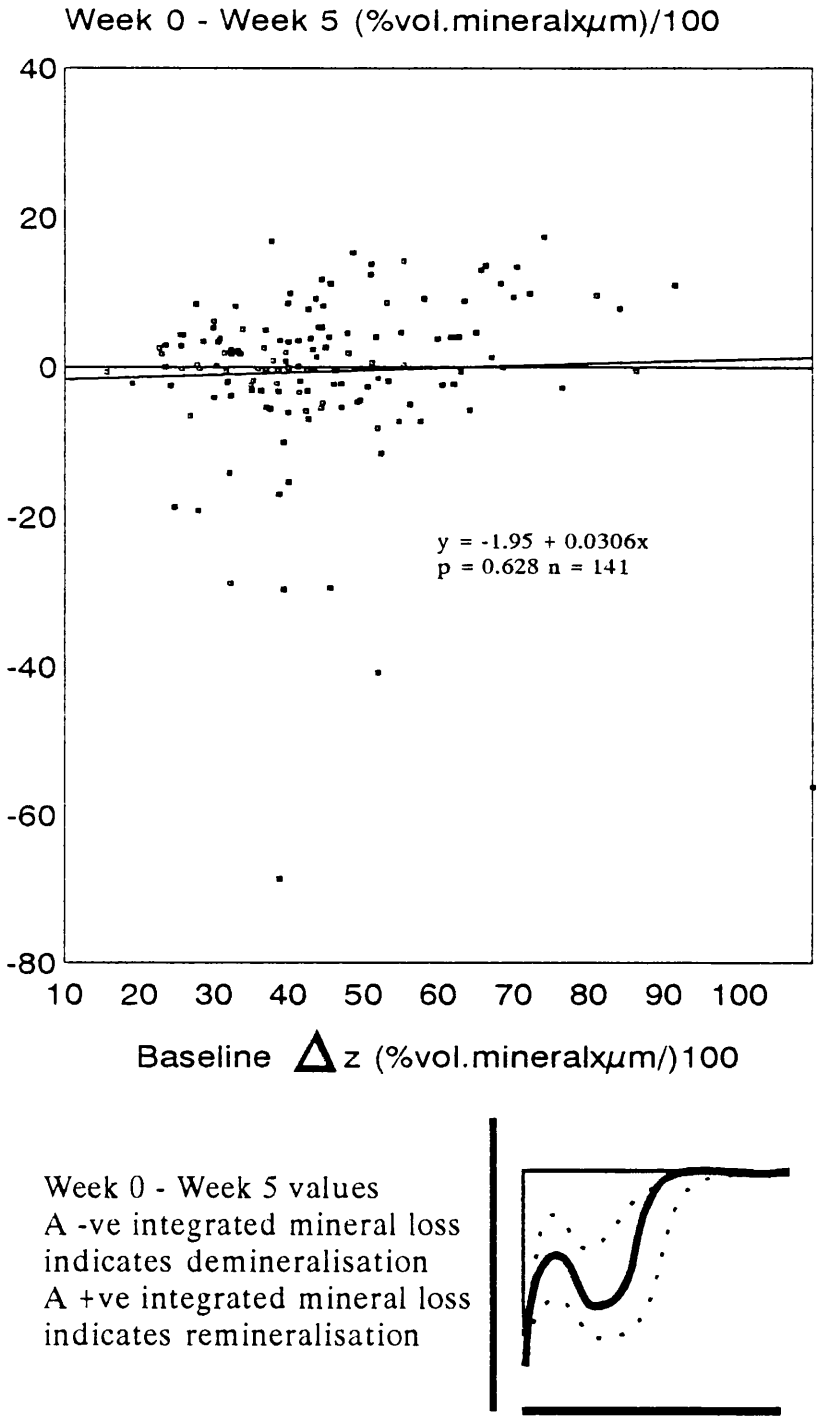


Figure 5.3. Regression plot of baseline Δz against Δz mineral change over 5 weeks for non-fluoride dentifrice only.

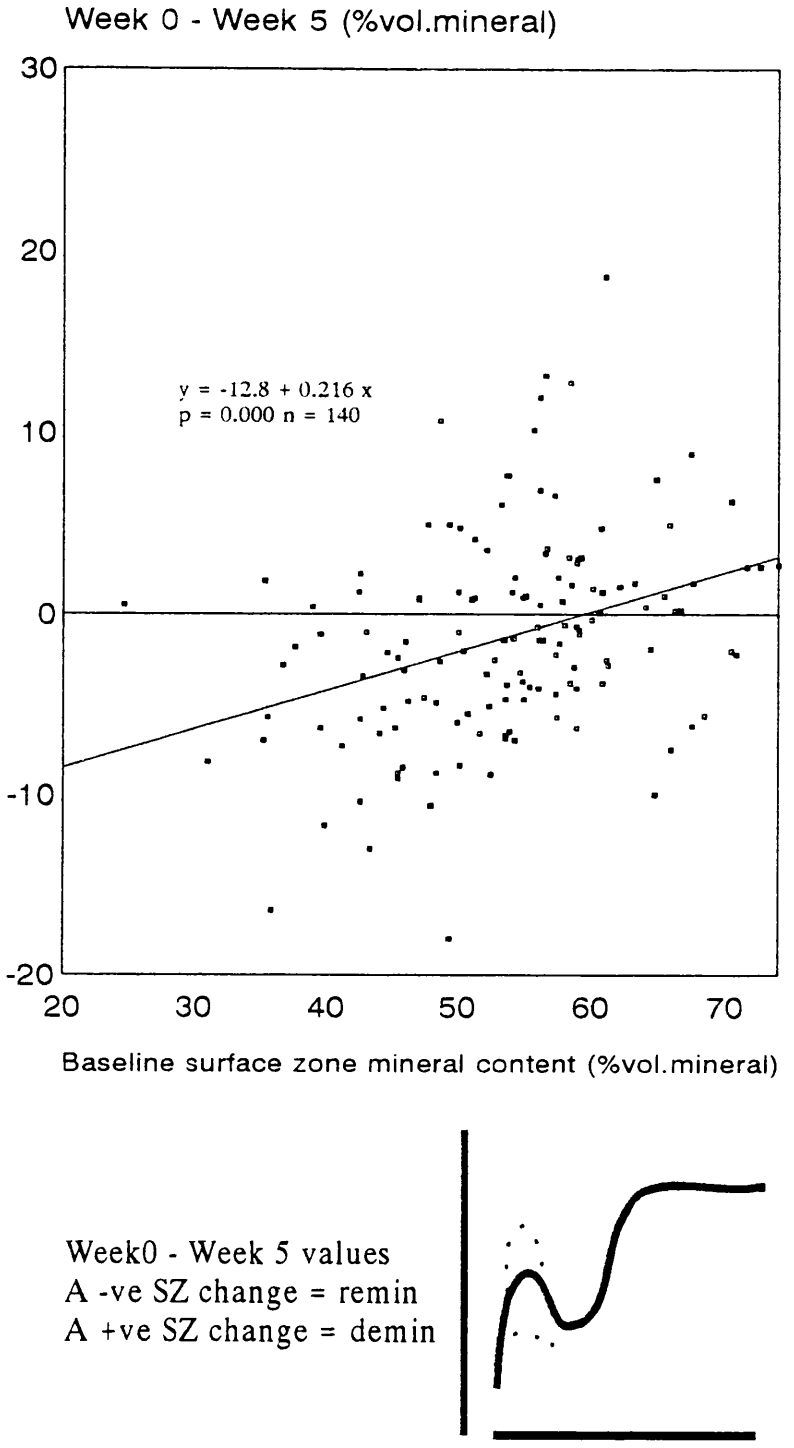


Figure 5.4 **Regression plot of baseline surface zone mineral content against mineral change over 5 weeks for fluoridated dentifrice.**

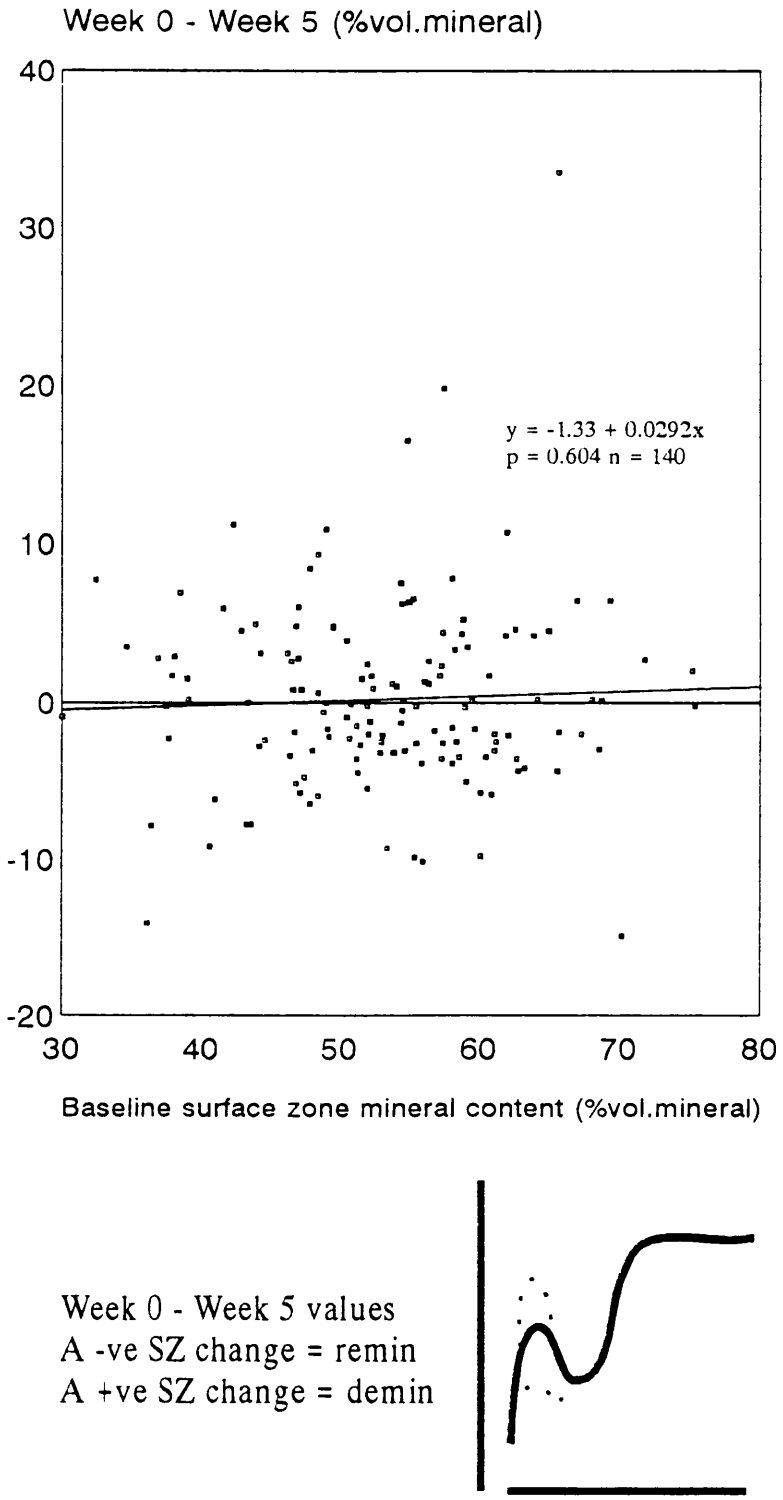


Figure 5.5 Regression plot of baseline surface zone mineral content against mineral change over 5 weeks for non-fluoridated dentifrice and sorbitol gum.

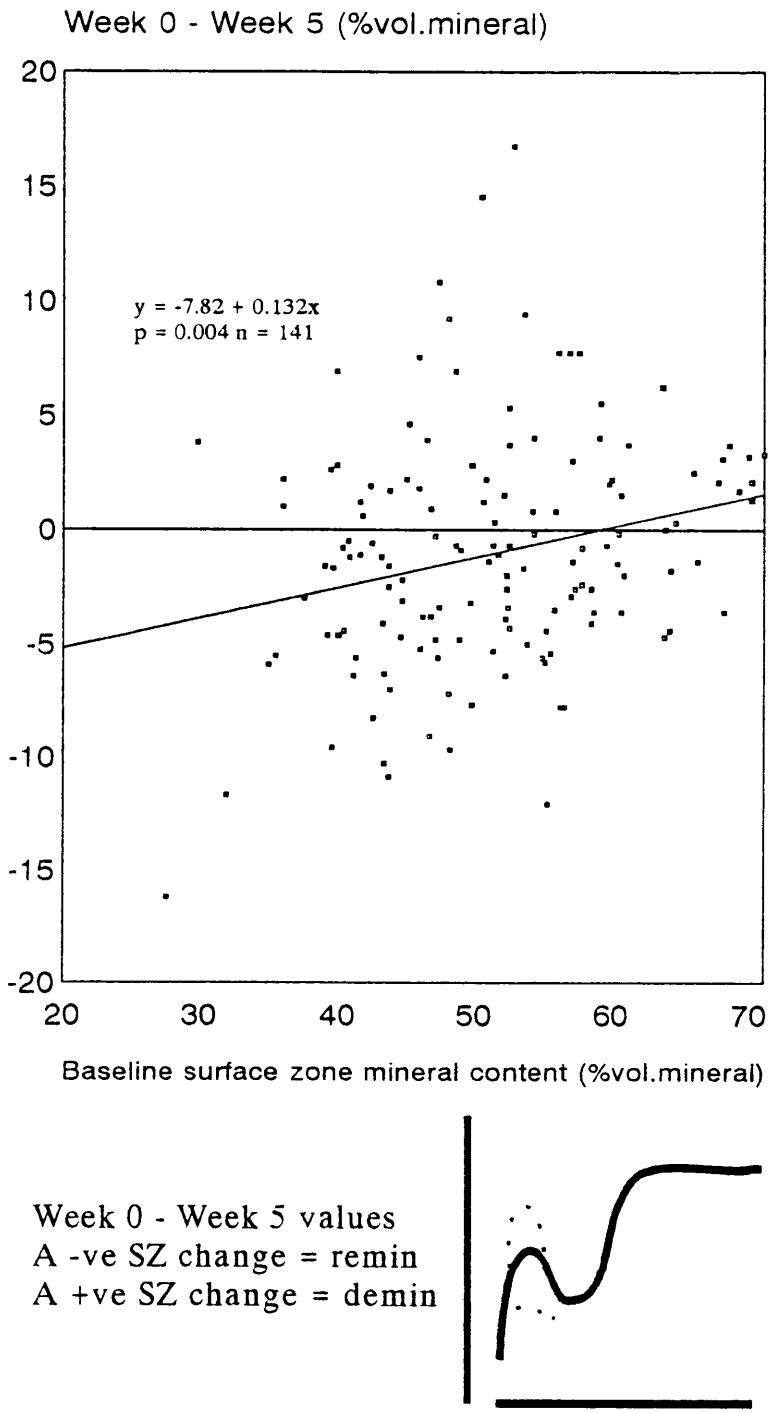


Figure 5.6 Regression plot of surface zone baseline mineral content against mineral change over 5 weeks for non-fluoridated dentifrice.

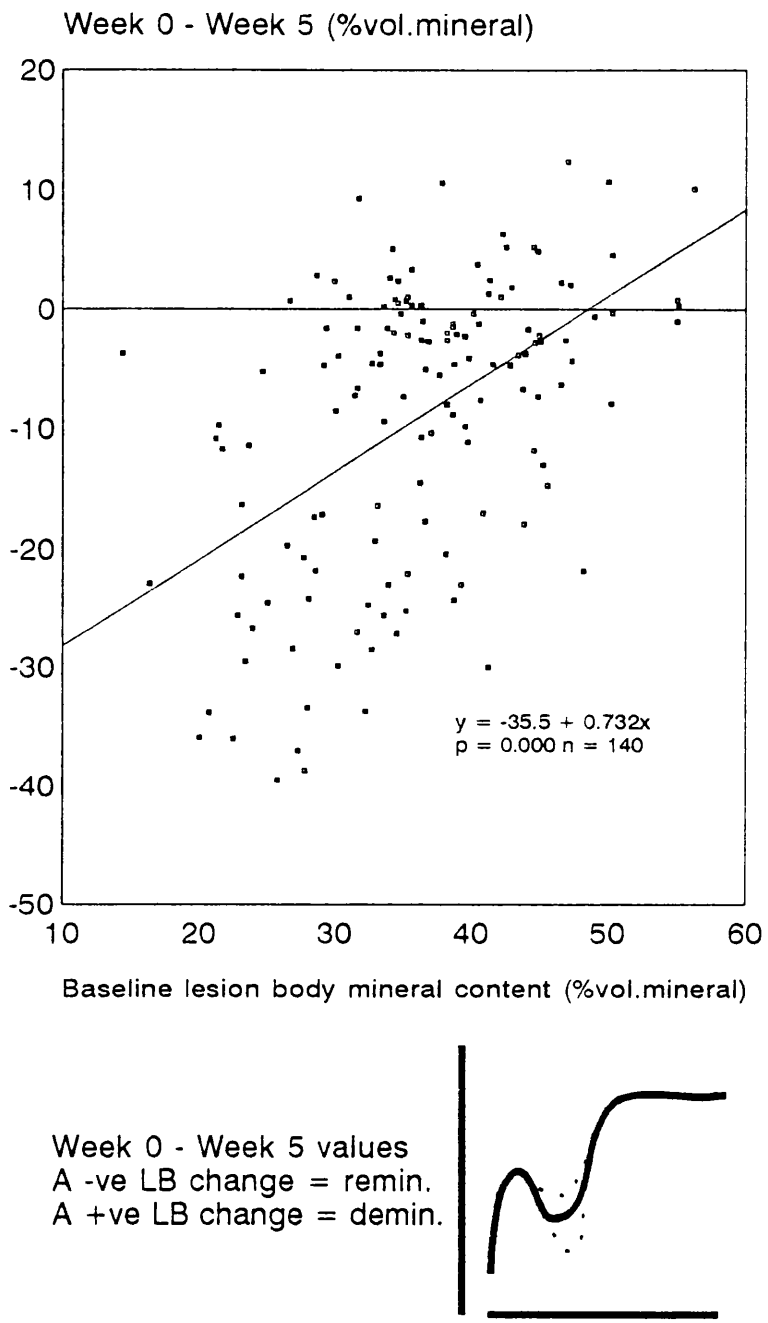


Figure 5.7 Regression plot of lesion body baseline mineral content against mineral change over 5 weeks for fluoridated dentifrice.

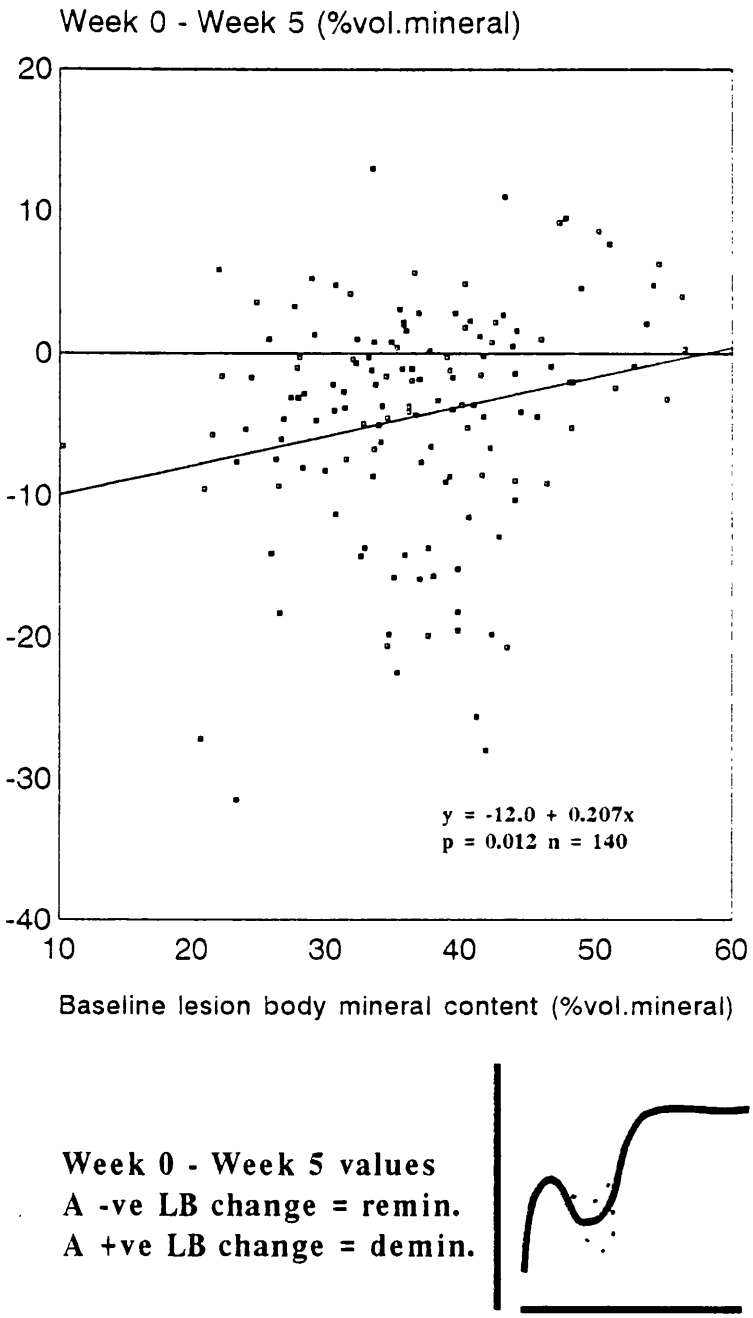


Figure 5.8 Regression plot of lesion body baseline mineral content against mineral change over 5 weeks for non-fluoridated dentifrice and sorbitol gum.

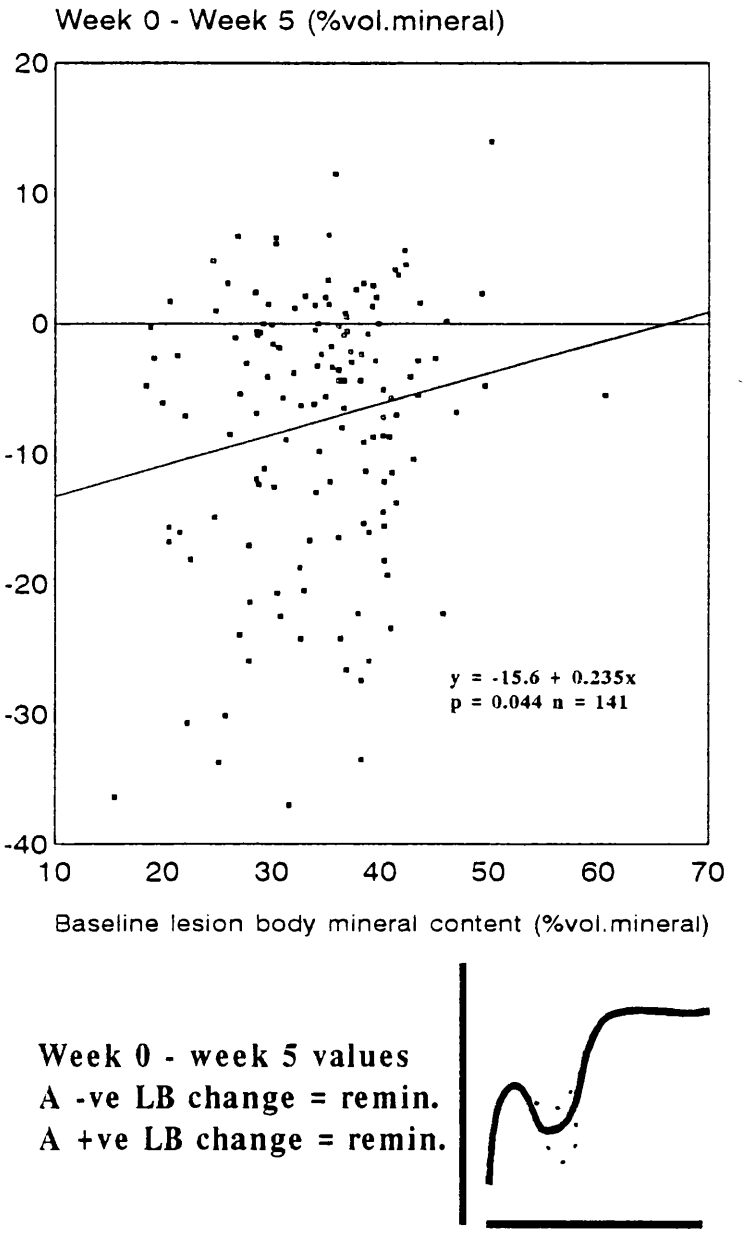


Figure 5.9 Regression plot of lesion body baseline mineral content against mineral change over 5 weeks for non-fluoridated dentifrice.

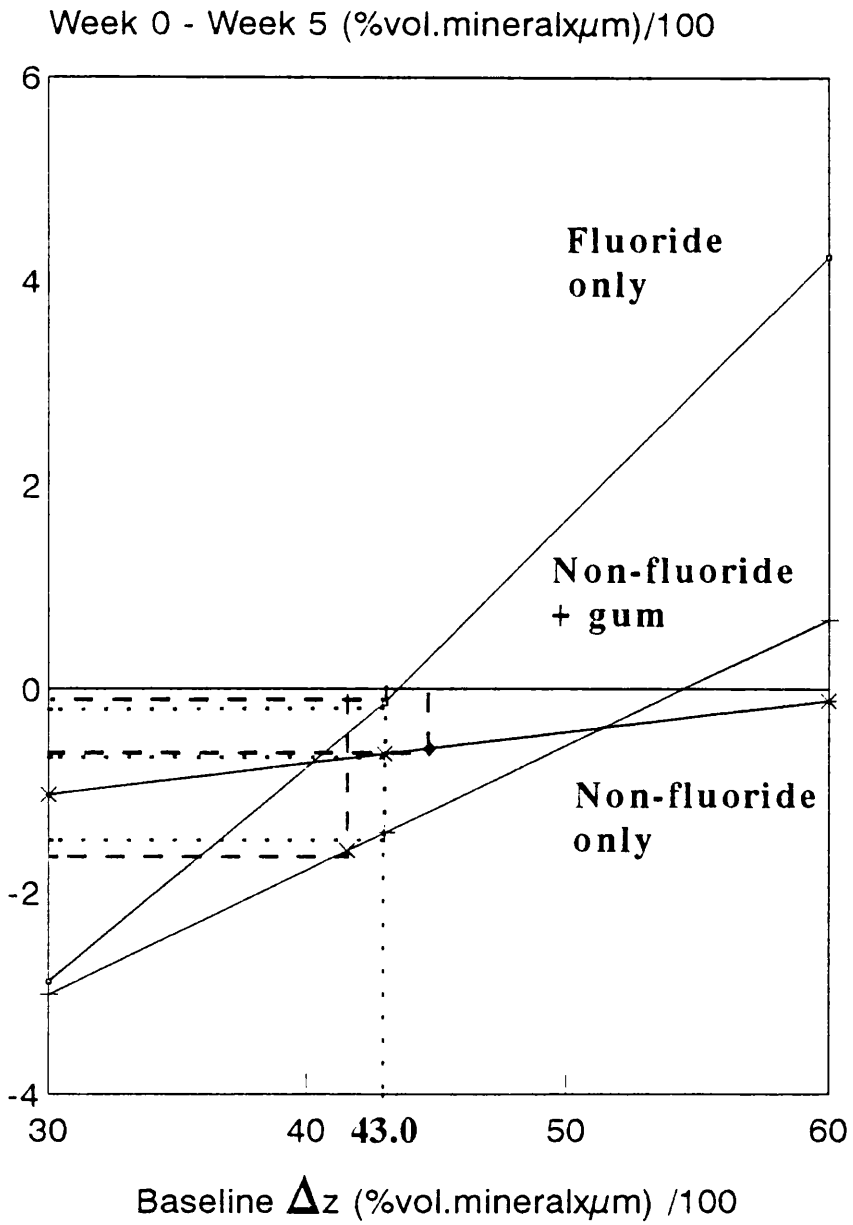


Figure 5.10 **Plot to demonstrate how variation in mean baseline Δz for different protocols may affect predicted lesion change over a 5 week period of intra-oral wear.** The dotted lines represent the predicted change for a lesion of mean trial baseline Δz (43.0). The dashed lines represent the predicted change for lesions where mean baseline Δz was determined according to protocol.

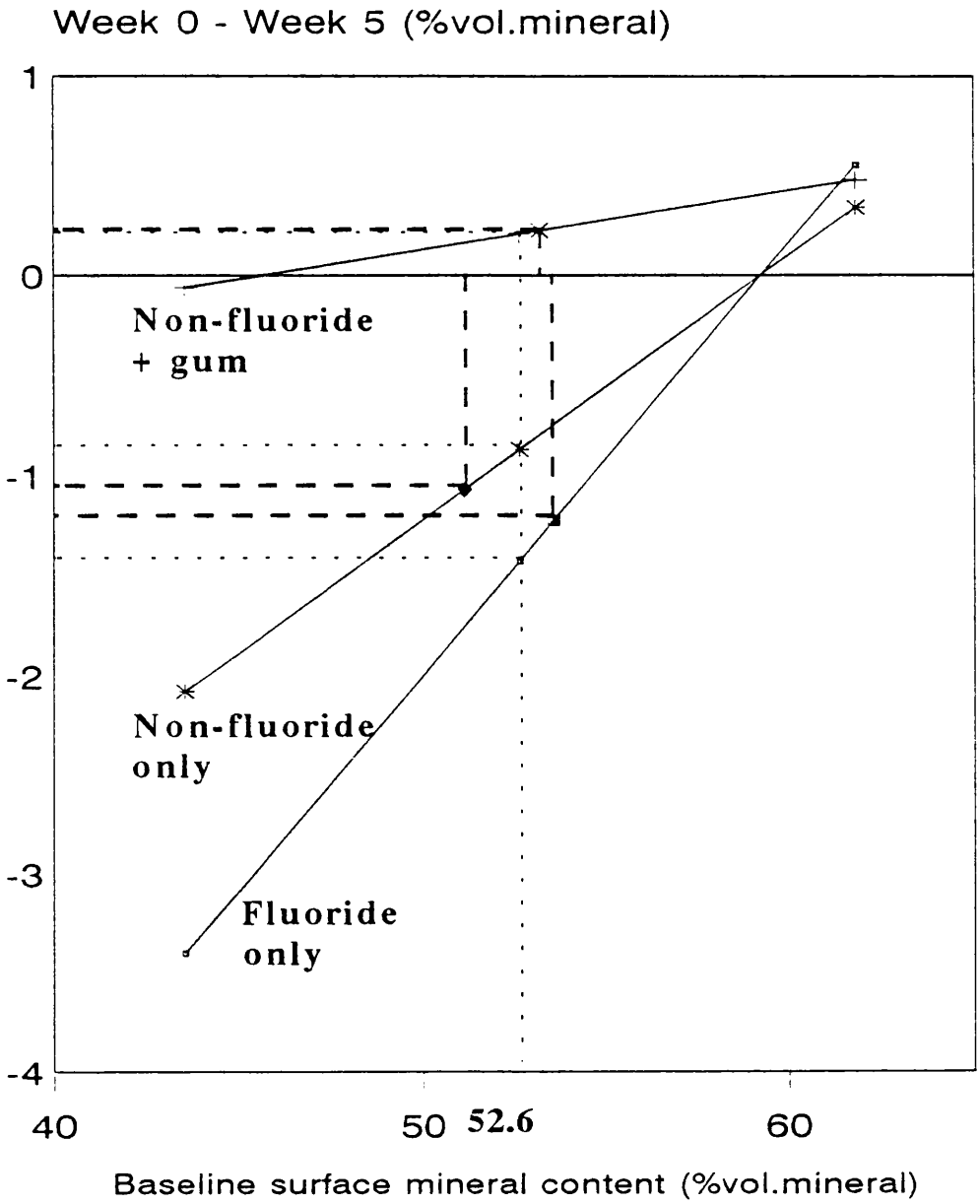


Figure 5.11 Plot to demonstrate how variation in mean baseline surface zone measurement for different protocols may affect predicted change over a 5 week period of intra-oral wear. The dotted lines represent the predicted change for a lesion of mean trial baseline surface zone mineral content (52.6). The dashed lines represent the predicted change for a lesion of mean baseline surface zone mineral content determined according to protocol.

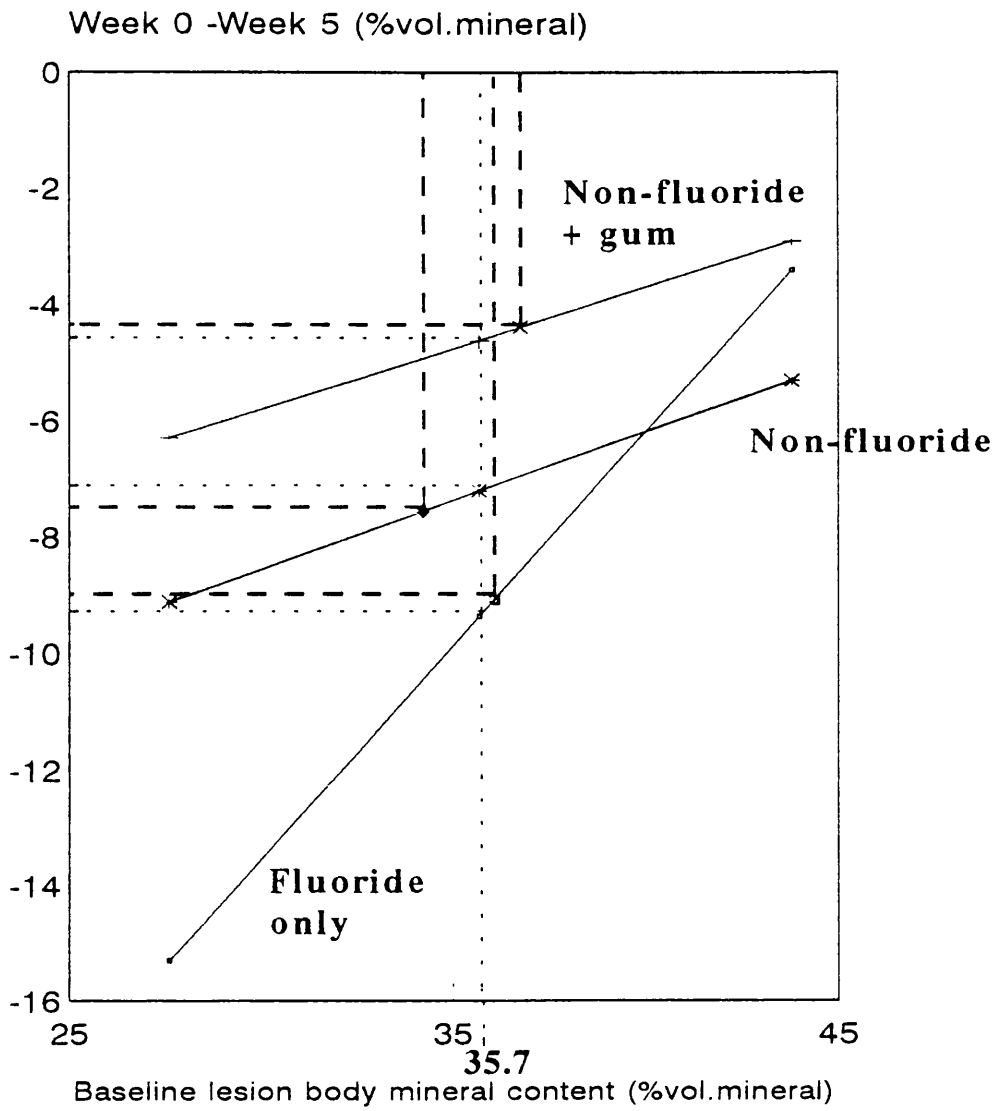


Figure 5.12 **Plot to demonstrate how variation in mean baseline lesion body measurement for different protocols may affect predicted change over a 5 week period of intra-oral wear.** The dotted lines represent the predicted change for a lesion of mean trial baseline lesion body mineral content (35.7). The dashed lines represent the predicted change for a lesion of mean baseline mineral content determined according to protocol.

Table 5.7

Predicted changes for lesion measurements of mean protocol size and mean trial size

Measurement	Protocol	Protocol mean size	Predicted change	Trial mean size	Predicted change	Difference
Δz	Fluoride only	43.0	0.14	43.0	0.14	0
	Non-fluoride + gum	41.6	-1.58	43.0	-1.4	0.14 in favour of remin.
	Non-fluoride only	44.7	-0.58	43.0	-0.63	0.05 in favour of demin.
SZ	Fluoride only	53.6	-1.22	52.6	-1.44	0.22 in favour of remin.
	Non-fluoride + gum	53.2	0.22	52.6	0.20	0.02 in favour of remin.
	Non-fluoride only	51.1	-1.07	52.6	-0.88	0.19 in favour of demin.
LB	Fluoride only	36.1	-9.07	35.7	-9.37	0.3 in favour of remin.
	Non-fluoride + gum	36.8	-4.38	35.7	-4.61	0.23 in favour of remin.
	Non-fluoride only	34.2	-7.56	35.7	-7.21	0.35 in favour of demin.

Δz (%vol.mineral x μm) / 100
SZ %vol.mineral
LB %vol.mineral

5.5.3 The Predicted Change Model

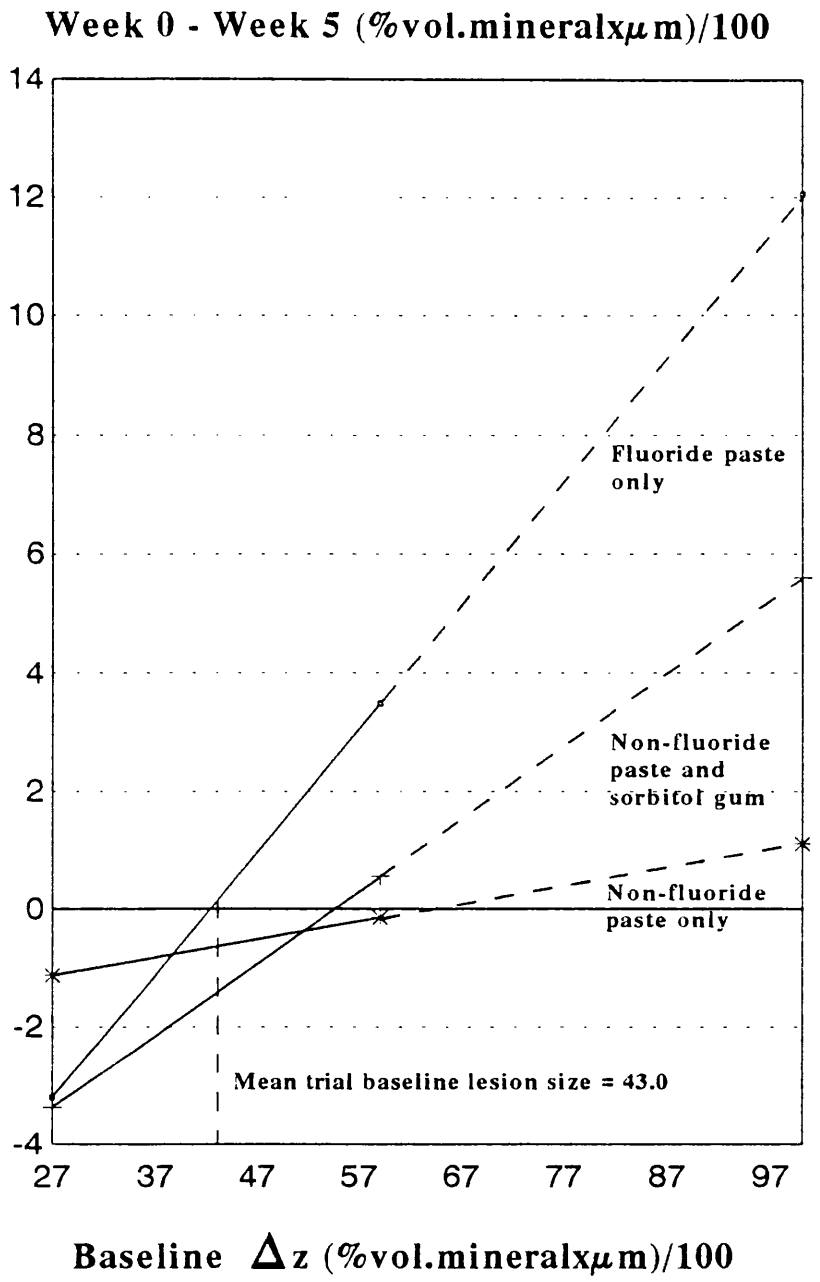
The predicted changes for each subject were based on regression lines through plots of baseline lesion measurement against the change over 5 weeks for up to 10 lesions. An impression of the accuracy of the fit of the regression line to the data can be gained by the p - value of the regression equation. In the large majority of cases in this trial, these p - values were greater than 0.05. This raises the question of the validity of the predicted value produced using such lines. Results using this data were compared to data obtained from the mean change model. The mean change model is perhaps a more conventional method of analysis but does not take into account the mean baseline lesion size. The mean change model used the arithmetical mean change in lesions over the five week intra-oral period for each subject. Comparison of statistical results from the two models is shown in Table 5.5. In all cases, results from data calculated using both models were similar and in no cases were significant differences between protocols detected. This does not necessarily validate the predicted change model as a method of analysis but does show it produces results not unrelated to other models.

5.5.4 Lesion-Size-Effect Regression Equations

The regression equations for each protocol have been demonstrated in figures 5.1 to 5.9. The baseline lesion sizes for Δz were normally distributed and, accordingly, one standard deviation either side of the mean accounted for 95%

of all lesions. This range of baseline lesion sizes was between 27 and 59 %vol. mineral x μm / 100. This range of baseline Δz lesion sizes was perhaps not ideal to demonstrate significant differences between protocols. Extrapolation of regression lines to larger baseline Δz sizes is shown in figure 5.13. Strang *et al.* (1987) have demonstrated a linear lesion size effect using a 1000 ppm NaF dentifrice for lesions up to 200 %vol.mineral x μm / 100. However, it is not known if a similar relationship would be true for a non-fluoridated protocol. Examination of the data from this experiment would suggest that there was a lesion size effect for non-fluoridated protocols but that it was not as strong as with fluoridated dentifrice use.

An estimate of the validity of the regression equations may be gained from the p - values attached to the regression analysis. For the fluoride only and non-fluoride and gum protocols this p - value was < 0.05. For the non-fluoride only protocol this p - value was 0.62. Extrapolation of lesion-size-effect regression lines to baseline lesion sizes greater than 50 %vol.mineral x μm / 100 indicated that the fluoridated dentifrice would result in the greatest amount of remineralisation whereas the non-fluoride dentifrice and chewing gum would result in an intermediate amount of remineralisation and the non-fluoride dentifrice alone would remain largely unchanged. In all probability, surface zone and lesion body measurements contribute to the reactivity of a lesion in addition to Δz measurements. However this data is very complex and such analysis has not been attempted here. From this data, it would appear that the range of lesion sizes used was not of sufficient magnitude to detect the



Note: solid line represents one standard deviation either side of the mean baseline lesion size whilst the dotted line indicates extrapolation to larger lesion sizes

Figure 5.13 Graph to demonstrate the effect of extrapolation to lesions of larger baseline size on the relative effects of different protocols. In this graph a +ve value on the *y*-axis indicates remineralisation.

changes between protocols. Additionally, this could be turned around indicating that the protocols used were not sufficiently distinct to be detected by this range of lesion sizes.

5.5.5 Comparison With Other Data

Data were available for 3 intra-oral model trials which have been described in Chapters 3, 4 and 5. There appeared to be a general trend for volunteers to produce similar results under similar protocols but this trend has not been formally examined.

The sugar-gum trial in Chapter 3 and the three way crossover trial discussed in this Chapter both contain a fluoridated dentifrice only protocol. On both occasions the dentifrice used was Crest Decay Prevention Formula (Proctor and Gamble, Eggham, Surrey) and had a fluoride content of 0.34% (1330 ppmF). Ten volunteers had taken part in both of these studies and the data were compared. Predicted change data for both studies are displayed in Table 5.7 and the data plotted on a graph in figure 5.14. It can be observed that the correlation between trials was excellent apart from two outliers. Closer examination of the data revealed most volunteers remineralised to a lesser extent on the present trial compared to data from the sugar gum trial in Chapter 3. The two outliers did not follow this trend. One outlier remineralised to approximately the same extent on each occasion and the other outlier remineralised more in the present study than in the study reported in

Table 5.8

Predicted changes for 10 volunteers who took part in two studies using a fluoridated dentifrice and no gum chewing

Subject	Integrated Mineral Loss Predicted Change (%vol.mineral x μm) / 100	
	Sugar-Gum Trial (Chapter 3)	3-way Crossover Trial (Chapter 5)
1	-7.09	-0.86
2	-6.25	0.40
3	-0.94	-7.70
4	-4.39	2.05
5	-14.05	-5.24
6	3.03	4.01
7	-6.31	-7.12
8	-10.20	-3.65
9	0.51	2.01
10	-11.81	-2.86

Note: A negative change indicates remineralisation

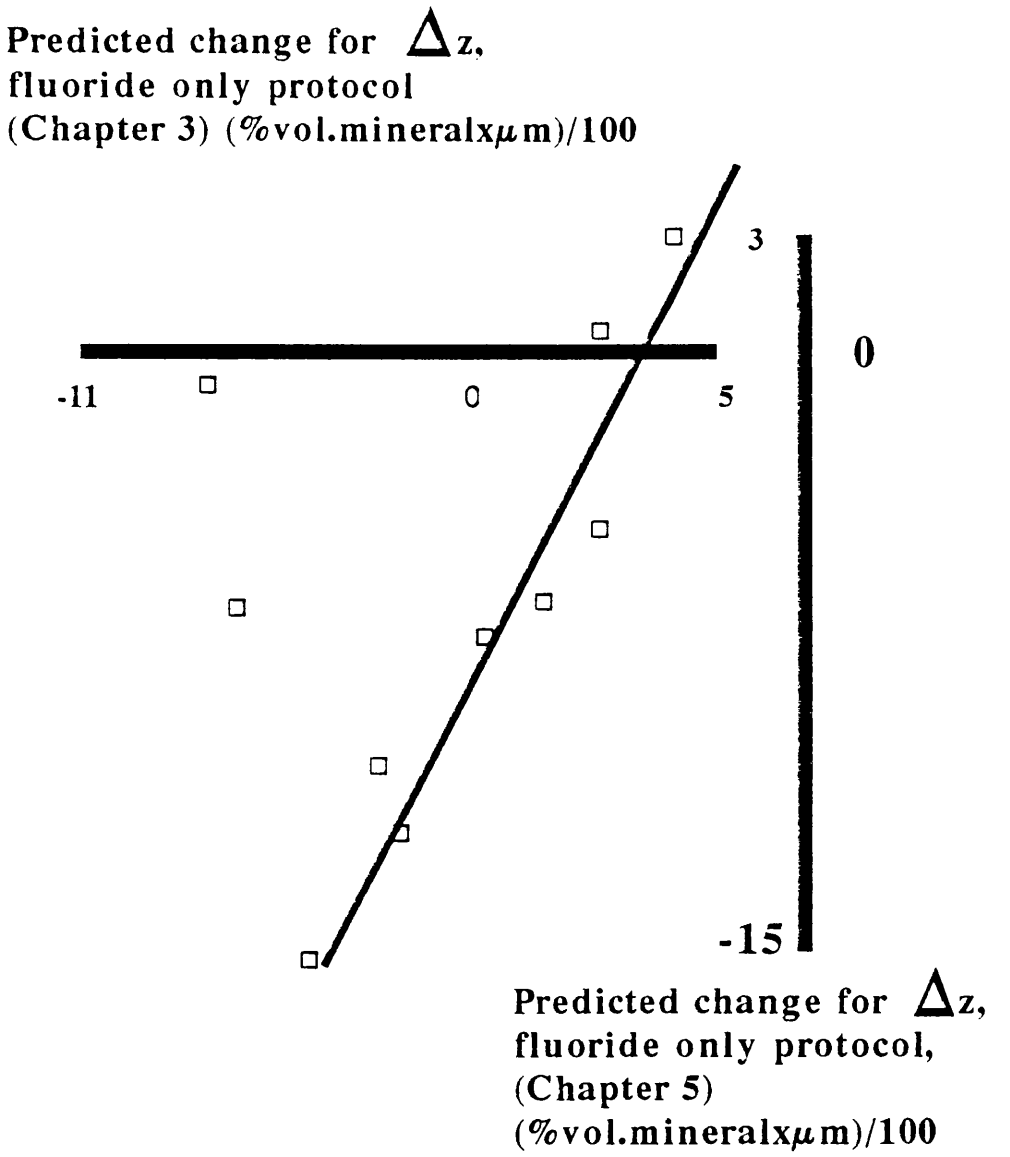


Figure 5.14 **Comparison of predicted changes for 10 volunteers undertaking two fluoride dentifrice / non-chewing protocols in two separate studies.** The regression line was drawn excluding the two outliers.

Chapter 3. If these two subjects were removed from the analysis the correlation now becomes 0.914.

5.5.6 Summary

The matching of baseline Δz measurements between the three protocols was successful although some differences were observed between surface zone and lesion body measurements. The significance of these differences between protocols was minimal but may have helped to separate the predicted changes between fluoride and non-fluoride protocols.

The predicted change model is questionable on the grounds of the accuracy of fit of the regression line to the data for each individual. However, comparison with the results obtained using a mean change model produced similar conclusions indicating no significant difference between the protocols.

The extrapolation of lesion-size-effect regression equations for Δz measurements would indicate that the range of lesions chosen for this study was inappropriate to demonstrate a significant difference between protocols. Lesions with a Δz of greater than 50 (%vol.mineral x μm) / 100 would have been better suited to demonstrate any differences between protocols.

CHAPTER 6 INDIVIDUAL VOLUNTEER CHARACTERISTICS RECORDED DURING THE THREE PHASE TRIAL DESCRIBED IN CHAPTER 5

6.1 INTRODUCTION

Results from previous experiments using the Glasgow intra-oral model (Creanor *et al.*, 1992, Chapter 3,) have suggested certain remineralisation and demineralisation characteristics peculiar to individual volunteers. Some volunteers consistently achieved high levels of remineralisation under a variety of conditions, whereas some volunteers characteristically demineralised under the same conditions. Comparative analysis of results from several different trials for individual volunteers has been discussed in Chapter 5. It is reasonable to discount the enamel sections used in each of the experiments as a reason for this observation, as each experiment used sections from many different teeth all from the Greater Glasgow area. It is possible, therefore, that there are other factors inherent within the volunteer to account for these results.

The use of such a small sample size precludes the use of data such as DMFS, which are usually used to describe populations, as a firm means to account for these findings. Indeed, subject number 14 in Chapter 5 has averaged between 50% and 85% demineralisation in each of the three phases of this experiment, and has also achieved similar results in previous trials, yet has a controlled,

minimal caries rate.

Therefore, it was decided to determine if certain measured parameters on a selected group of volunteers may help to explain the results obtained in the experiments reported in Chapters 3 and 5.

Measurements were made according to the availability of facilities in the laboratory and existing techniques which, in some cases, were modified for use within the intra-oral appliance. These measurements were not meant to provide a comprehensive list of all possible factors which may account for caries susceptibility, but to address a number of relevant aetiological factors in the caries process. Three factors were assessed and included:-

1 The mean levels of fluoride in plaque and saliva during a four week "washout" period, immediately prior to the five week period of appliance wear. Volunteers changed from fluoridated to non-fluoridated dentifrices at the beginning of this period.

2 The ability of saliva to clear substances from the appliance trough.

3 The ability of plaque, within the appliance trough, to produce acid in response to a standard sucrose challenge and the subsequent ability of saliva and deionised water to affect the fall

in pH as a result of such acid production.

The method and materials for each of these investigations are presented separately along with the results and a discussion of the findings.

6.2 PLAQUE AND SALIVARY FLUORIDE ESTIMATIONS

6.2.1 Introduction

Plaque and salivary fluoride levels are discussed at length in Chapter 1 (Section 1.6.3). Resting salivary fluoride levels are generally about 1 µmol/l (Gron *et al.*, 1968; Shannon, 1977), whereas resting plaque fluoride levels tend to be somewhat higher (Tatevossian, 1990, 1991). Venkateswarlu (1990) discussed some of the various analytical methods for determination of fluoride in biological materials. He commented that inorganic fluoride in biological material could now be determined accurately using an ion specific, fluoride electrode with the use of a suitable buffer. However, resting salivary levels of fluoride are at the limits of detection of such methods (Venkateswarlu, 1990) and care should be taken with the interpretation of these results.

Levels of fluoride in plaque and saliva may change with varying dentifrice use or with time, after a change in dentifrice use. Duckworth *et al.* (1987) and Jacobsen *et al.* (1990, 1992) have demonstrated a dose response relationship between resting salivary fluoride levels and the use of dentifrice with variable

concentrations of fluoride. In an effort to address this problem, Damato (1990), Stephen (1992) and Damato & Stephen (1994) have suggested the use of a four week washout period between dentifrices of differing fluoride concentrations with intra-oral trials. To the author's knowledge, data on saliva and plaque fluoride levels have not been collected and compared to remineralisation or demineralisation data from subjects taking part in an *in situ* trial. The aims of this work were:-

1 To determine the change in levels of fluoride in plaque and saliva over a four week washout period with the use of a non-fluoridated dentifrice after a period of fluoridated dentifrice use.

2 To determine the differences in plaque and salivary fluoride levels with the use of fluoridated and non-fluoridated dentifrices.

3 To determine if levels of plaque and salivary fluoride measured during the washout period bore any relationship to subsequent *in situ* de- or re- mineralisation.

6.2.2 Method And Materials

During the course of the three phase *in situ* trial described in Chapter 5, the volunteers were asked to use both fluoridated and non-fluoridated toothpastes for periods of up to nine weeks. This nine week period included a four week "washout" phase prior to the five week periods of appliance wear. Fourteen of the eighteen volunteers taking part in the three phase *in situ* trial described in

Chapter 5 worked at the Dental Hospital and could attend easily for collection of plaque and saliva samples. This afforded an ideal opportunity to study the change in plaque and salivary fluoride with the use of different dentifrices. Plaque and saliva were collected at the same time each week during the 4-week "washout" periods when no appliances were worn. Volunteers were asked to refrain from tooth brushing the night before, and the morning of, plaque collection. Furthermore, volunteers were asked to allow at least 1 hour to elapse between the last intake of food and provision of plaque and saliva samples. This was to reduce the impact of previous ingestion of food on the stimulation of saliva production and plaque metabolism.

Resting saliva was collected by asking volunteers to sit quietly and drool into a plastic tube to a graduated line which represented a production of approximately 2.0 ml (figure 6.1). The individual samples of saliva were then mixed vigorously using a vortex mixer (Jencons Miximatic, Leighton Buzzard, England) before pipetting approximately 1 ml into an Eppendorf tube for storage at -18°C.

Plaque samples from each individual were collected from all surfaces of the lower teeth, using a sterile metal spatula (figure 6.1), and placed in a pre-weighed plastic Eppendorf tube. Initially, plaque samples were collected only from the lingual surfaces of the lower molar teeth. The amount collected, however, was insufficient on some occasions to provide a valid estimation of the fluoride content as pilot studies had indicated in excess of 3 mg of plaque

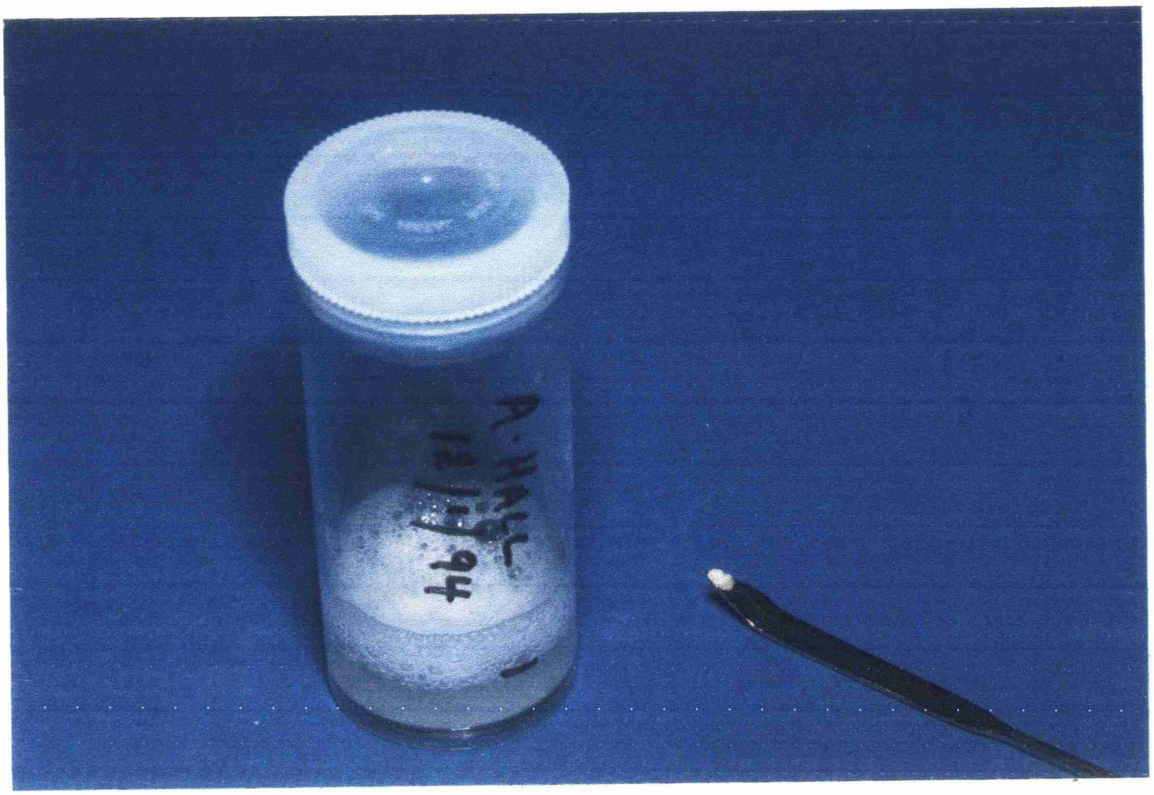


Figure 6.1 Photograph of typical plaque and saliva samples collected for fluoride measurement.

were required to attain reproducible results. Plaque samples were weighed using a Sartorius MC1 balance (Sartorius, Gottingen, Germany) (figure 6.2) which had a resolution of 0.1 mg. All plaque samples were handled with gloved hands to avoid sweat contamination, which may have had the undesired effect of artificially increasing the weight of the sample. Samples were frozen immediately at -18°C until fluoride analysis was performed.

Fluoride analysis was undertaken using a combination ionselective fluoride electrode (Orion Research Electrode No. 9609BN) and ionanalyser (Orion Research Expandable ion Analyzer EA940, Boston Massachusetts)(figure 6.3). Saliva samples (200 µl) were vortexed prior to mixing with equal parts of low level total ionic strength adjustment buffer (TISAB). This buffer adjusts the ionic strength of the sample to prevent drifting of potentiometric measurements by the electrode. In addition, TISAB adjusts the pH to between 5 and 5.5, producing standardised conditions under which fluoride is measured. The chemical formula for the total ionic strength adjustment buffer is given in Appendix IV. For each sample, the fluoride electrode was allowed to stabilise for 5 min before a reading was taken. All samples had a total volume of less than 1 ml and so were covered with clingfilm to reduce sample loss by evaporation. Two aliquots were measured from the saliva sample provided by each volunteer and the arithmetical mean fluoride content of the two samples was calculated. The lanthanum fluoride membrane of the fluoride electrode was carefully cleaned, using a little non-fluoridated dentifrice on a small sponge, between analysis of saliva samples from different volunteers.

Figure 6.2 Photograph of Sartorius MC1 balance for determining the weight of plaque samples.

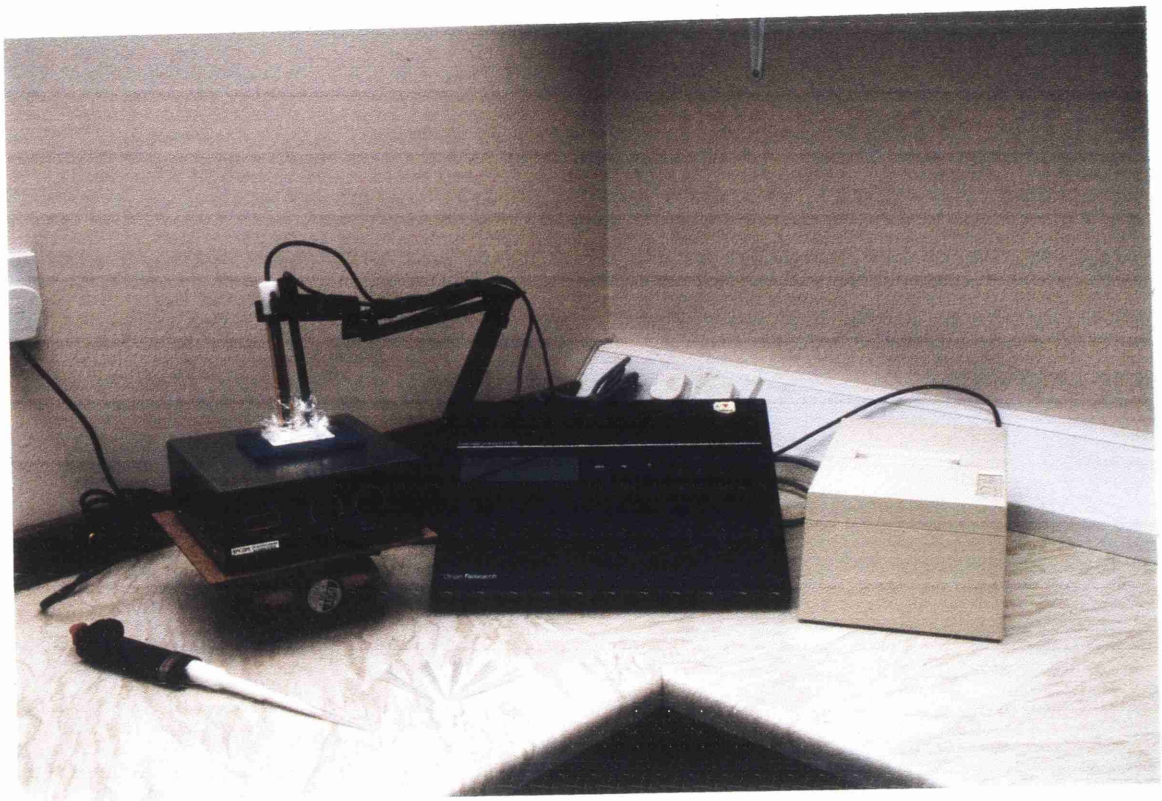


Figure 6.3 **Apparatus for fluoride determination in plaque and saliva samples.**

Electrode cleaning was necessary to remove any protein deposits that may affect the electrode response and has previously proved to enhance reproducibility of results in this laboratory (Robertson *et al.*, 1990).

Plaque fluoride measurements were undertaken in a similar manner. The plaque was permitted to come to room temperature before adding 350µl of deionised water. The individual Eppendorf tubes containing plaque and water for each subject were mixed thoroughly on the vibromixer to produce a homogeneous suspension suitable for analysis. Two 150 µl samples of plaque suspension were mixed with equal quantities of low level TISAB for each volunteer. The mean fluoride concentration was determined in the manner described previously. The concentration of fluoride in the sample was expressed subsequently in units of parts per million fluoride per milligram wet weight plaque using the following calculation:-

$$\text{ppmF/mg wet weight plaque} = \frac{\frac{M_{DIW}}{M_{PI}} \times \text{meter reading(ppmF)}}{M_{PI}}$$

Where M_{DIW} = Mass of deionised water (mg)

M_{PI} = Mass of plaque (mg)

This calculation assumes that the density of plaque is 1 g/cm^3.

Mean predicted change values for each subject for each phase of the concurrent 3-way crossover trial described in Chapter 5 were used to

determine the relationship between these values and measured plaque and salivary fluoride levels. Most results were determined using t-tests and regression analyses with the aid of a computer based Minitab statistical package.

6.2.3 Results

The results of plaque and salivary fluoride measurements are presented in figures 6.4 to 6.9. Results were not available for all fourteen subjects in all cases due to failure to attend or, in one case, a consistent failure to produce enough plaque for analysis in a 24 hr period. As this was a randomised three-way crossover trial, the order in which the results are presented does not reflect the order in which volunteers undertook each phase of the study.

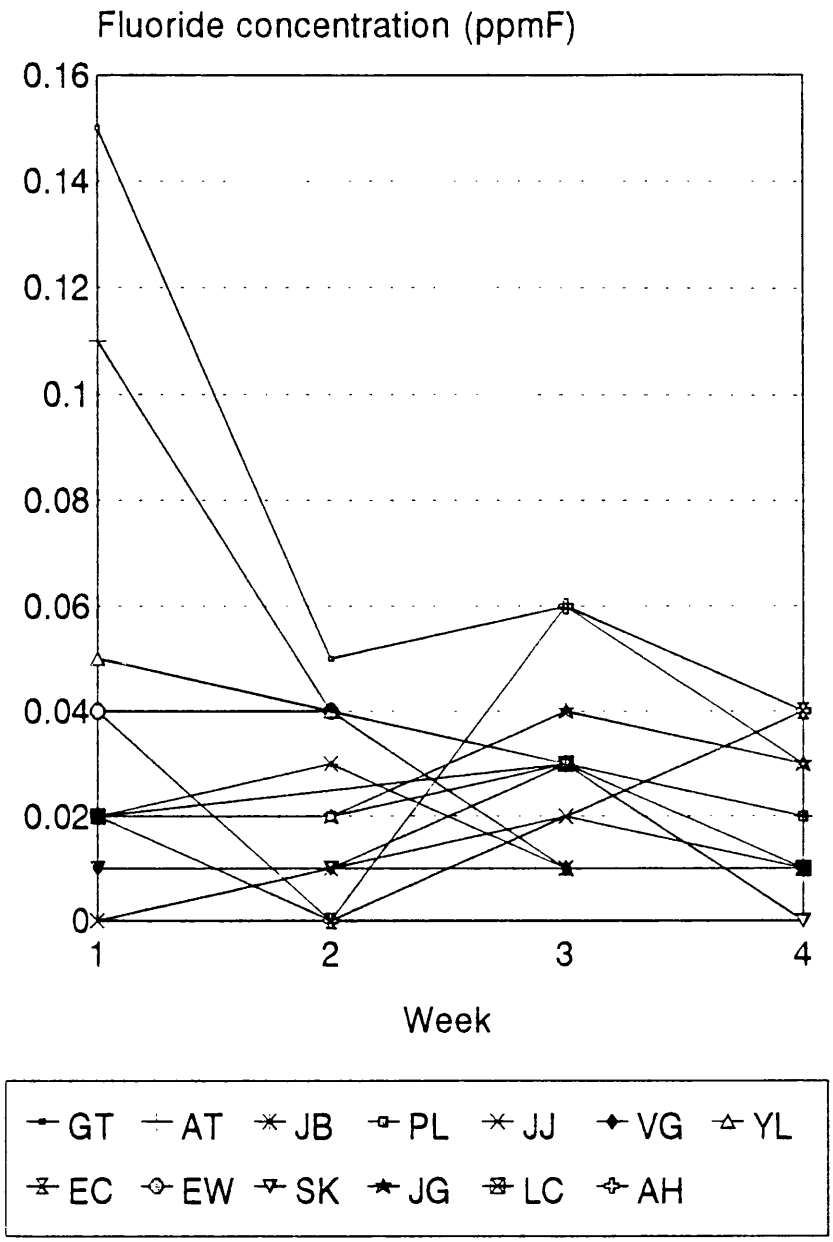


Figure 6.4 Salivary fluoride levels over a 4 week washout period for subjects using a 0.32% sodium fluoride dentifrice. These subjects subsequently went on to the fluoride dentifrice, no gum chewing protocol in the 3 way crossover trial described in Chapter 5.

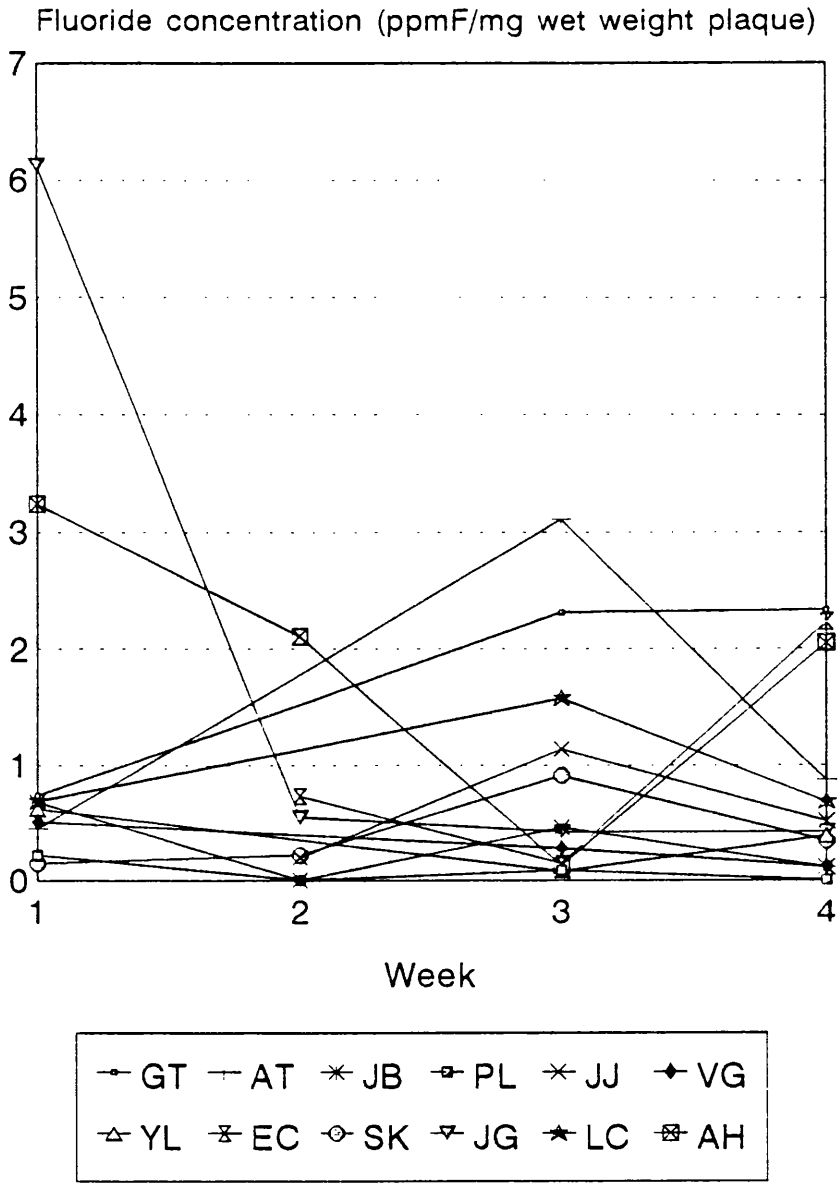


Figure 6.5 Plaque fluoride levels over a 4 week washout period for subjects using a 0.32% sodium fluoride dentifrice. These subjects subsequently went on to the fluoride dentifrice, no gum chewing protocol in the 3 way crossover trial described in Chapter 5.

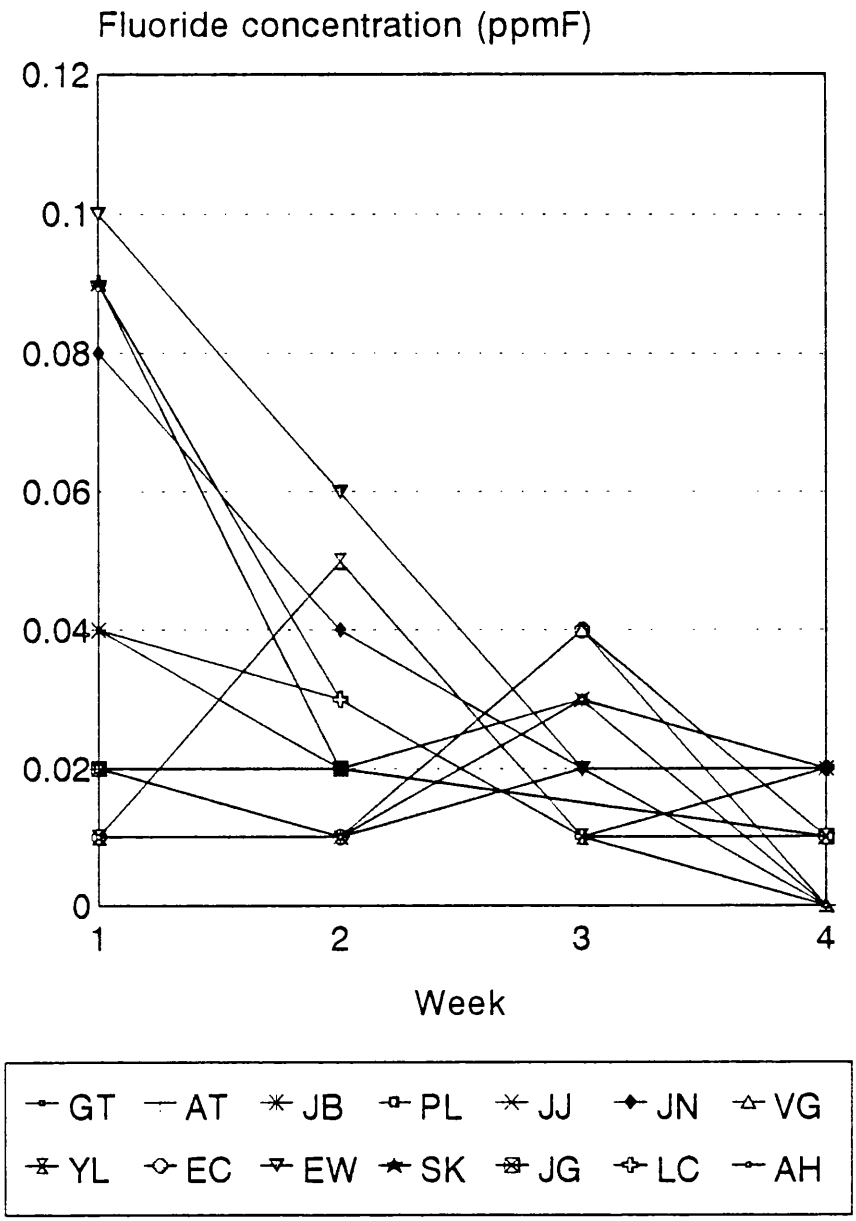


Figure 6.6 Salivary fluoride levels over a 4 week washout period for subjects using a non-fluoride dentifrice subsequent to a period of fluoridated dentifrice use. These subjects went on to the non-fluoride, no gum chewing protocol in the 3 way crossover trial described in Chapter 5.

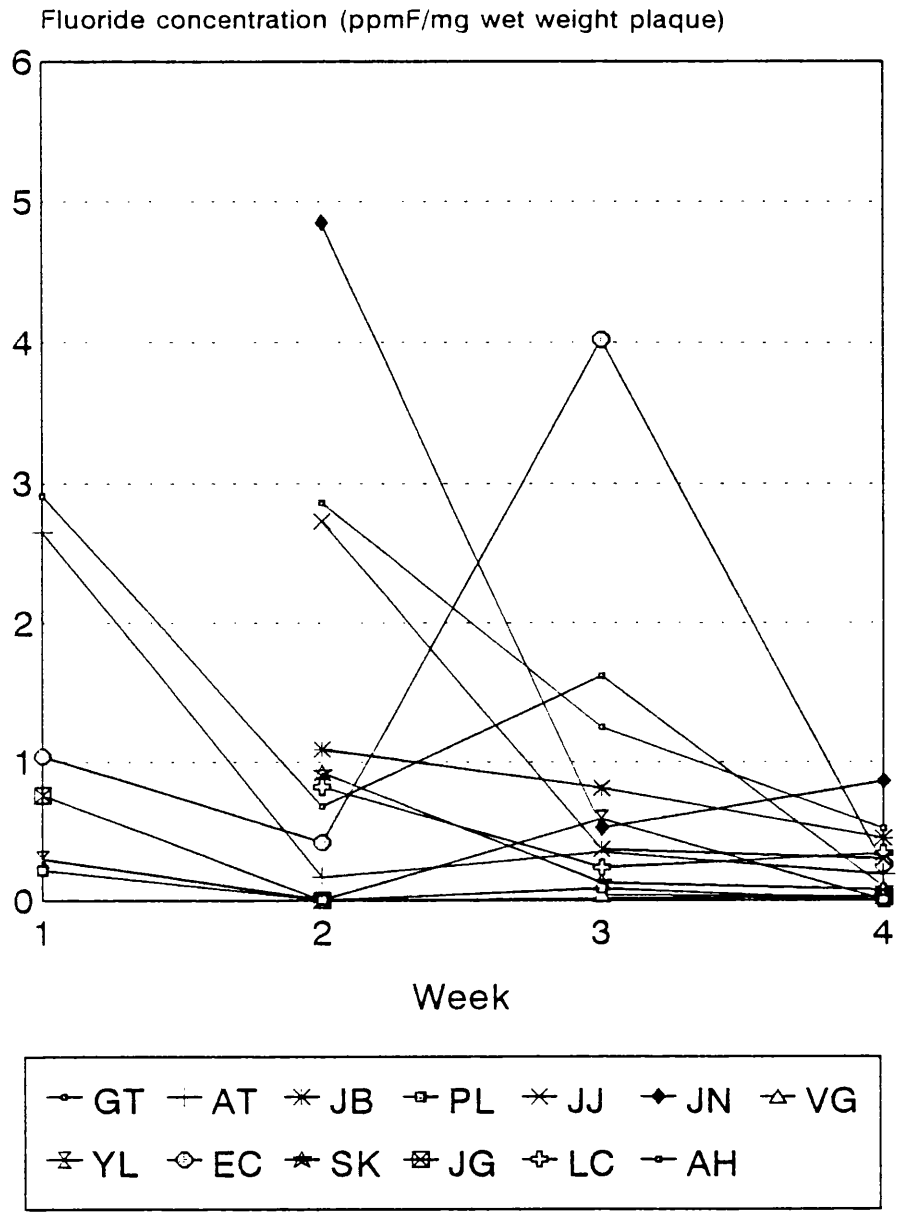


Figure 6.7 Plaque fluoride levels over a 4 week washout period for subjects using a non-fluoridated dentifrice subsequent to a period of fluoridated dentifrice use. These subjects went on to the non-fluoride, no gum chewing protocol in the 3 way crossover trial described in Chapter 5.

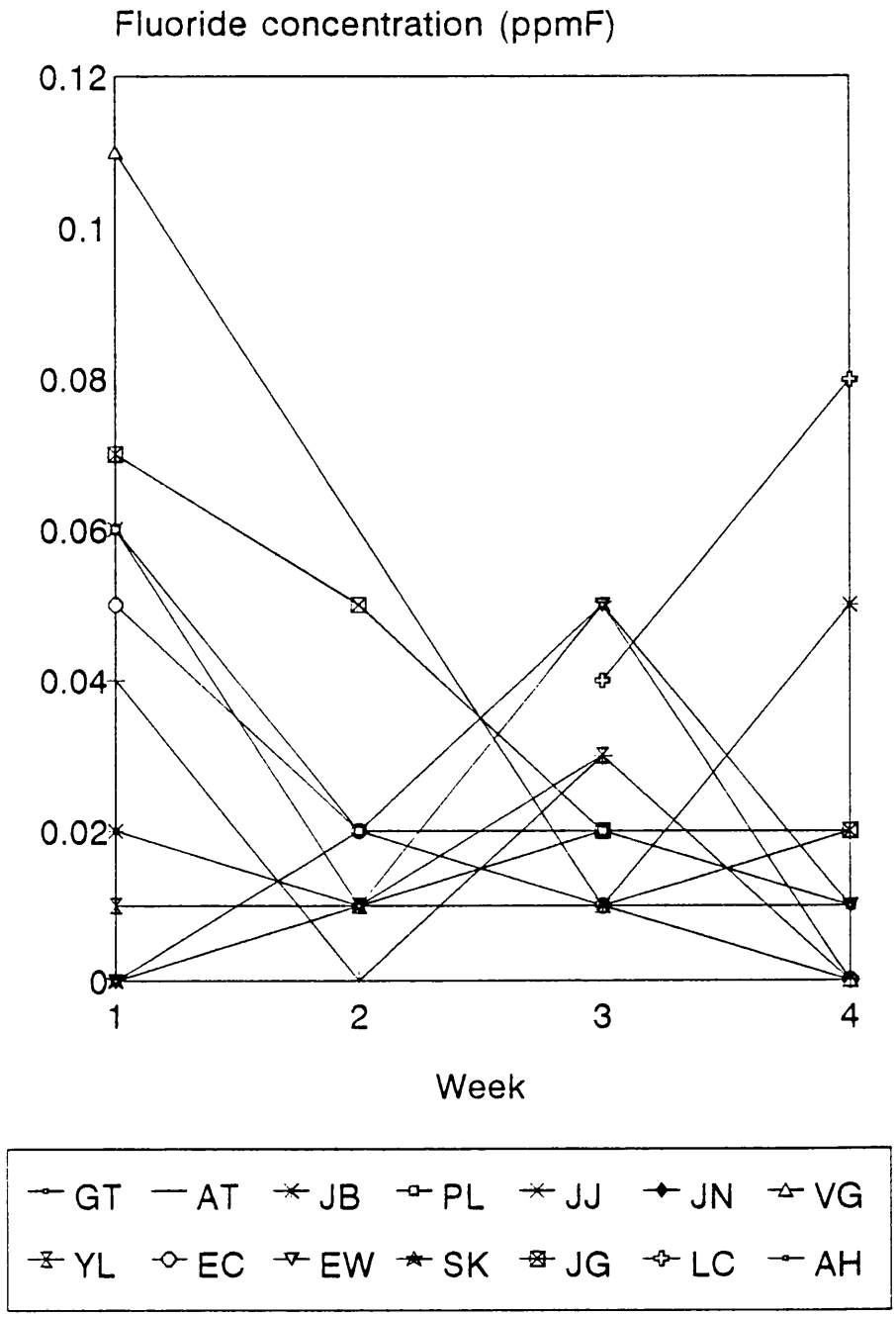


Figure 6.8 Salivary fluoride levels over a 4 week washout period for subjects using a non-fluoridated dentifrice subsequent to a period of fluoride dentifrice use. These subjects went on to the non-fluoride and sorbitol gum chewing protocol in the 3 way crossover trial described in Chapter 5.

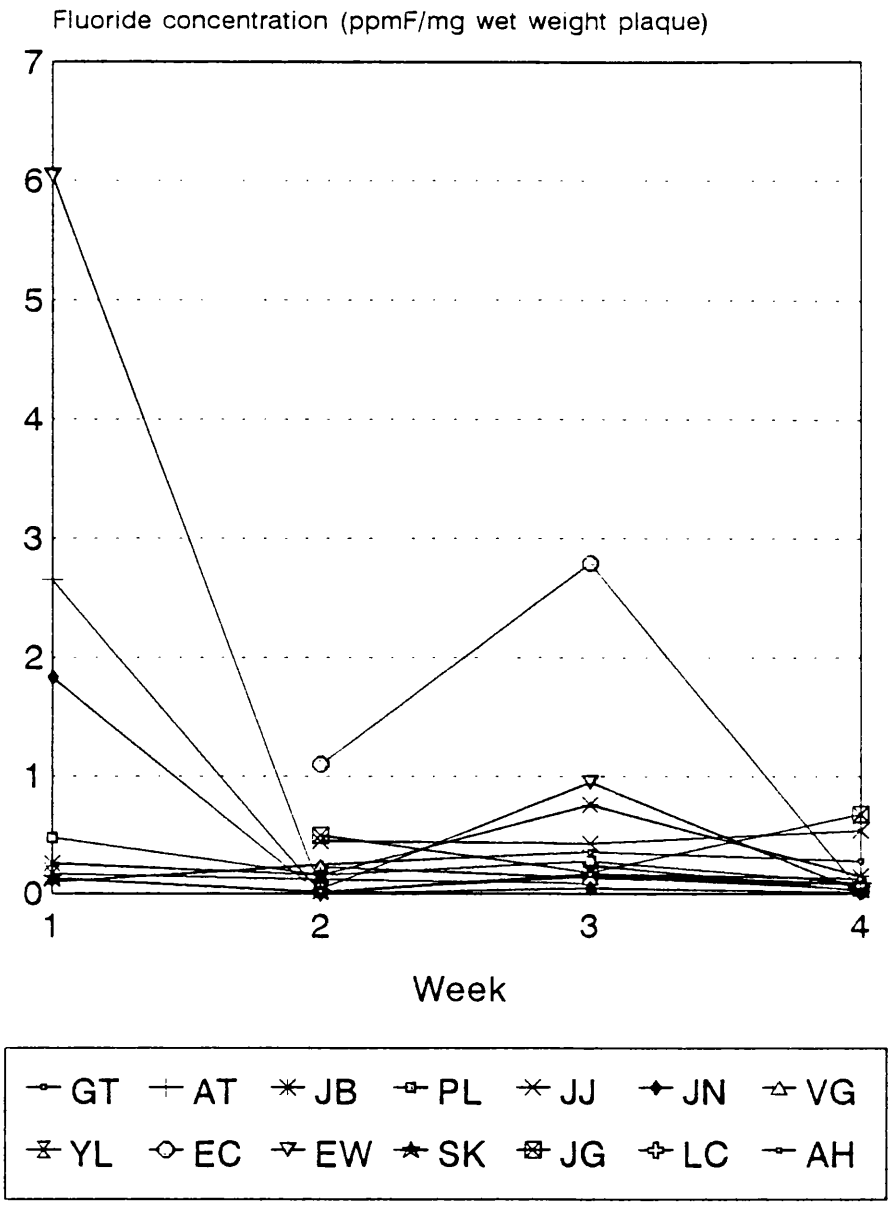


Figure 6.9 Plaque fluoride levels over a 4 week washout period for subjects using a non-fluoridated dentifrice subsequent to a period of fluoridated dentifrice use. These subjects went on to the non-fluoride and sorbitol gum chewing protocol in the 3 way crossover trial described in Chapter 5.

Change in salivary or plaque fluoride concentration over the 4 week washout period.

The mean salivary and plaque fluoride levels are shown in figures 6.10 to 6.15 and represent an arithmetical mean of up to four independent samples taken at the same time each week over the four week "washout" period for each volunteer. The error bars indicate 1 standard deviation either side of the arithmetical mean value. Two sample t-tests did not demonstrate a statistically significant difference between week 1 and week 4 saliva or plaque samples when subjects used a fluoridated dentifrice ($p = 0.12$ for saliva and 0.46 for plaque). As a result of the design of the 3-way crossover experiment, each subject had 2 washout periods where a non-fluoridated dentifrice was introduced subsequent to fluoridated dentifrice use. These washout periods have been described according to the experimental protocol each subject followed at the end of the washout period. These protocols were non-fluoridated dentifrice use and no gum chewing and non-fluoridated dentifrice use and sorbitol gum chewing. For the washout period subsequently followed by non-fluoridated dentifrice use and no gum chewing, a statistically significant difference was observed between week 1 and week 4 saliva samples ($p = 0.008$) but not for plaque samples ($p = 0.079$). For the washout period, subsequently followed by non-fluoridated dentifrice use and sorbitol gum chewing, no statistically significant differences were observed between week 1 and week 4 saliva or plaque samples ($p = 0.16$ for saliva and $p = 0.12$ for plaque). In addition, generally no statistically significant differences were

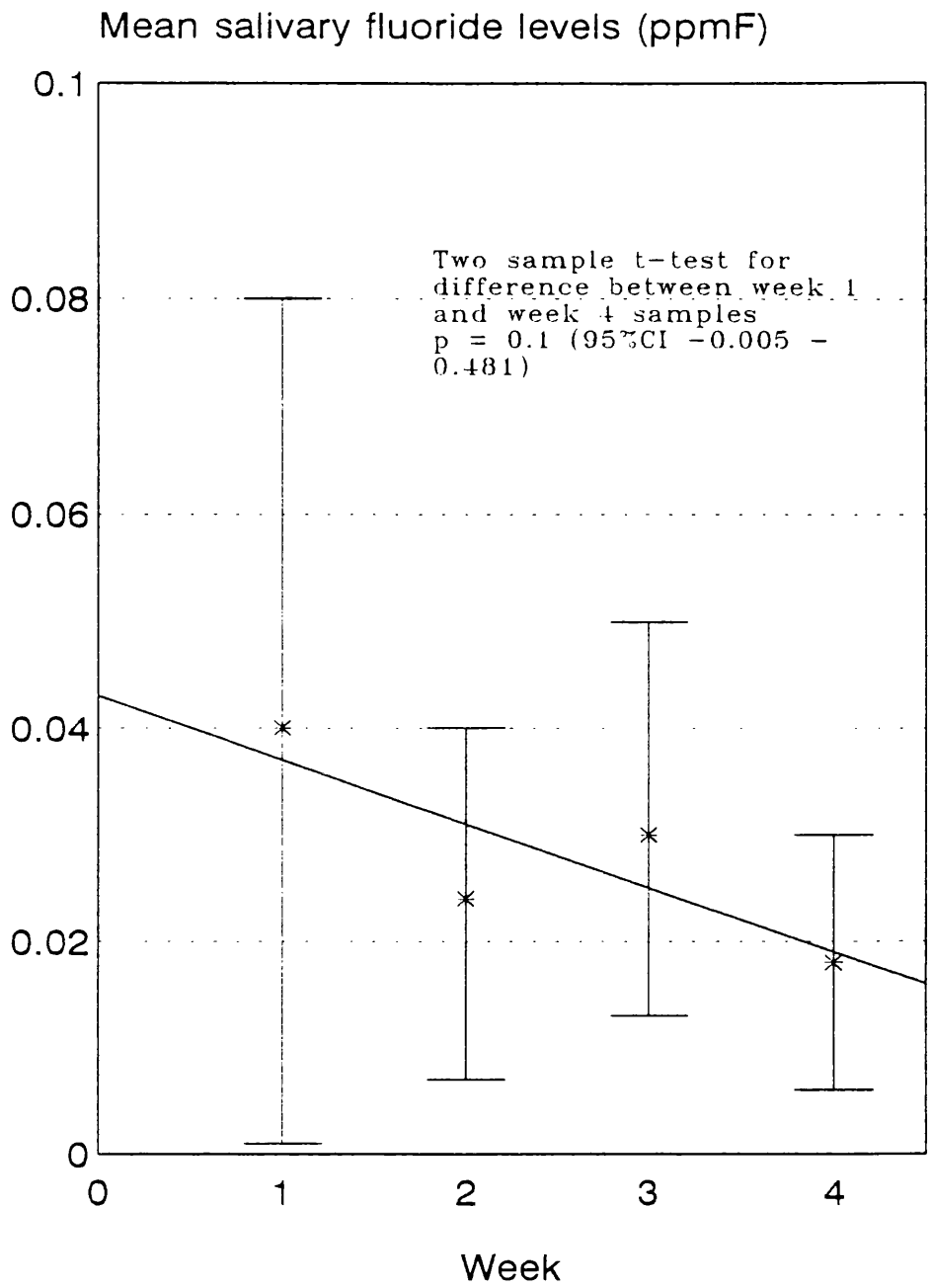


Figure 6.10 Change in mean saliva fluoride levels over a 4 week washout period for subjects using a 0.32% sodium fluoride dentifrice. The error bars indicate 1 standard deviation either side of the mean.

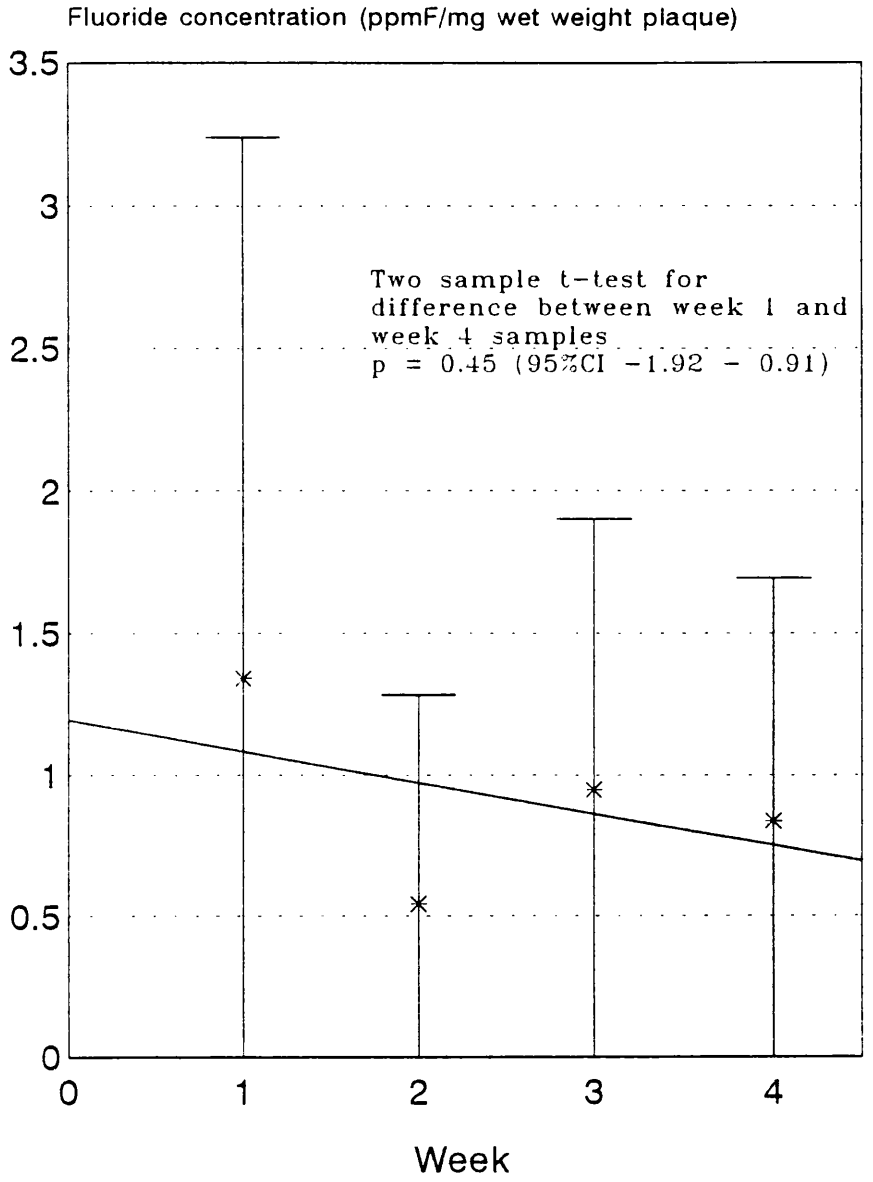


Figure 6.11 Change in mean plaque fluoride levels over a 4 week washout period for subjects using a 0.32% sodium fluoride dentifrice. The error bars indicate 1 standard deviation either side of the mean.

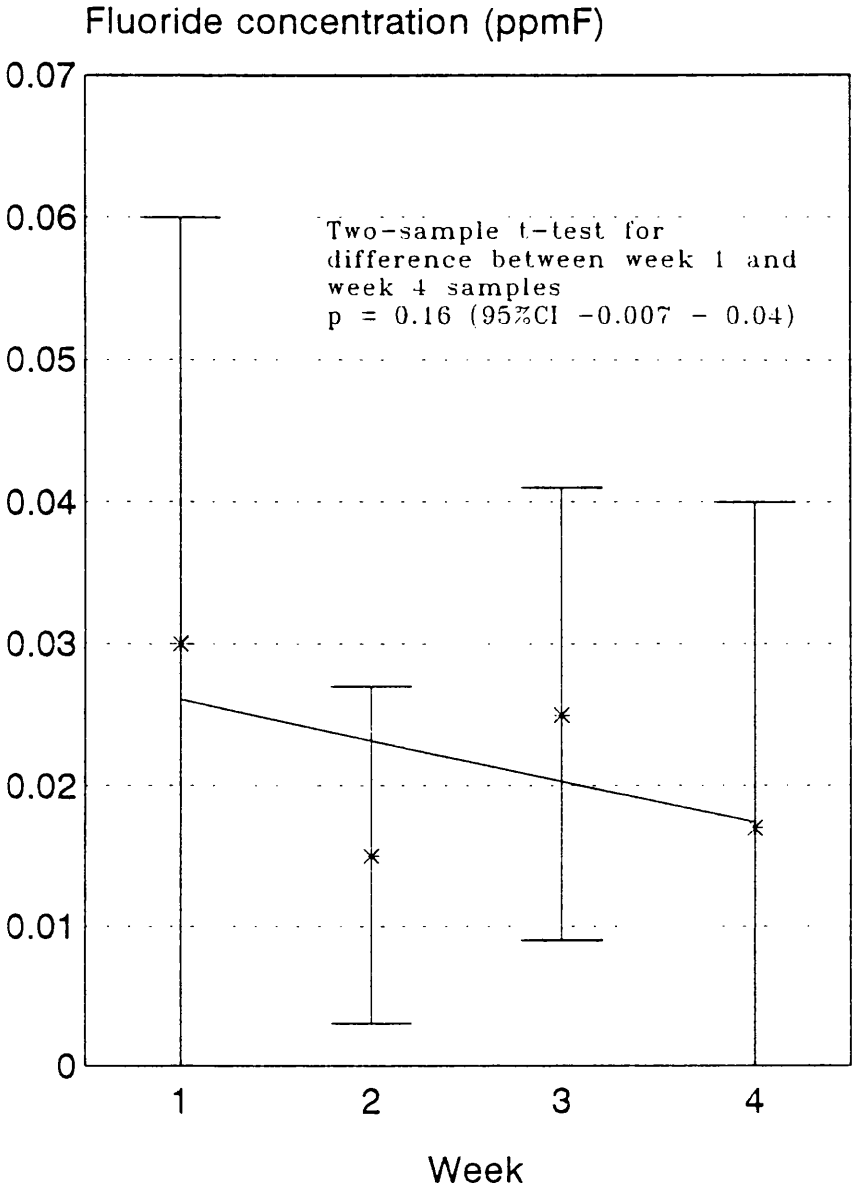


Figure 6.12 Change in mean saliva fluoride levels over a 4 week washout period for subjects using a non-fluoridated dentifrice subsequent to fluoridated dentifrice use. These subjects went on to a non-fluoride dentifrice, no gum chewing protocol in the 3 way crossover trial described in Chapter 5. The error bars indicate 1 standard deviation either side of the mean.

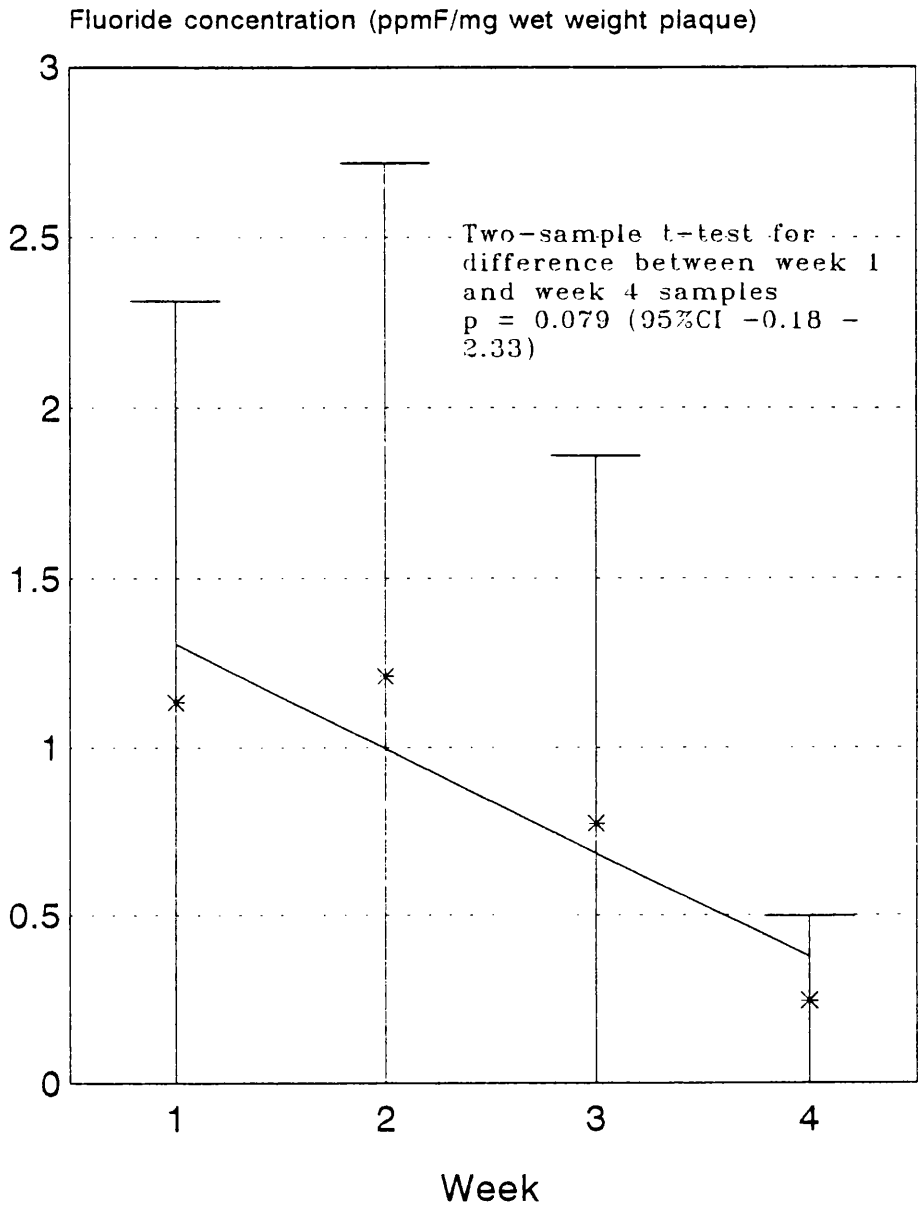


Figure 6.13 Change in mean plaque fluoride levels over a 4 week washout period for subjects using a non-fluoridated dentifrice subsequent to fluoride dentifrice use. These subjects went on to a non-fluoride dentifrice, no gum chewing protocol in the 3 way crossover trial described in Chapter 5. The error bars indicate 1 standard deviation either side of the mean.

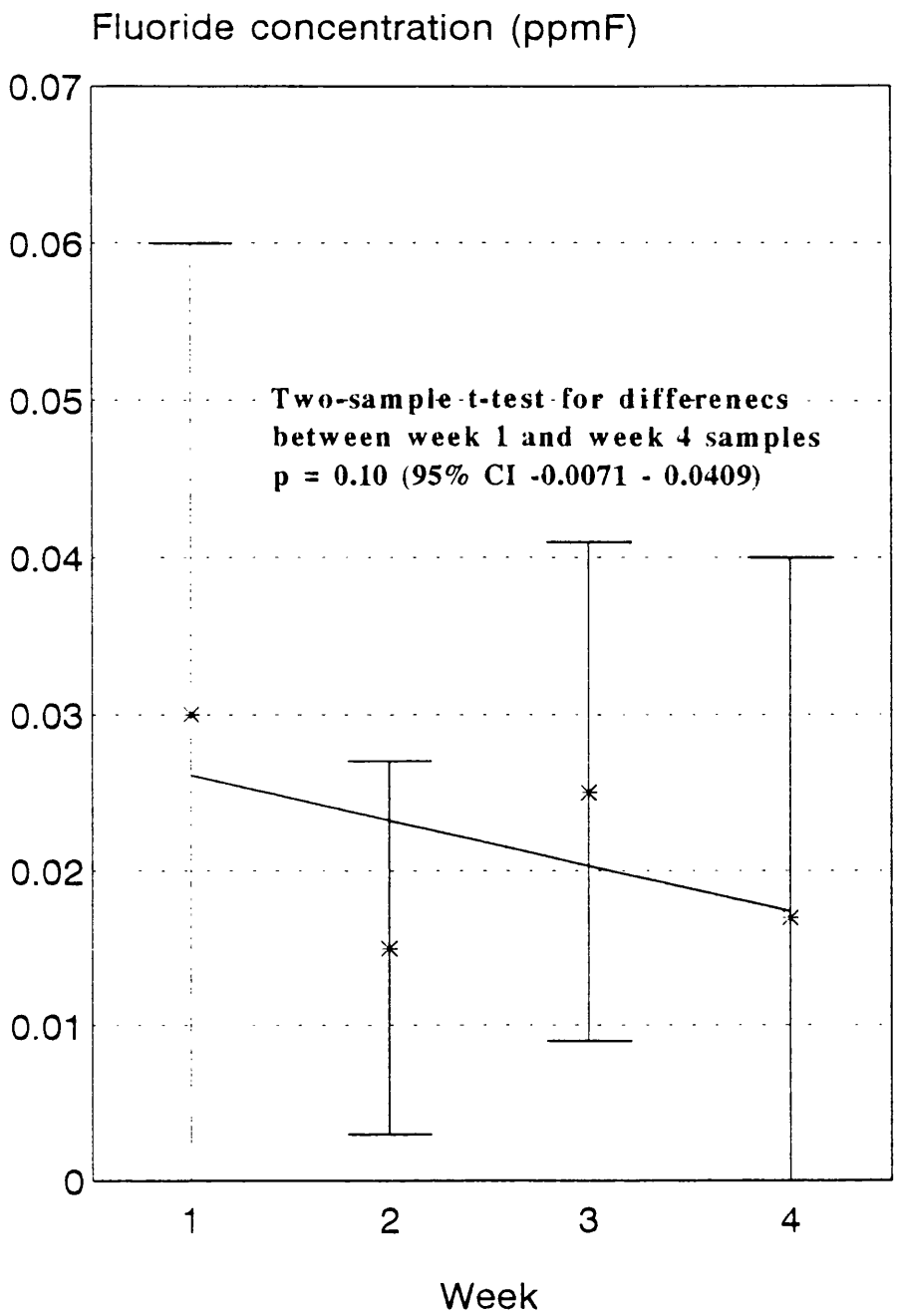


Figure 6.14 Change in mean saliva fluoride levels over a 4 week washout period for subjects using a non-fluoridated dentifrice subsequent to fluoride dentifrice use. These subjects went on to a non-fluoride dentifrice, sorbitol gum chewing protocol in the 3 way crossover trial described in Chapter 5. The error bars indicate 1 standard deviation either side of the mean.

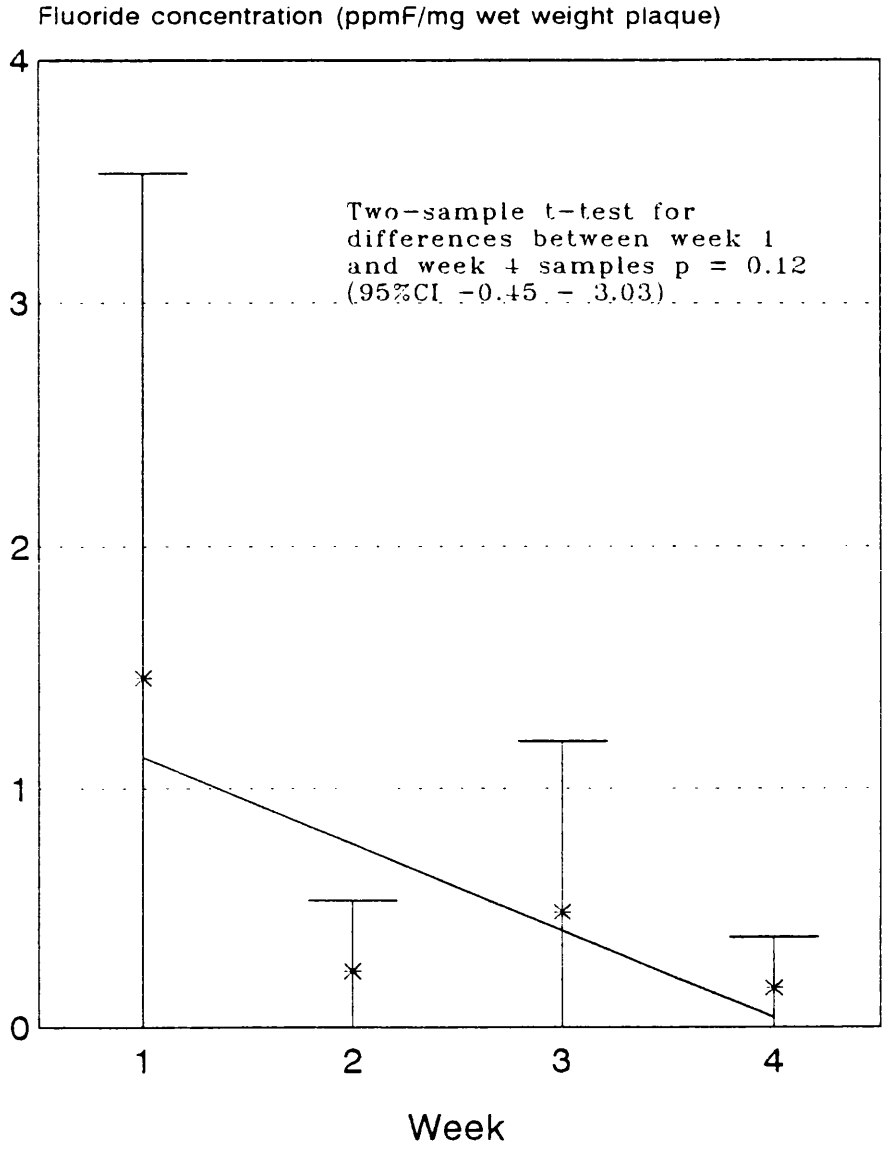


Figure 6.15 Change in mean plaque fluoride levels over a 4 week washout period for subjects using a non-fluoridated dentifrice subsequent to fluoride dentifrice use. These subjects went on to a non-fluoride dentifrice, sorbitol gum chewing protocol in the 3 way crossover trial described in Chapter 5. The error bars indicate 1 standard deviation either side of the mean.

observed for either plaque or salivary fluoride levels between the two non-fluoride protocols. The only exception to this being the comparison of plaque fluoride levels during the second week of the washout period ($p = 0.05$). This is illustrated in figures 6.16 and 6.17 by comparison of arithmetical mean values for each week of plaque and salivary fluoride measurements.

Comparison of plaque and salivary fluoride levels in subjects using fluoridated and non-fluoridated dentifrices

Figures 6.18 and 6.19 show the relationship between the arithmetical mean plaque and salivary fluoride levels for each protocol over the 4 week washout period. Two-sample t-tests revealed statistically significant differences in salivary fluoride levels between week 4 values for fluoride and non-fluoride/no gum protocols ($p = 0.045$). In addition two-sample t-tests revealed significant differences in plaque fluoride levels between fluoride and both non-fluoride protocols at week 4 ($p = 0.03$ for non-fluoride/no gum and $p = 0.01$ for non-fluoride/gum).

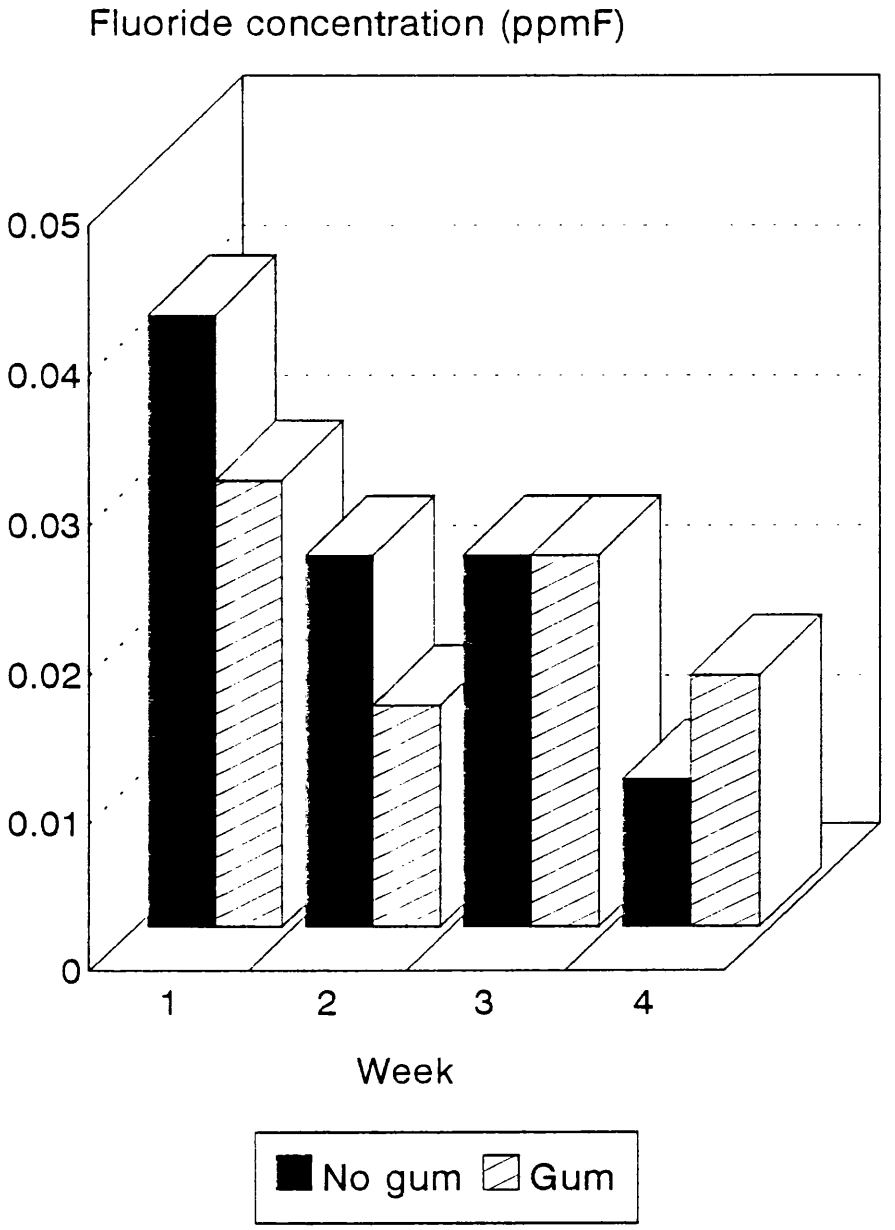


Figure 6.16 Histogram to show the comparison of mean saliva fluoride levels over a 4 week washout period in subjects using a non-fluoride dentifrice subsequent to fluoride dentifrice use. The subjects are divided according to the protocol they went on to undertake during the 3 way crossover trial described in Chapter 5.

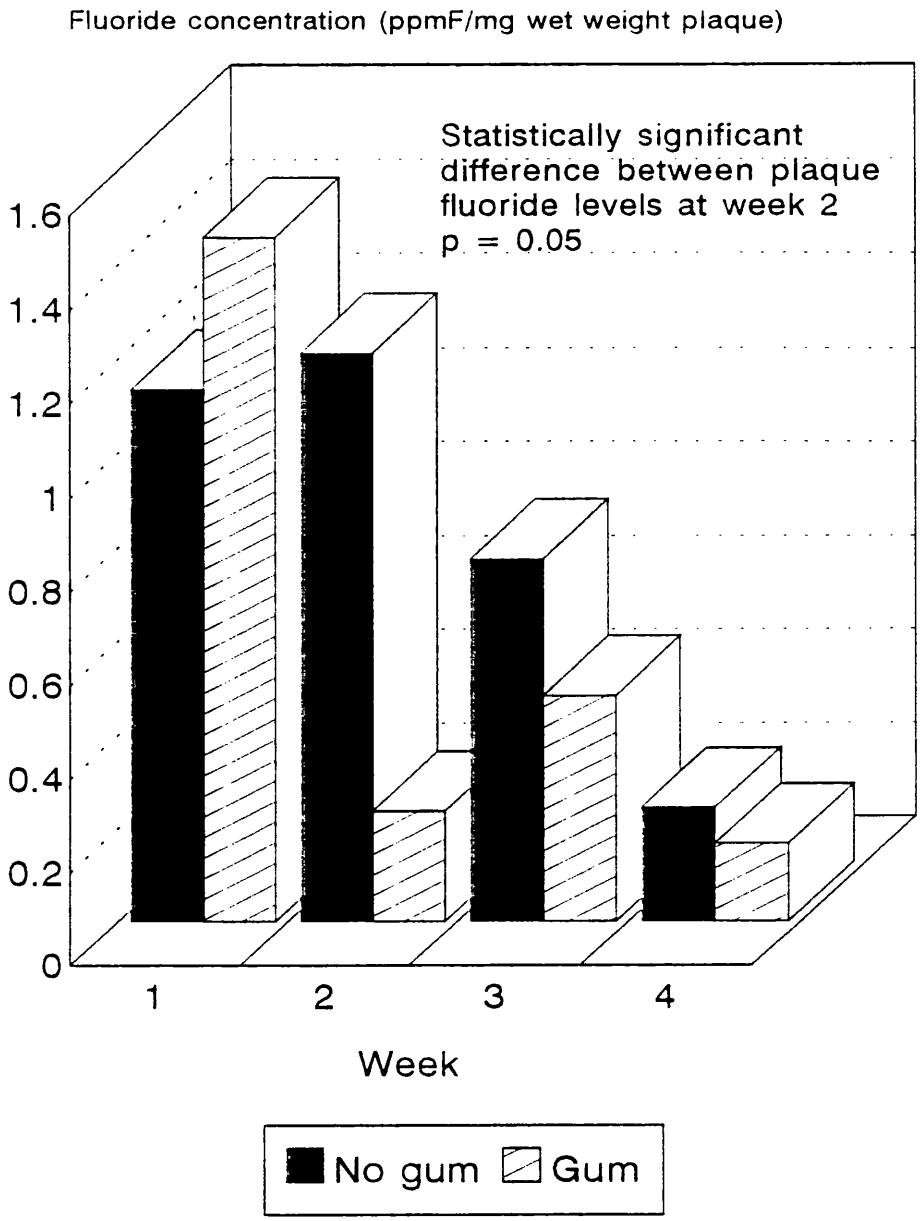


Figure 6.17 Histogram to show the comparison of plaque fluoride levels over a 4 week washout period in subjects using a non-fluoride dentifrice subsequent to fluoride dentifrice use. The subjects are divided according to the protocol they went on to undertake during the 3 way crossover trial described in Chapter 5.

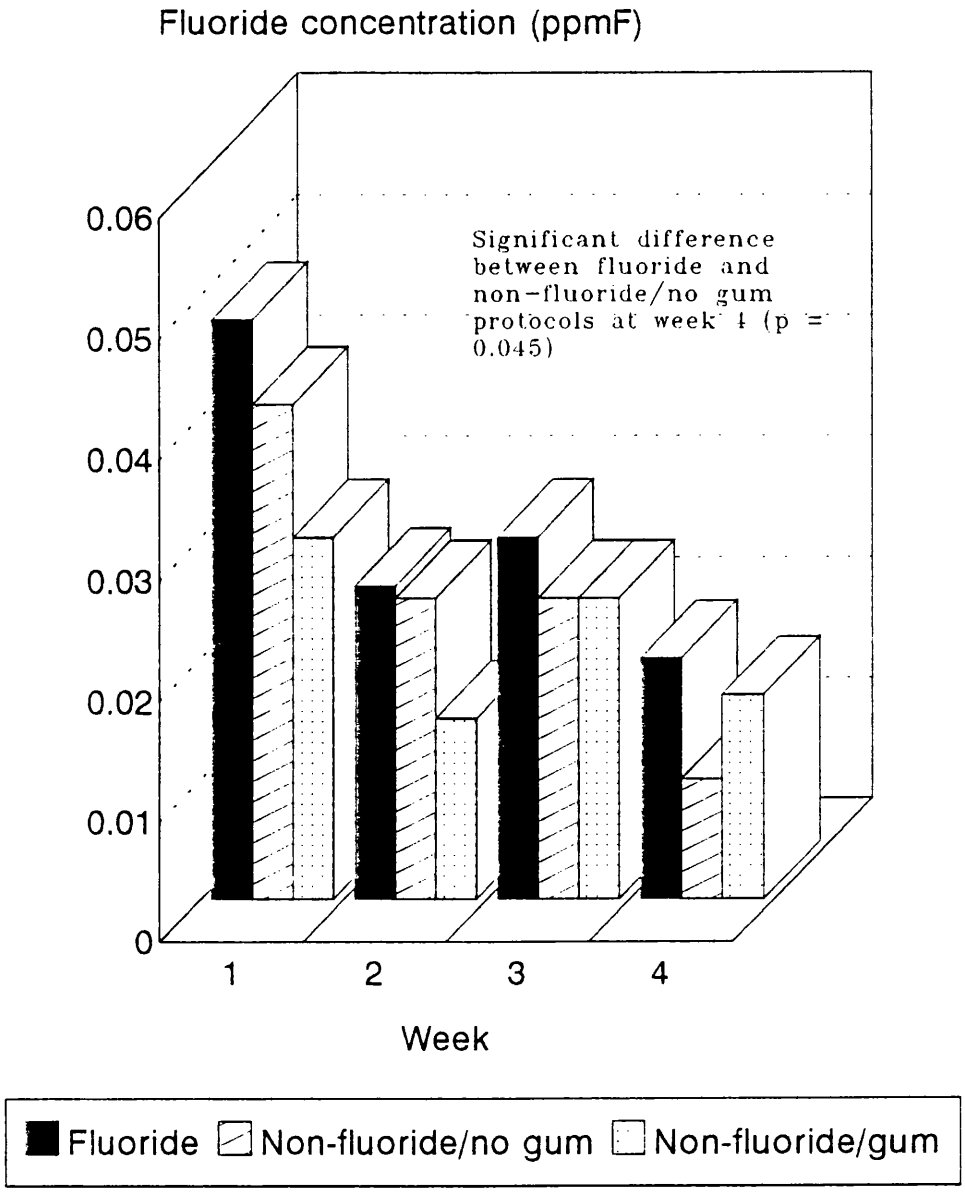


Figure 6.18 **Histogram to show the comparison of mean saliva fluoride levels for all subjects over a 4 week washout period. Subjects are divided according to the protocol they followed during the subsequent phase of the 3 way crossover trial described in Chapter 5.**

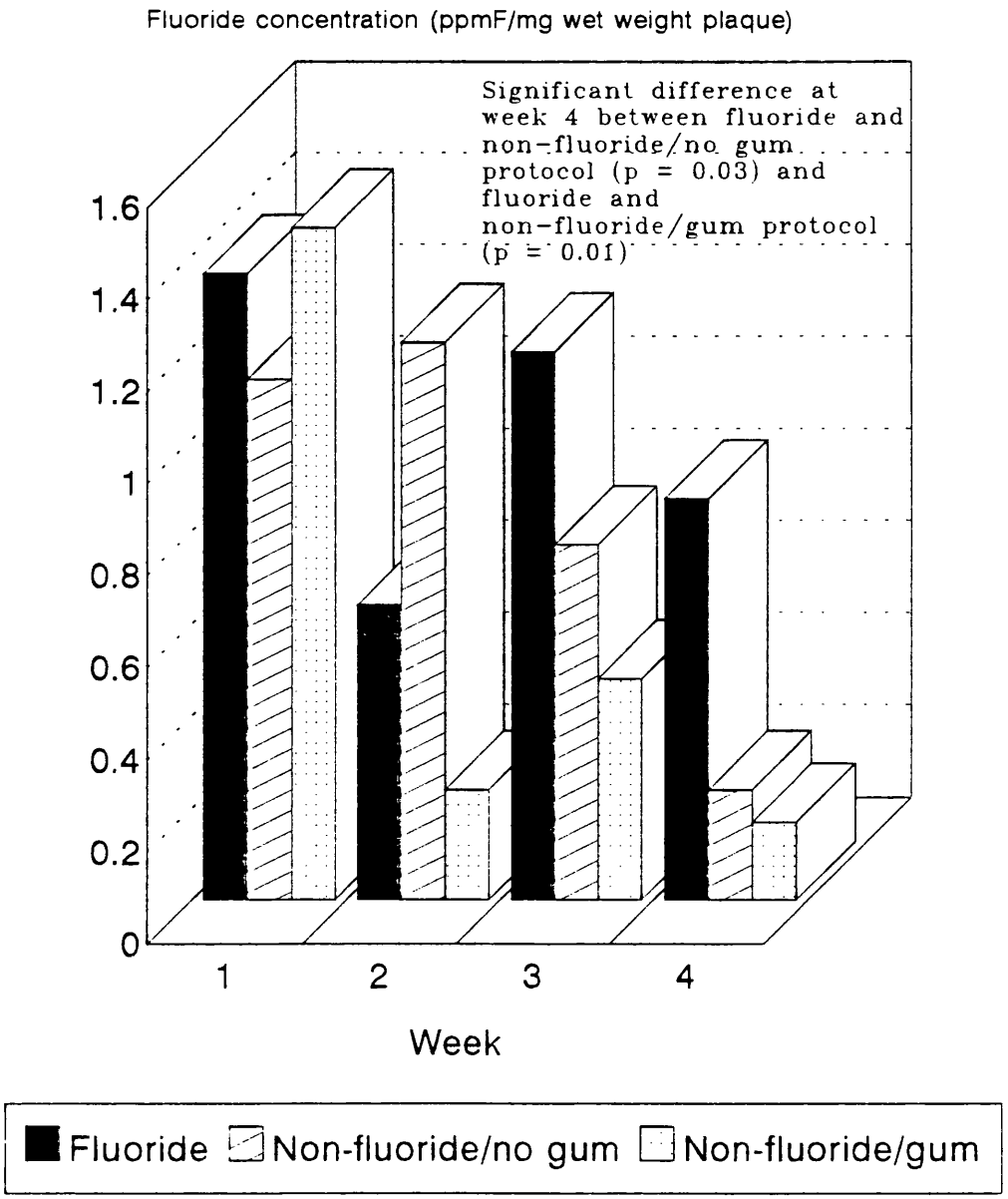


Figure 6.19 Histogram to show the comparison of mean plaque fluoride levels for all subjects over a 4 week washout period. Subjects are divided according to the protocol they followed during the subsequent phase of the 3 way crossover trial described in Chapter 5.

Comparison of plaque and salivary fluoride levels and predicted mineral changes in subsequent *in situ* trials.

Mean washout period plaque and salivary fluoride levels were compared to predicted mineral changes from subsequent *in situ* work. Data used in this analysis are shown in Table 6.1. Linear regression analysis was performed to determine the relationship between mean plaque and salivary fluoride levels over the 4 week washout period and the predicted mineral changes in the subsequent phase of the 3 way crossover trial described in Chapter 5. Eighteen analyses were performed. One analysis demonstrated a significant relationship ($p = 0.02$) between the mean salivary fluoride levels and the predicted surface zone mineral change for the non-fluoridated dentifrice/sorbitol gum protocol. A further analysis demonstrated a relationship of borderline significance ($p = 0.06$) between mean salivary fluoride levels and the predicted lesion body mineral change for the non-fluoridated dentifrice/no gum protocol. These analyses are demonstrated in figures 6.20 and 6.21.

Table 6.1

Mean plaque and salivary fluoride values from a 4 week washout period and associated predicted mineral changes from a subsequent *in situ* trial

Subject	Fluoride dentifrice only					Non-fluoridated dentifrice and sorbitol gum					Non-fluoridated dentifrice no gum				
	Mean fluoride level over 4 week washout period		Predicted change			Mean fluoride level over 4 week washout period		Predicted change			Mean fluoride level over 4 week washout period		Predicted change		
	Saliva (ppmF)	Plaque (ppmF/mg)	Δz	LB	SZ	Saliva (ppmF)	Plaque (ppmF/mg)	Δz	LB	SZ	Saliva (ppmF)	Plaque (ppmF/mg)	Δz	LB	SZ
1	0.07	1.78	-0.857	5.647	-1.342	0.02	0.10	-0.804	1.206	-0.452	0.01	1.33	2.987	-6.090	-6.799
2	0.05	1.48	0.398	0.395	4.832	0.02	0.73	-0.173	2.317	-0.092	0.01	0.84	3.754	4.715	2.251
3	0.02	0.31	-5.245	6.638	3.890	0.02	0.33	0.061	-0.490	0.303	0.03	0.78	-7.334	9.010	3.550
4	0.04	0.13	-1.534	4.764	2.892	0.03	0.25	-0.562	0.804	0.495	0.02	0.08	-6.979	7.244	5.988
5	0.01	1.85	-3.653	4.934	3.537	0.02	0.47	-6.957	4.034	4.824	0.02	1.13	-2.750	3.560	2.778
6			2.009	0.753	-3.167	0.01	0.47	2.148	-1.844	2.724	0.04	2.08	5.247	0.481	3.199
7	0.01	0.30	-1.038	0.425	0.594	0.04	0.44	1.799	-2.624	-3.845	0.02	0.04	1.679	-1.140	0.089

Δz - (%vol.mineralxμm)/100
LB - %vol.mineral
SZ - %vol.mineral

Table 6.1 (Contd.)

Mean plaque and salivary fluoride values from a 4 week washout period and associated predicted mineral changes from a subsequent *in situ* trial

Subject	Fluoride dentifrice only					Non-fluoridated dentifrice and sorbitol gum					Non-fluoridated dentifrice no gum				
	Mean fluoride level over 4 week washout period		Predicted change			Mean fluoride level over 4 week washout period		Predicted change			Mean fluoride level over 4 week washout period		Predicted change		
	Saliva (ppmF)	Plaque (ppmF/mg)	Δz	LB	SZ	Saliva (ppmF)	Plaque (ppmF/mg)	Δz	LB	SZ	Saliva (ppmF)	Plaque (ppmF/mg)	Δz	LB	SZ
8	0.03	0.60	-5.353	5.448	4.031	0.01	0.13	-2.659	0.439	2.996	0.02	0.22	1.728	3.253	4.960
9	0.02	1.04	4.007	-6.801	-7.172	0.02	1.31	1.272	-3.779	-1.009	0.002	1.44	-2.612	-0.630	0.546
10	0.03		-7.118	7.916	3.812	0.02	1.76	-0.776	1.607	2.159	0.06		-8.335	12.088	3.709
11	0.01	0.40	-7.699	6.282	1.488	0.02	0.08	14.67	2.513	0.168	0.03	1.13	10.36	-0.866	-3.9
14	0.03	1.88	44.12	3.208	0.807	0.04	0.45	23.08	0.897	2.585	0.02	0.20	31.16	0.908	-1.11
17	0.02	0.98	-4.540	2.634	0.185	0.03	0.17	0.345	-1.512	-5.200	0.04	0.47	-1.977	2.520	1.375
18	0.04	1.88	-2.862	8.312	5.592	0.01	0.25	-9.100	9.634	7.233	0.02	1.54	-26.67	4.740	4.338

Δz - (%vol.mineralxμm)/100
LB - %vol.mineral
SZ - %vol.mineral

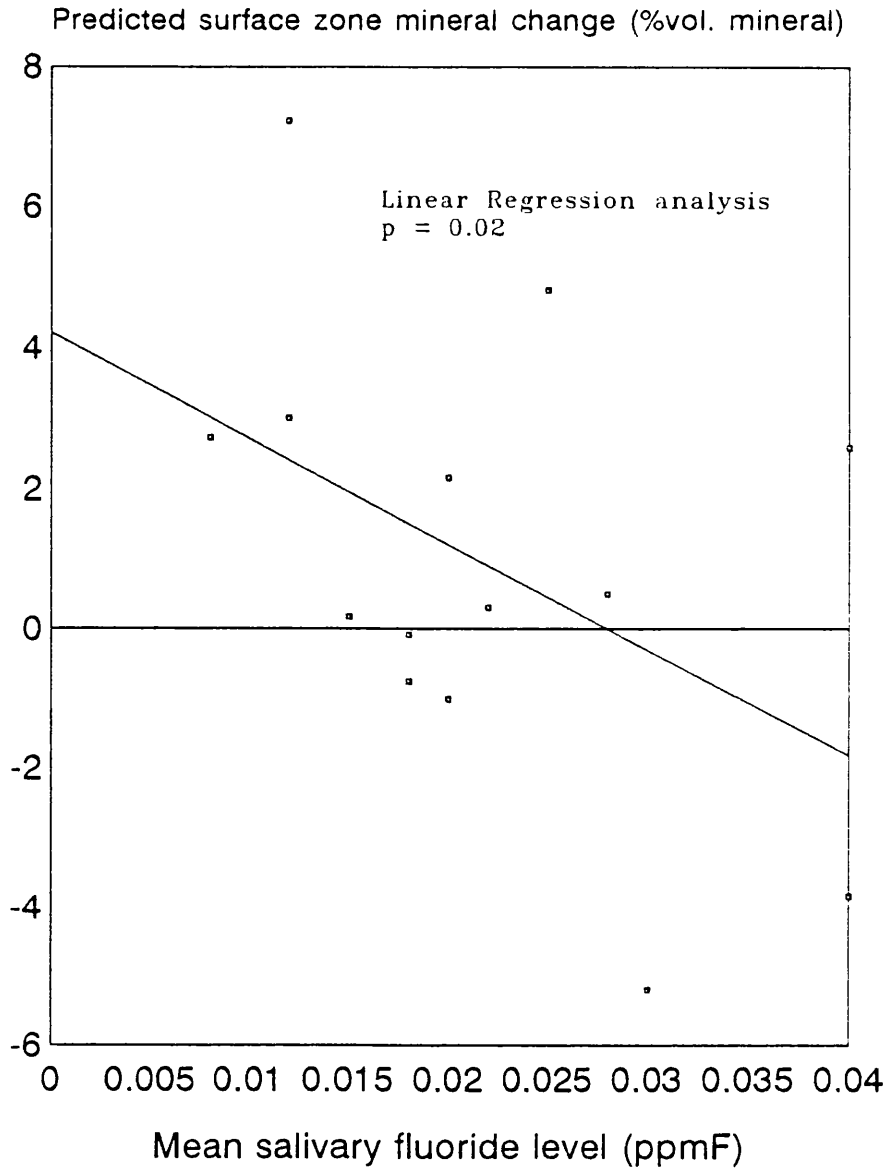


Figure 6.20 Graph to demonstrate the relationship between mean salivary fluoride levels over a 4 week washout period, for subjects using a non-fluoridated dentifrice, and the predicted surface zone change over a subsequent 5 week protocol of non-fluoride dentifrice use and sorbitol gum chewing.

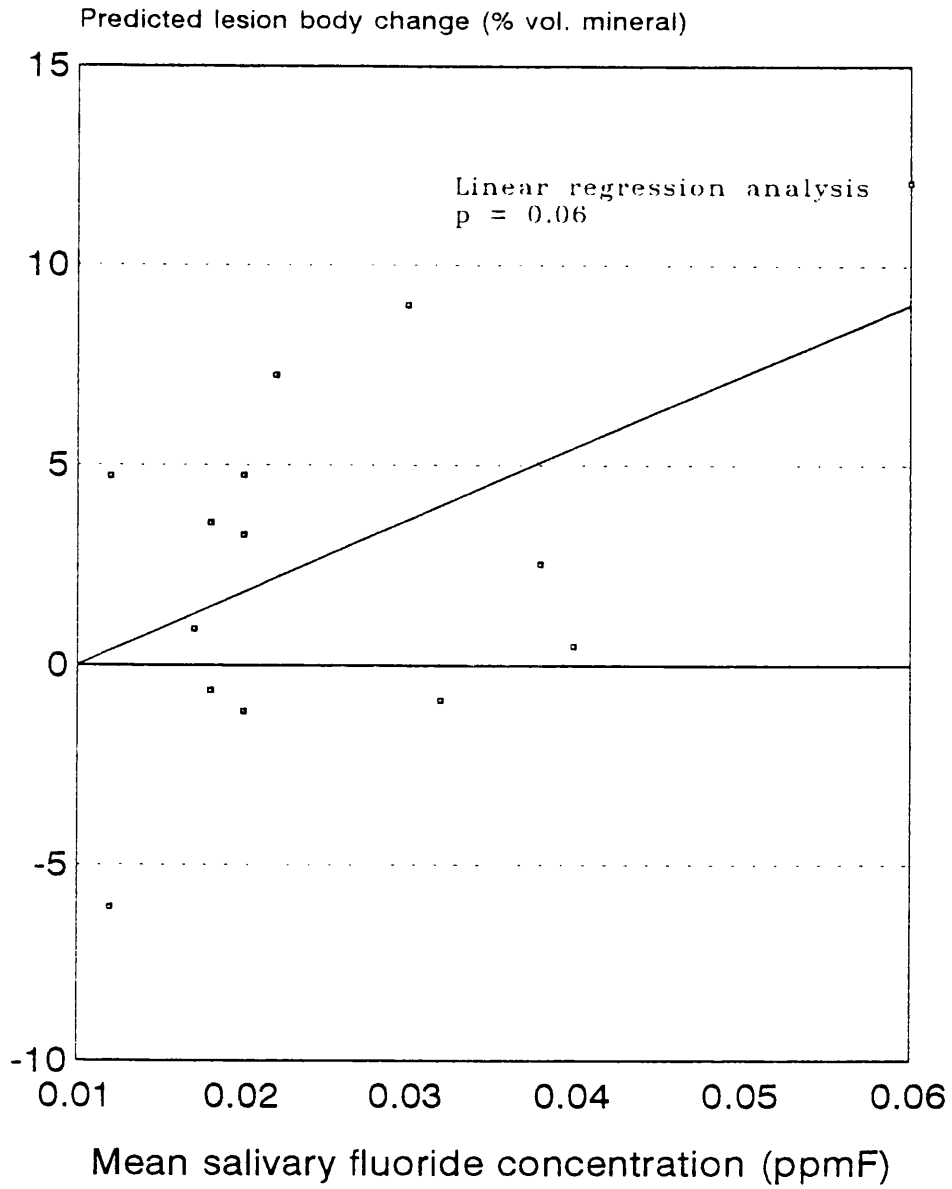


Figure 6.21 Graph to demonstrate the relationship between mean salivary fluoride levels over a 4 week washout period, for subjects using a non-fluoridated dentifrice, and the predicted lesion body changes over a subsequent 5 week protocol of non-fluoride dentifrice use and no gum chewing.

6.2.4 Discussion

The measured levels of fluoride in saliva were in agreement with the work of Shannon (1977) and Gron *et al.* (1968). The values recorded for plaque fluoride tended to be somewhat higher than those reported by Tatevossian (1990) although the fluoride status of those samples analyzed is not recorded.

The data have demonstrated statistically significantly lower salivary fluoride levels during week 4 of the washout period compared to week 1 for subjects using a non-fluoridated dentifrice in the group that went on to complete the non-fluoride / no gum protocol. There was a trend for a reduction in salivary fluoride levels over the 4 week washout period for the non-fluoride / sorbitol gum protocol but this was not statistically significant. Data for plaque fluoride failed to show any significant decrease in fluoride concentration over the 4 week washout periods when non-fluoride dentifrice was used. However, a downward trend was observed.

The standard deviation of the saliva results was much less than that of the plaque fluoride levels and, accordingly, differences between groups of salivary data were easier to detect. In addition, it is probably easier to obtain a homogeneous saliva sample than to obtain a plaque sample which may be contaminated with retained food debris or saliva.

The non-fluoride plaque and saliva data was analyzed further by comparison

with the data from subjects using a fluoridated dentifrice. It was apparent that significant differences between fluoride and non-fluoride protocols only developed towards the end of the 4 week period. This would support the suggestions by Damato (1990), Stephen (1992) and Damato & Stephen (1994) that there should be a minimum 4 week washout period between fluoride and non-fluoride dentifrice use in a *in situ* trial.

The regression analysis of mean salivary and plaque fluoride levels and predicted lesion changes using the *in situ* model requires further comment. There was a failure to observe any significant differences in plaque fluoride levels under any protocol over the 4 week washout period. In addition, only the salivary fluoride data for the non-fluoride / no gum protocol demonstrated a significant drop over the 4 week washout period. In order that all the data be treated in the same manner it was considered justified to use an arithmetical mean of each subject's data over the 4 week washout period.

Graphic representation of the two significant relationships between mean plaque and salivary fluoride levels and predicted changes (see figures 6.20 and 6.21) suggested that linear regression analysis was a suitable method of analysis although it did yield some results which are difficult to interpret.

The relationship between mean salivary fluoride levels and the predicted surface zone changes for the non-fluoride / sorbitol gum protocol suggested an increase in predicted surface zone mineralisation was associated with a

decrease in salivary fluoride concentration. This is difficult to explain in the light of work by Arends & Christofferson (1986) who demonstrated an increase in the mineral content of the surface zone with increasing levels of fluoride. However, this work was concerned with the formation of sub-surface lesions *de novo* rather than a dynamic change mineral content over the period of an *in situ* trial.

The relationship between salivary fluoride levels and the predicted lesion body changes for the non-fluoride / no gum protocol suggested an increase in salivary fluoride was associated with an increase in remineralisation of the lesion body. This was in line with results by Duckworth *et al.* (1992) who observed a decreased caries incidence with increased salivary levels of fluoride.

In summary fluoride levels in plaque and saliva tended to decrease when subjects changed from a fluoridated to a non-fluoridated dentifrice. This was more easily demonstrated in saliva than plaque which may have been due to the inhomogeneity of the plaque samples. Significant differences between saliva and plaque fluoride levels when comparing fluoridated and non-fluoridated dentifrices use became apparent at the end of a 4 week washout period. This suggests a significant carryover effect when changing from a fluoridated to a non-fluoridated dentifrice. Significant relationships between plaque and salivary fluoride levels and predicted mineral changes using an *in situ* model were difficult to demonstrate.

6.3 STUDIES TO DETERMINE THE ABILITY OF SALIVA TO CLEAR SUBSTRATE FROM THE APPLIANCE TROUGH

6.3.1 Introduction

The rate at which a film of saliva passes over the oral tissues varies at different sites within the oral cavity (Dawes *et al.*, 1989). The rate of movement of such a film will effect the rate of clearance of bacterial substrate and other organic and inorganic ions from any given site. This may have a profound effect on the local biochemistry which may, in turn, affect the caries process. The thickness of the film of saliva flowing over the various surfaces of the oral cavity has been estimated to be between 0.07 and 0.1 mm (Collins & Dawes, 1987). The rate of flow of such a film is known as the salivary film velocity. Dawes *et al.* (1989) argued that a low salivary film velocity will retard the clearance of diffusants, such as organic acids, from plaque thereby prolonging the low pH portions of the Stephen curve. In addition, the work of Lagerlof (1983) would tend to support this theory as a higher flow rate of saliva is associated with increased saturation with respect to calcium and phosphate salts. However, it is also possible to argue that, in the presence of an ionic gradient from plaque to saliva such as exists for fluoride (Tatevossian, 1991), a high salivary film velocity may deplete plaque fluoride concentrations which may have a profound effect on local saturation conditions.

The determination of salivary film velocity requires further comment. Initial estimations were made of the rate of clearance of known quantity of substrate

by saliva from an artificial plaque. Studies by Dawes (1989) have shown an exponential relationship between the rate of flow of saliva over the surface of an artificial plaque and the clearance half-time of a chemical marker within the plaque. In this way, the clearance half-time can be directly related to the salivary film velocity. However, much of the present work relates to clearance half-times rather than salivary film velocity.

The intra-oral appliance used in the work described in Chapter 3 supports varnished sections of teeth in the lower lingual sulcus. This sulcus area has been shown to be the site of highest film velocity in the oral cavity (Dawes & Macpherson, 1993). The appliance was designed to try and limit the amount of saliva flowing over the sections by mounting them in a protected recess, or trough, with a salivary ingress point at the superior aspect of the trough and a salivary egress inferiorly. The preliminary blocking out of undercuts, in order to remove the appliance from the model, makes it impossible to ensure the fitting surface of the lingual flange is always in contact with the lingual mucosa. This may result in a higher rate of flow of saliva than was originally intended.

Before continuing with a description of the methodology, it is perhaps appropriate to define some terms in relation to this work:-

1 Salivary Clearance

The passage of saliva from the salivary duct opening through the oral cavity to the pharynx.

2 Salivary film velocity

The rate at which a film of saliva travels over a given site in the oral cavity.

3 Salivary clearance of substrate "*x*"

The rate at which saliva depletes the amount of "*x*" at a given site by the physical act of washing it away or by chemical dilution.

4 Clearance half-time of "*x*" by saliva

The time taken for saliva to reduce the amount of "*x*" by half at a given site.

With these definitions in mind, the following experimental aims were proposed:-

1 -to devise a method of estimating the rate at which saliva would clear a substrate from the appliance trough.

2 -to compare this estimate with earlier work measuring clearance of the same substrate from the lingual sulcus.

3 -to determine how this estimate varies between volunteers.

4 -to determine if this estimate bore any relation to the results achieved during remineralisation experiments.

6.3.2 Method And Materials

The principle of this work relies on the diffusion of potassium chloride (KCl) from an artificial plaque, into saliva. When an artificial plaque, containing KCl, is placed at different sites within the oral cavity, different rates of diffusion of

KCl, from the plaque, are observed (Lecomte & Dawes, 1987). KCl was chosen as a diffusant as it has a low molecular weight, it is soluble and it diffuses easily from the artificial plaque. The concentration of potassium can be measured easily by spectrophotometric methods. KCl also has little taste and is, therefore, unlikely to stimulate salivary flow (Lecomte & Dawes, 1987). Agarose was used as the artificial plaque. It acted as a matrix to hold the KCl and had sufficient mechanical strength to resist damage in the mouth. In addition, the samples were easily removed for analysis (Lecomte & Dawes, 1987).

Before this methodology could be transferred to an *in situ* device, a decision was required on the positioning of the agarose gel to determine accurately the effect of saliva flowing through the *in situ* device trough on the clearance of KCl. The intra-oral appliance was designed to support sections of teeth mounted just above the base of the trough cut in the fitting surface of the lingual flange. The assumption was made that saliva would flow as a film over the sections and the base of the trough. Therefore, this appeared to be the most appropriate place to site the agarose gel. The size, shape and diffusion characteristics of the gel had to be calculated, as well as undertaking modifications to the intra-oral appliance.

Tile Construction

Previous studies (Lecompte & Dawes, 1987) used machined acrylic tiles with

a well diameter of 6 mm and a depth of 1.5 mm. In those studies, tiles were tied to the teeth with dental floss. For this study, however, it was decided to use stainless steel tiles (figure 6.22) with the same well dimensions to ensure comparability between this and previous studies (Lecompte & Dawes, 1987; Dawes & Macpherson, 1993). The tiles were machined from stainless steel blocks for improved rigidity, dimensional stability and ease of handling. The tiles were planoparallel with a surface area of 1 cm^2 and a thickness of 2 mm. The well dimensions were accurate to within 0.1 mm. Seven tiles were machined for calibration.

Calibration

The clearance half-time of KCl from a pellet of agarose gel in a stainless steel tile flush with the base of the trough on the intra-oral appliance was determined simultaneously in the right and left troughs of the appliance. Therefore, for each subject, two stainless steel tiles were required, with matched diffusion characteristics.

In keeping with previous experimental work (Lecompte & Dawes, 1987), a 1% by weight agarose gel was made up by mixing 0.5 g agarose (Bio-Rad Laboratories, Richmond, California, USA) with 49.5 ml of 1M KCl solution. The suspension was heated gently in a covered conical flask placed in a water bath over a bunsen burner. Once the gel had become clear, it was ready to use. The wells were filled by placing them on a flat surface and overfilling with

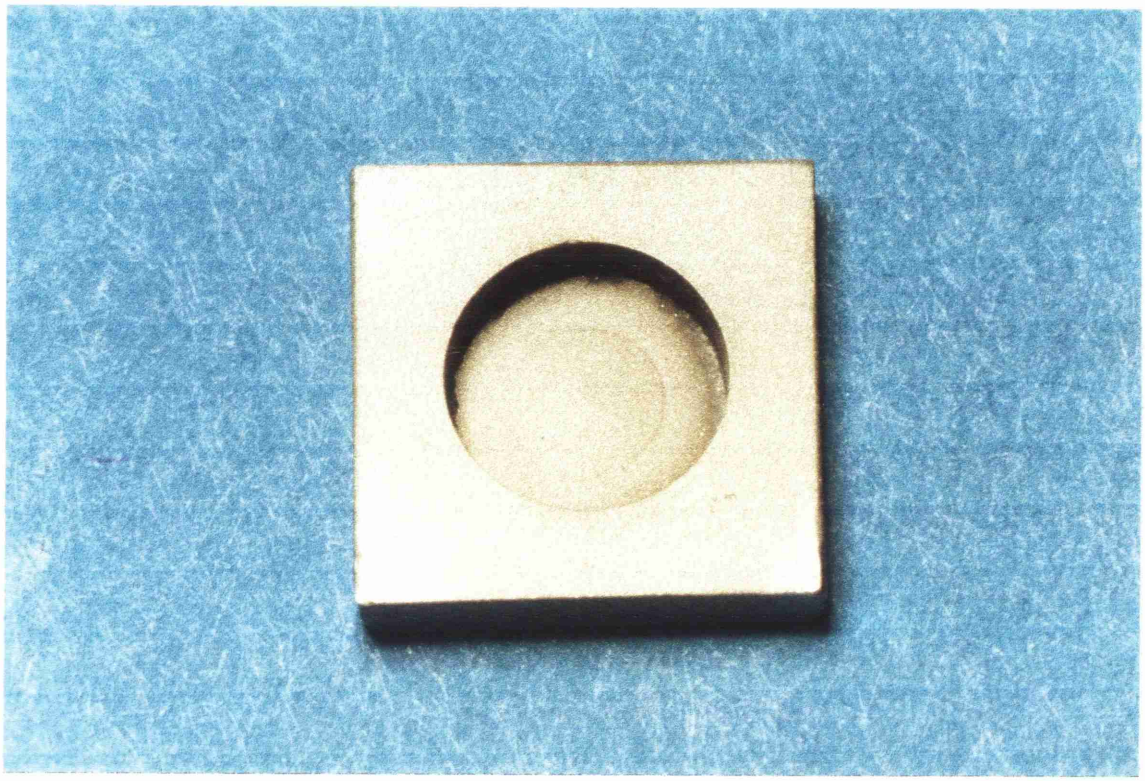


Figure 6.22 Photograph of stainless steel tile used for creation of agarose pellets. Internal diameter = 6 mm. Depth = 1.5 mm

molten agarose. The excess was removed by placing a glass microscope slide over the tiles for one minute. This produced a smooth surface to the gel, flush with the surface of the tile.

To determine the initial amount of KCl in the well, control agarose pellets were required. The tiles were, therefore, placed on ice cubes to chill the agarose and to facilitate removal of the agarose pellet (figure 6.23). Pellet removal was aided by the use of a needle to coax the pellet out of the well. Each pellet was placed in a separate conical flask containing a measured 500 ml of 100 ppm Na^+ solution. The flask was covered with parafilm and occasionally shaken gently over the next one and a half hours whilst the KCl leached out of the pellet.

The construction of a clearance curve required information on the rate of loss of KCl from the agarose with time. Further agarose pellets were created for this purpose. Each tile, which contained a fresh agarose pellet, was placed in 1 l of rapidly stirred 100 ppm Na^+ solution at 37°C for a timed 1.5 min. Once again, the tiles were chilled on ice, the pellets were removed and each placed in 500 ml of 100 ppm Na^+ solution for a minimum of one and a half hours. The procedure was repeated a final time when tiles containing fresh pellets were placed in the same rapidly stirred solution for 6 min. before being placed in 500 ml 100 ppm Na^+ solution.

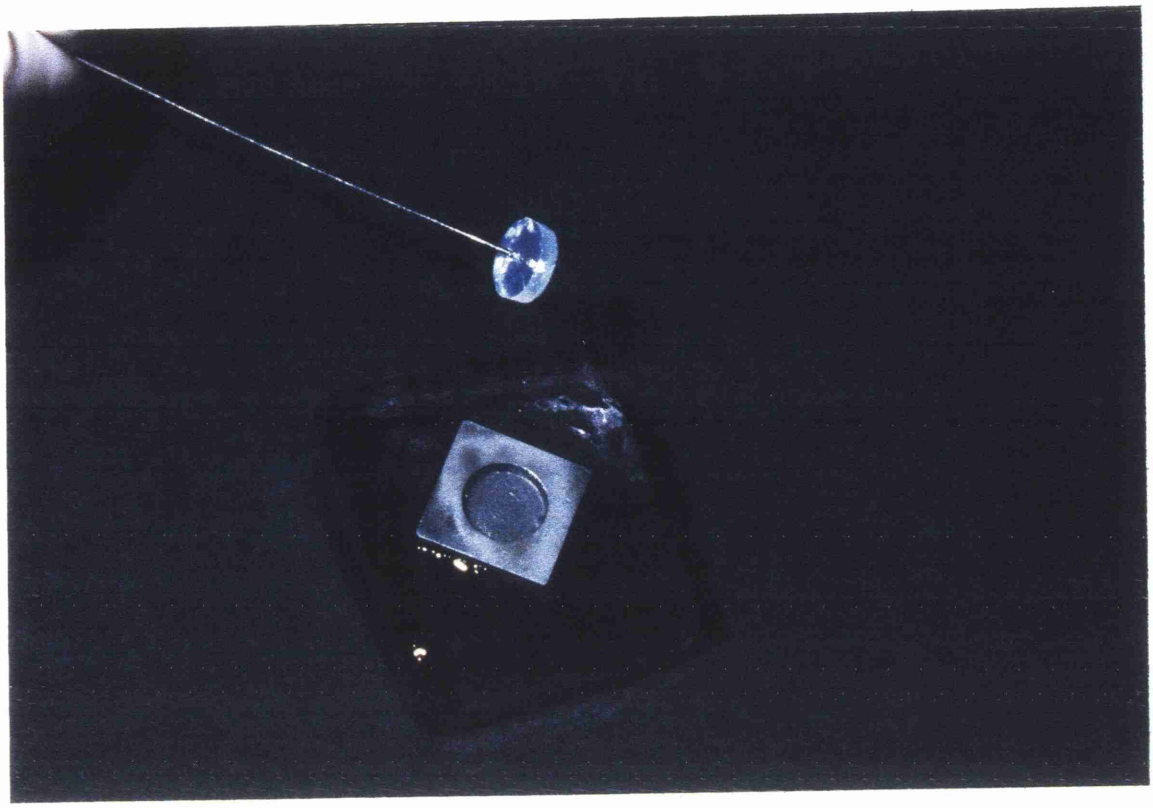


Figure 6.23 Photograph of chilled stainless steel tile on an ice cube to facilitate agarose pellet removal with a needle

Potassium Measurement

A minimum of one and a half hours were required to leach all the available KCl out of the agarose pellets (Lecompte & Dawes, 1989). A sample of fluid was withdrawn then for potassium measurement using a flame photometer (Corning 410 Flame Photometer, Corning Science Products, Halstead, Essex, England)(figure 6.24).

The flame photometer was adapted for a natural gas supply and calibrated using a 100 ppm Na^+ solution as a blank and a 1M KCl solution as a positive control. The control, 1.5 min. and 6 min. samples were measured for each tile. Plots were made of the potassium concentration against the square root of time and a least squares regression line fitted to each plot (figure 6.25). The clearance half time of potassium from each tile was calculated by the equation:-

$$\text{Clearance Half Time} = \left(\frac{\text{intercept}}{2 \times \text{slope}}\right)^2$$

The whole procedure was repeated three times to establish reproducibility of the experiment and two tiles were chosen with the closest clearance half-times. The difference in the clearance half-times of these tiles was approximately 12 sec. These tiles were used for all subsequent work.

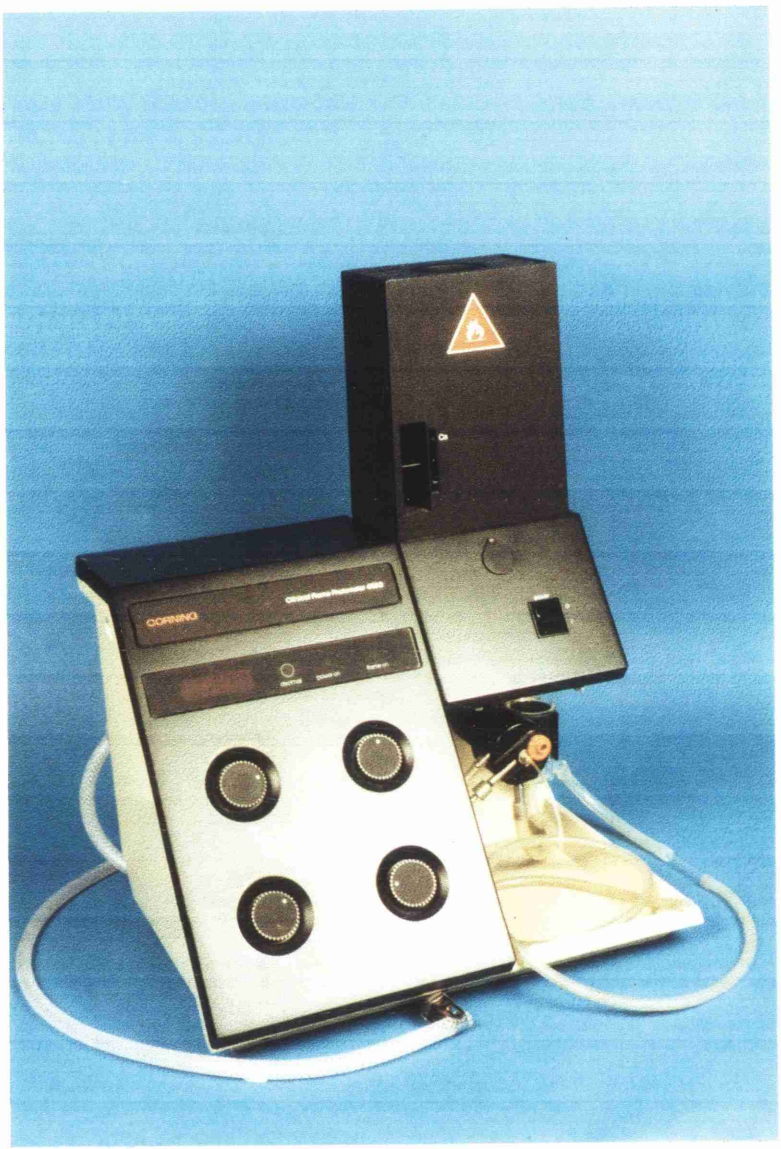
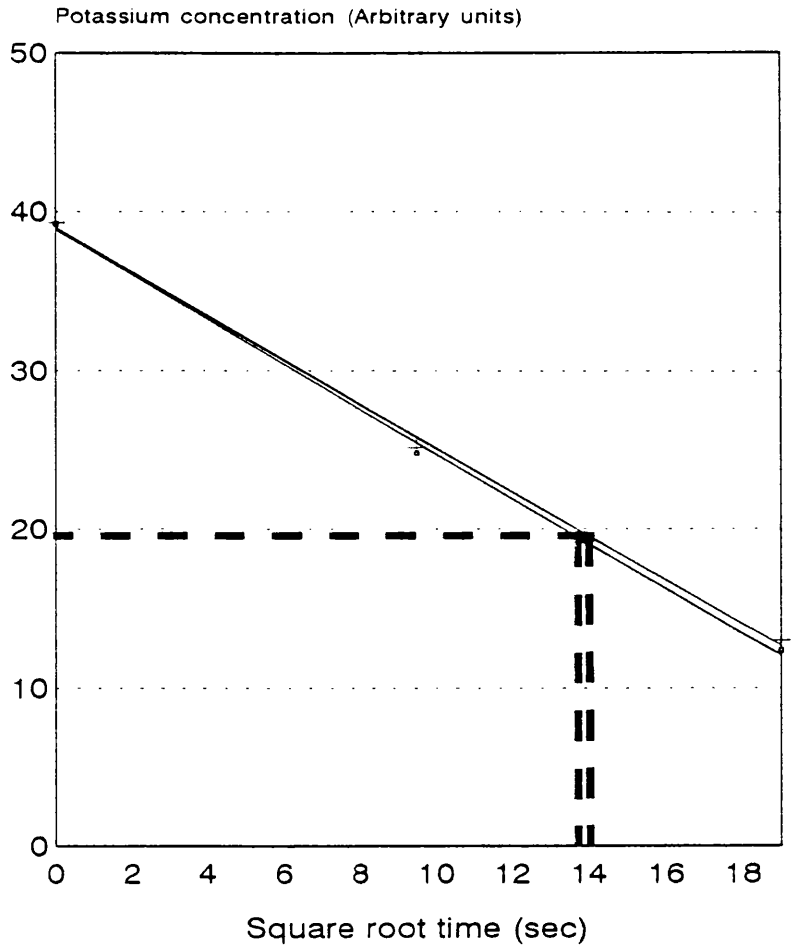


Figure 6.24 **Photograph of Corning 410 flame photometer for determination of potassium concentration.**



Tile 1	Tile 2
r = -0.999	r = -0.999
p = 0.03	p = 0.03
$t_{1/2}$ = 3.14 min	$t_{1/2}$ = 3.29 min

Figure 6.25 Graph of the potassium leached out of the agarose pellets plotted against the square root of the time the pellets were exposed to 1 l of rapidly stirred 100 ppm Na^+ solution. The data shown is for the two matched tiles used in subsequent experimental work.

Appliance Construction

New appliances had to be created to house these tiles so that the surface of the pellet was level with the base of the trough. The new appliances were of a slightly different design to those used previously (figure 6.26). The posterior lingual trough was of the same size and in the same position as the original appliance, as were the salivary ingress and egress. The main difference was the replacement of the wrought lingual bar between the right and left posterior lingual flanges by an acrylic plate. It was felt that this slight change in design may stimulate salivary production making it very difficult to determine a resting saliva clearance rate of potassium from the agarose gel. In an attempt to counteract this effect, this experiment took place on the last day of one of the five week *in situ* phases. The new appliances were placed in the mouth immediately after removal of those appliances supporting the artificial lesions used in the three-way crossover trial. With this protocol, the volunteers would, therefore, not have a period without an intra-oral appliance. This appliance was used to study the effect of both resting and stimulated salivary flow on the clearance of KCl from agarose gel at the base of the trough.

Experimental Protocol

Six subjects were chosen to participate in this experiment which took place at the end of the second appliance-wearing phase of the three-way crossover trial described in Chapter 5. The choice of subjects was influenced by their

Figure 6.26 Photograph of new appliance to hold the stainless steel tiles which were recessed in the base of the lingual trough.

performance in a previous *in situ* trial reported in Chapter 3. An attempt was made to use volunteers who had previously shown considerable demineralisation or considerable remineralisation. In addition, a subject representative of a result somewhere between these extremes was also included.

To determine the effect of resting salivary flow on KCl clearance from the agarose pellet, the appliance, loaded with stainless steel tiles containing agarose, was placed in the oral cavity. The subject was asked to remain seated quietly and given reading material to provide some distraction. The appliance was left in the mouth for a timed 15 min. The stainless steel tiles were then removed from the appliance and placed on an ice cube to chill the agarose pellet and facilitate its removal. Each agarose pellet was placed into a separate 1 l conical flask containing 500 ml of 100 ppm Na^+ solution. The flasks were covered with paraffin wax sealing film and labelled according to which side of the mouth each pellet had originated. The tiles were re-filled with agarose and placed back into the appliance which was placed back into the mouth for a timed 5 min. Once again, the agarose pellets were removed and placed into 500 ml of 100 ppm NaCl solution. This experiment was repeated three times. All flasks containing the agarose pellets were left for at least one and a half hours, agitated occasionally, before a sample was withdrawn for analysis.

The methodology for determination of stimulated salivary flow on clearance was similar to that for resting salivary flow. However, on this occasion,

volunteers were asked to chew sorbitol-containing chewing-gum whilst wearing the appliance. Appliance wearing times were reduced to 5 min and 2 min respectively. Once again, the experiment was repeated three times.

Twenty eight pellets were used for each volunteer, including right and left control pellets, for both the resting and stimulated saliva experiments. A sample was taken from each conical flask containing an agarose pellet and 500 ml 100 ppm Na^+ solution. This was analyzed for potassium using the flame photometer as described previously. Each sample was analyzed on three separate occasions to ensure accuracy and reproducibility. A simple arithmetical mean of flame photometer values was calculated for each of the resting and stimulated salivary experiments for both right and left sides. The mean values were used to plot the concentration of potassium against the square root of time. Clearance half-times were calculated according to the equation given previously.

Dr Colin Dawes (Department of Oral Biology, University of Manitoba, Winnipeg, Canada) was kind enough to calculate the salivary film velocity values for the unstimulated salivary clearance half times, based on previous work undertaken in his department. The use of the same methodology as that of Dawes *et al.* (1989) facilitated these calculations. Salivary film velocities for stimulated salivary clearance half time have not been calculated as, in many cases, these are equivalent to the clearance observed in a well stirred solution and therefore, at the maximum limit of detection of this model.

6.3.3 Results

Tile Calibration Results

The data for the calibration of the tiles used for this work are shown in figure 6.25. The data for each tile represents a mean of five repeated experiments. The clearance half-times for these tiles were 3.14 and 3.29 min respectively.

Choice of volunteers

The volunteers for this experiment were chosen on the basis of their behaviour in a previous trial. It has been suggested in Chapter 5 that past performance is no indicator of future performance with this *in situ* model. However, on this occasion, an attempt was made to stratify subjects according to previous performance. All volunteers who took part in this experiment had completed two *in situ* trials which involved a fluoride / non-chewing protocol. There was, however, a two week difference in the length of the appliance wearing period between the two trials. It would, therefore, seem appropriate to compare the predicted changes from both of these experiments to determine if volunteer behaviour was comparable on both occasions. Table 6.2 shows the mean predicted changes for lesions for the studies reported in Chapters 3 and 5. In order to compare such small data sets, analysis of performance within the group, rather than regression analysis, is more appropriate. A Spearman's Rank Correlation Coefficient is shown in Table 6.3 and shows an excellent

Table 6.2

Comparative results for predicted lesion changes for a set of six volunteers in two unrelated *in situ* trials

Subject (Identification number from three-way crossover trial)	Predicted Change					
	Fluoridated dentifrice, two-way crossover trial, Chapter 3			Fluoridated dentifrice, three-way crossover trial, Chapter 5		
	Δz	Lesion body	Surface zone	Δz	Lesion body	Surface zone
2	-6.25	4.60	0.36	0.398	0.395	4.832
3	-14.5	14.93	6.45	-5.245	6.638	3.890
6	0.51	2.26	0.57	2.009	0.753	-3.167
9	3.03	2.66	-2.10	4.007	-6.801	-7.172
14	7.7	-4.0	-2.2	44.118	3.208	
18	-11.81	12.54	10.16	-2.862	8.312	5.591

Δz - (%vol.mineralxμm)/100
LB - %vol.mineral
SZ - %vol.mineral

Table 6.3

Spearman's Rank Correlation Coefficient of predicted values from two unrelated *in situ* trials

		Fluoridated dentifrice, two-way crossover trial, Chapter 3		
		Δz	Lesion body	Surface zone
Fluoridated dentifrice, three-way crossover trial, Chapter 5	Δz	1.000		
	Lesion body		0.429	
	Surface zone			0.600

relationship between volunteers' performance in relation to one another for predicted changes in Δz ($r = 1.0$) although the ranking of other parameters was less successful ($r = 0.429$ for LB and 0.6 for SZ).

Unstimulated and stimulated clearance half times and salivary film velocities

Data for salivary clearance half times and salivary film velocity were obtained for five out of the original six volunteers. Data from one volunteer (Volunteer no. 2) were not analyzed due to a technical error with agarose gel preparation.

The arithmetical mean value for the five volunteers for unstimulated clearance half time was 7.0 min (SD 2.5), the mean value for stimulated clearance half time was 4.2 min (SD 1.0) and the mean value for unstimulated salivary film velocity was 25.2 mm/min (SD 23.4).

Table 6.4 shows the data obtained for the determination of salivary clearance half time and salivary film velocity. The predicted changes for each volunteer in each phase of the three-way crossover trial reported in Chapter 5 are displayed in Table 6.1.

Table 6.5 shows the results of paired t-tests to compare clearance half time and salivary film velocities from the right and left sides of the mouth. No significant differences were seen between left and right sides of the oral cavity

Table 6.4

Data for unstimulated and stimulated salivary clearance half times and unstimulated salivary film velocities for a group of 5 volunteers.

Subject	Salivary Clearance Half Time				Salivary Film Velocity	
	Unstimulated Right Side (min)	Unstimulated Left Side (min)	Stimulated Right Side (min)	Stimulated Left Side (min)	Unstimulated Right Side (mm/min)	Unstimulated Left Side (mm/min)
3	6.83	6.38	4.12	3.96	12.94	16.45
6	4.55	4.94	4.09	3.68	66.69	45.52
9	12.19	9.93	4.39	3.84	3.94	6.68
14	4.62	5.88	2.98	3.31	60.34	21.11
18	9.97	8.15	4.99	6.64	9.97	8.17

Table 6.5

P values for paired t-tests to compare salivary clearance half times and salivary film velocities on the right and left sides of the mouth.

		Clearance half times				Salivary film velocity		Combined RIGHT and LEFT stimulated clearance half times
		Unstimulated RIGHT side	Unstimulated LEFT side	Stimulated RIGHT side	Stimulated LEFT side	Unstimulated RIGHT side	Unstimulated LEFT side	
Clearance half time	Unstimulated RIGHT side		0.43	0.054				
	Unstimulated LEFT side	0.43			0.033			
	Stimulated RIGHT side	0.054			0.25			
	Stimulated LEFT side		0.033	0.25				
Salivary film velocity	Unstimulated RIGHT side						0.25	
	Unstimulated LEFT side					0.25		
Combined RIGHT and LEFT unstimulated clearance half times								0.0023

for either unstimulated or stimulated salivary clearance half time or unstimulated salivary film velocity. This data is displayed as a histogram in figure 6.27 and justifies combining the left and right data sets by calculating a mean of the two sides for each volunteer. Comparison of combined unstimulated and stimulated clearance half times shows a highly statistically significant difference ($p = 0.0023$). In addition, statistically significant differences were also observed for uncombined right and left data between stimulated and unstimulated salivary flow ($p = 0.03$, left side and $p = 0.05$, right side).

Comparison of salivary data with predicted mineral changes

Table 6.6 shows the p-values obtained for linear regression analysis of salivary parameters measured in this experiment and predicted changes for all five volunteers from the three-way crossover trial reported in Chapter 5. This table contains a large number of regression analyses and great care must be taken with the interpretation of these results.

Out of a total of 90 regression analyses only 4 had $p < 0.05$. It was decided that these data should form the basis of further analysis. These were for comparisons of stimulated saliva and non-fluoride Δz predicted changes. Plots of data together with their regression lines are displayed in figure 6.28.

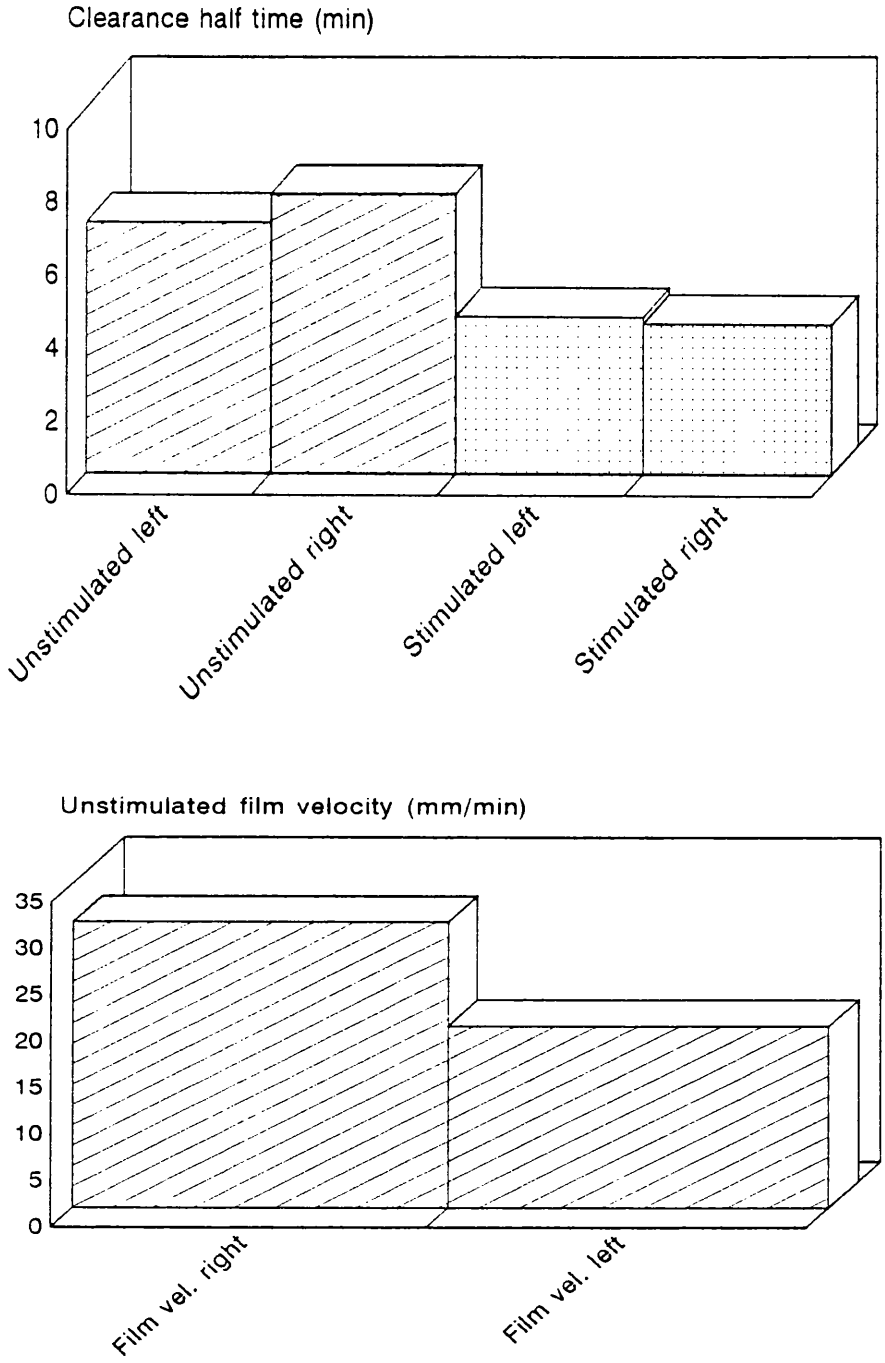


Figure 6.27 Histogram to show the difference between right and left unstimulated and stimulated clearance half times and unstimulated salivary film velocity.

Table 6.6

P - values for linear regression analysis of measured salivary parameters compared to predicted lesion changes in the 3 way crossover trial described in Chapter 5.

		Fluoridated dentifrice alone			Non-fluoridated dentifrice and sorbitol gum			Non-fluoridated dentifrice alone			Combined Δz,I.B,SZ data from each of the three protocols		
		Δz	I.B	SZ	Δz	I.B	SZ	Δz	I.B	SZ	Δz	I.B	SZ
Clearance half time	Unstimulated RIGHT side	0.651	0.551	0.723	0.311	0.843	0.877	0.256	0.923	0.643			
	Unstimulated LEFT side	0.865	0.469	0.654	0.500	0.912	0.797	0.418	0.807	0.445			
	Stimulated RIGHT side	0.139	0.869	0.824	**0.004**	0.477	0.182	**0.006**	0.675	0.446			
	Stimulated LEFT side	0.334	0.358	0.303	0.167	**0.042**	0.116	**0.086**	0.570	0.743			
Salivary film velocity	Unstimulated RIGHT side	0.306	0.977	0.832	0.245	0.737	0.843	0.157	0.488	0.661			
	Unstimulated LEFT side	0.790	0.988	0.754	0.697	0.592	0.974	0.489	0.694	0.206			
Combined RIGHT and LEFT unstimulated values		0.728	0.518	0.696	0.376	0.868	0.846	0.311	0.879	0.566	0.380	0.795	0.588
Combined RIGHT and LEFT stimulated values		0.228	0.505	0.797	**0.063**	0.131	0.887	**0.026**	0.586	0.477	**0.086**	0.308	0.843
Combined RIGHT and LEFT film velocities		0.443	0.998	0.449	0.369	0.677	0.226	0.238	0.542	0.624	0.294	0.739	0.843

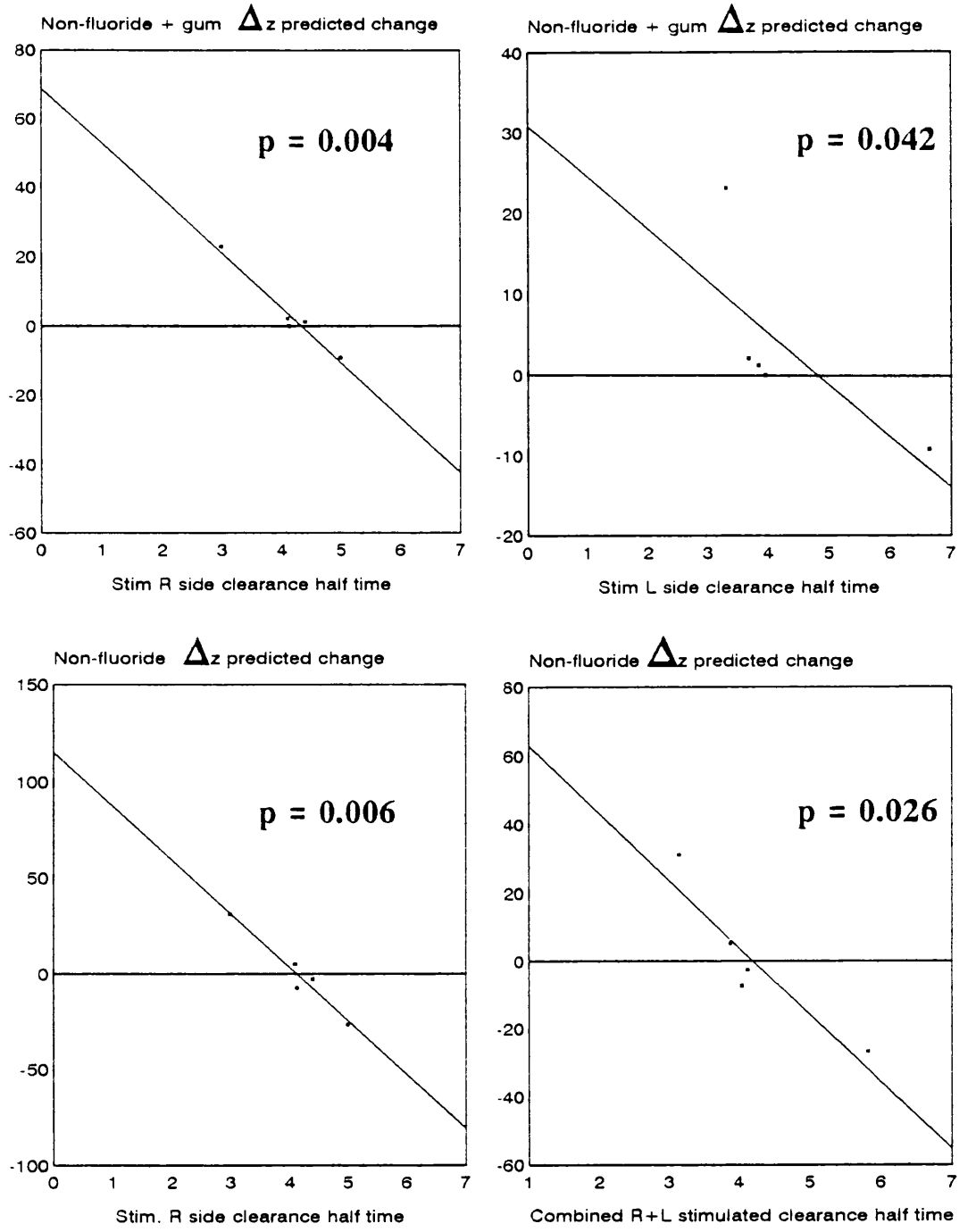


Figure 6.28 Graphs of regression analyses of clearance half times against Δz predicted changes, from the 3 way crossover trial described in Chapter 5, where $p < 0.05$.

Δz predicted change - (%vol.mineralxμm)/100
Clearance half time - minutes

NOTE: A negative Δz predicted change indicates remineralisation over the 5 week appliance wearing period.

In all cases, the slope of the regression line indicated an increased stimulated salivary clearance half time was associated with increased remineralisation implying that a reduced stimulated salivary flow through the trough is associated with increased remineralisation. This is contrary to the hypothesis of Dawes *et al.* (1983).

Analysis of an increased data set, by increasing the arbitrary cut-off p-value to 0.1, included a further 4 cases. This inclusion is potentially controversial but may support the trend in the data described in the previous paragraph. These cases, together with their regression lines are presented in figure 6.29. Once again the relationships between stimulated salivary clearance half time and predicted Δz followed a similar trend. In addition a plot of the relationship between the combined right and left clearance half time and predicted SZ produced the same result.

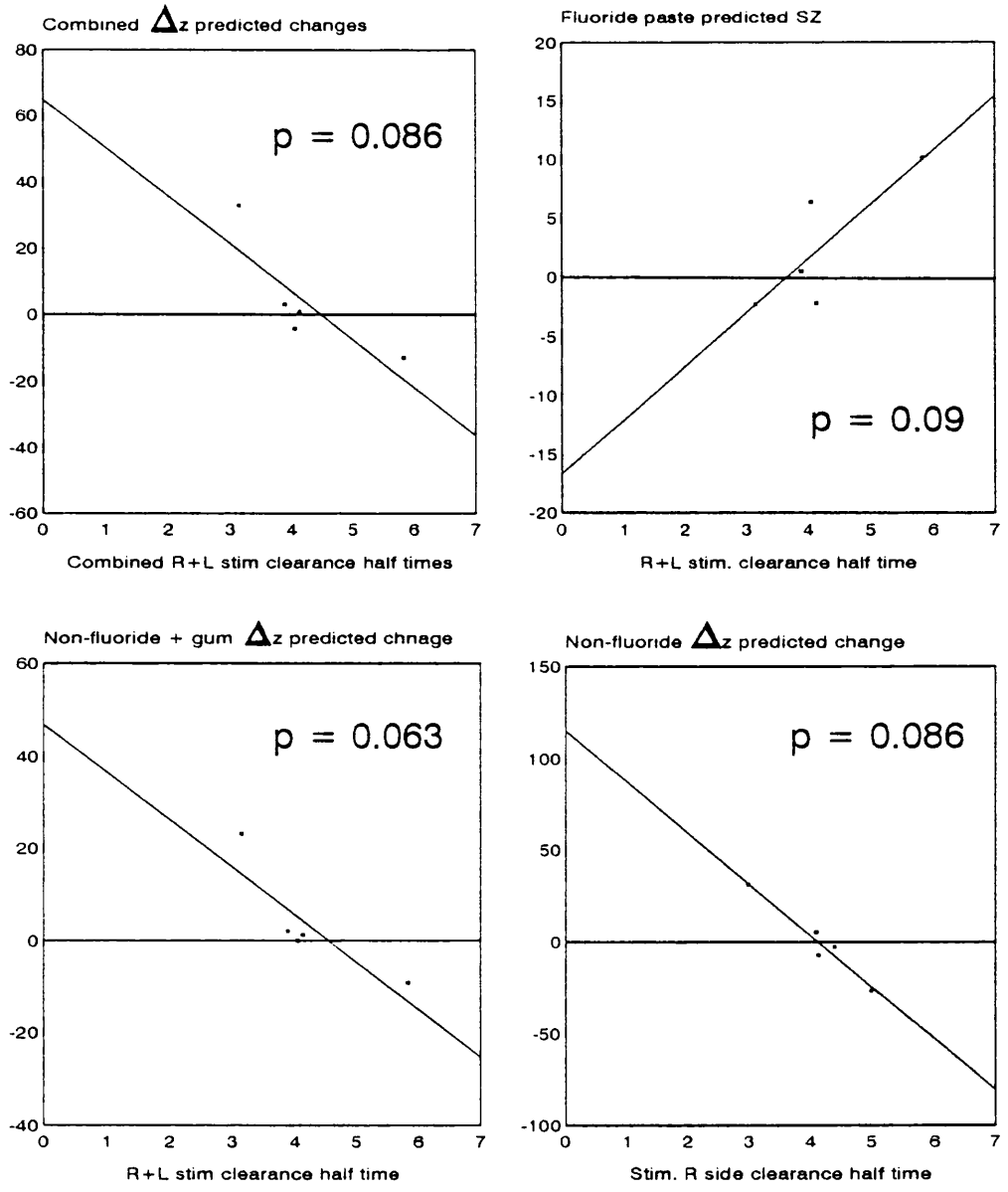


Figure 6.29 Graphs of regression analyses of clearance half times against predicted mineral changes, from the 3 way crossover trial described in Chapter 5, where $p < 0.1$.

Δz - (%vol.mineralxμm)/100
SZ - %vol.mineral
Clearance half time - minutes

Note: A negative change in Δz indicates remineralisation whereas a negative change in SZ indicates demineralisation.

6.3.4 Discussion

The data for mean unstimulated and stimulated salivary clearance half times and unstimulated salivary film velocity may be compared to the data of Dawes & Macpherson (1993). Both groups have used the same protocol to determine salivary clearance half time. Mean data for stimulated and unstimulated salivary clearance half times and unstimulated salivary film velocities from both studies are shown below.

	Present study (n = 5)	Dawes & Macpherson (1993) (n = 33)
Unstimulated clearance half time	7.3 min.	9.7 min.
Stimulated clearance half time	4.2 min.	2.9 min.
Unstimulated salivary film velocity	25.2 ml/min.	6.5 ml/min.

Although there is a large difference in the numbers of participants between the two studies the values for clearance half times are comparable. The exponential relationship of salivary film velocity to clearance half time (Dawes *et al.*, 1989) accounts, in part, for the large difference in values between the two studies. In addition, the siting of the agarose gel may also account for this

difference. The study by Dawes & Macpherson (1989) places the agarose pellet at the level of the lingual surface of the lower molar teeth. In the present study the agarose pellet was placed further towards the base of the lingual sulcus. It is possible that the pellet was placed in a salivary channel created by the lateral border of the tongue and the lingual mucosa. The effect of such a channel may be to rapidly direct saliva to into the pharynx and also to "artificially" increase any measurement of salivary film velocity.

The comparability of clearance half times and salivary film velocities on the right and left sides of the mouth is in agreement with other studies (Dawes *et al.*, 1989; Dawes & Macpherson, 1993) as is the difference between unstimulated and stimulated salivary flow.

To the author's knowledge, comparisons have not been made between salivary clearance half times and predicted lesion changes using the same appliance. The large amount of data available for analysis from previous trials, in conjunction with the relatively small amount of salivary data suggests these results should be interpreted with caution. Each measurement of clearance half time was based on the mean of three repeated experiments on each volunteer undertaken on the same day and were essentially a measurement at one point in time. It is unknown how these estimations would vary with longer time intervals. Each predicted change for Δz, LB and SZ mineral content for each volunteer was based on the combined behaviour of between 8 and 10 lesions carried by the volunteer for between 5 and 7 weeks. Therefore, predicted

lesion changes were also a measurement at one point in time. The difference in this case was that the point had a duration of several weeks rather than several minutes. On the timescale for the development of a carious lesion compared with the timescale for a change in salivary flow rate it is possible that the two "points" were similar. Analysis of gradients of the regression lines for all regression where $p < 0.1$ indicated the same association between saliva and predicted lesion changes. A reduced rate of salivary flow is associated with a predicted lesion change indicating remineralisation. The same observation was recorded with data from different trials. This observation is difficult to explain and the only possibility, if the result is indeed real and not statistical artifact, would seem to be that rapidly flowing saliva may be detrimental to the underlying plaque such that remineralisation is inhibited or retarded.

It is interesting to note that unstimulated salivary measurements seldom demonstrated associations with lesion predicted changes. The sections would be in contact with unstimulated saliva for the majority of the time they were in the mouth. If saliva was to exert a major effect on the artificial lesions then it would be expected that unstimulated saliva would contribute most to this. Once again, the small sample size made it difficult to prove, or disprove this hypothesis.

6.4 PLAQUE AND SALIVA INTERACTIONS IN VITRO

6.4.1 Introduction

The growth of plaque in the trough of the intra-oral appliance provides an excellent opportunity to study the behaviour of the plaque in the local environment of the single section, artificial white spot lesions. Plaque is allowed to grow over and between the sections. The reaction of plaque to external stimuli and its interaction with saliva may provide some explanation of the re- / de- mineralisation potential achieved by the volunteers during the course of the experiment described in Chapter 3.

Much work on the reaction of plaque to external stimuli has been concerned with the study of pH changes and acid anion production. The ability of plaque to produce organic acids in response to an external stimulus, and the ability of saliva to both clear away the stimulus and buffer any change in pH, has been the subject of a considerable amount of research (**Stephan, 1944;**Ericsson, 1959; Gilmour & Poole, 1967; Geddes, 1972; Lagerlof *et al.*, 1984).

Recent work has concentrated on the acid anions produced by the plaque in the posterior lingual trough of the appliance. Both Creanor *et al.*(1986) and Macpherson *et al.* (1991) have shown that plaque produced at this site is identical in behaviour to that of plaque colonising the lingual surfaces of adjacent lower molar teeth. Whilst this is a very precise measurement of

plaque behaviour, it is very time consuming and is not practicable for repeat measurements on the same sample of plaque or for the analysis of plaque samples from several individuals. Other methods of analysis have involved the determination of pH using a sampling technique, and a one drop electrode, and touch electrode techniques (Schachtele & Jensen, 1982) as well as the use of telemetric or "indwelling" electrodes (Jensen & Wefel, 1989). The plaque sampling method results in a gross disturbance of the plaque and is not suitable for repeat measurement of the same sample, whereas previous work in this institution has encountered difficulties with the touch electrode techniques when placed in lingual trough of the appliance due to the difficulty in constructing a suitable circuit (Macpherson, personal communication, 1994). The recent acquisition of a combination microelectrode prompted some pilot work to determine the possibility of recording Stephen curves from *in situ* plaque grown in the appliance trough. Initial studies showed that a measurable and repeatable response could, indeed, be achieved in such a manner and formed the basis for the following experiments.

The aims of this work were:-

1 To measure the plaque pH reduction in the trough of an *in situ* appliance on exposure to a 10% sucrose solution for 20 minutes.

2 To measure the plaque pH reduction in the trough of an *in situ* appliance with exposure to a 10% sucrose solution and the subsequent change with exposure to stimulated saliva.

3 To determine the change in the pH of the saliva as it flows through the experimental system.

4 To determine if a change in the plaque pH induced by stimulated saliva was a result of salivary buffering capacity or simple dilution.

5 To devise a method of production of a sorbitol gum extract

6 To measure the plaque pH reduction in the trough of an *in situ* appliance with exposure to a solution of sorbitol gum extract and the subsequent change with exposure to stimulated saliva.

7 To compare the pH changes in plaque under different protocols.

8 To compare the plaque pH changes in the trough of the *in situ* appliance for each volunteer with the predicted mineral changes for varnished tooth sections with artificial carious lesions in the same trough.

6.4.2 Method And Materials

This experimental work was performed using the same six volunteers used for the salivary clearance experiments reported in section 6.3. Each experiment was repeated three times on each volunteer with an interval of at least 24 hrs to permit the plaque to recover from the trauma of removal from the mouth. Subjects were asked to refrain from eating or drinking for at least one hour prior to removing their appliance for all experimental work. It was felt that this time period would be sufficient to permit plaque pH changes to revert to near baseline levels. Furthermore, plaque pH measurements were undertaken

at the same time each day for each particular volunteer in order to measure a response in plaque of a similar metabolic state on each occasion.

The electrode used was a combination pH micro-electrode (MI-410 pH combination electrode. Microelectrodes Inc. Londonderry, New Hampshire, USA). This electrode has a 1 mm diameter tip with a reference junction 1.5 mm from the tip (figure 6.30). By placing the electrode at an angle within an area of relatively thin plaque it was often possible to record a baseline reading for resting plaque pH. The placement of a drop of liquid at the electrode tip facilitated pH measurement as the surface tension of the liquid was such that the reference junction was easily reached.

Either the right or left trough was selected depending on the distribution of plaque over the appliance. Pilot studies showed that the most stable and most reproducible pH measurement was achieved by placing the electrode in a relatively thin area of plaque, well away from any retained food debris and preferably not in a corner of the trough or immediately adjacent to a section. This prevented the pH electrode being buried deep in plaque, in which case the response was poor. In addition a thin layer of plaque would ensure maximal bacterial response to sucrose solution or saliva, whereas it is possible that with a thick layer of plaque only the surface bacteria would respond.

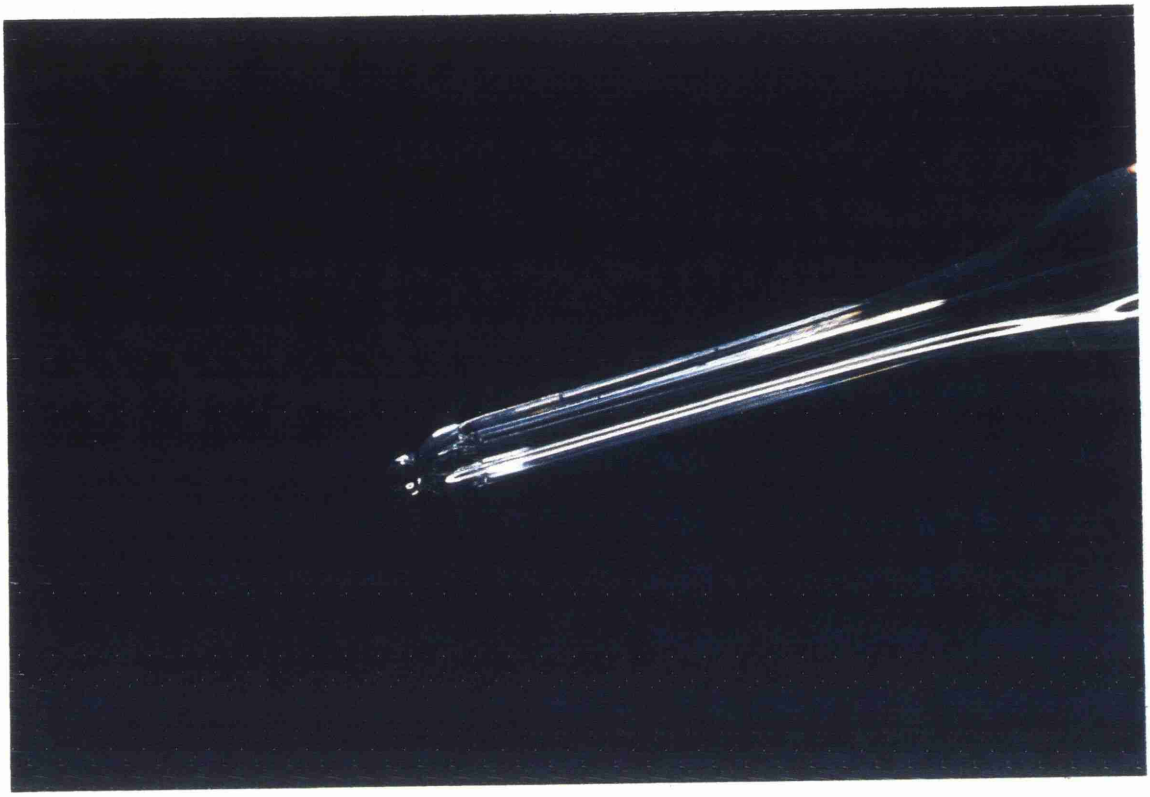


Figure 6.30 Photograph of the MI-410 pH combination electrode with 1 mm diameter tip and reference junction 1.5 mm from the tip.

Reduction of plaque pH with the addition of 10 sucrose solution

Initial experiments were designed to verify the nature and reproducibility of the plaque response to a 10% sucrose solution. The appliance was removed from the mouth and placed quickly in a retort stand so that the base of the chosen trough was at a slight incline. The trough on the other side of the appliance was wrapped in damp gauze to prevent the plaque from drying out. The electrode was placed immediately in a suitable area of plaque and a reading taken if possible. This was a fairly inaccurate reading. Only a general impression could be obtained of baseline plaque pH and there was often insufficient fluid to form a contact between the electrode tip and the reference junction. The next stage involved the addition of 100 µl of 10% (by weight) sucrose solution to the plaque in the region of the electrode tip. This permitted good conduction between the electrode tip and the reference junction. The pH was recorded every minute for the first 10 min and at 5 min intervals thereafter for 20 min. The trough and the electrode were both covered carefully with clingfilm to reduce evaporation.

Without the interaction of saliva with plaque, this is a grossly artificial system. The ingestion of sucrose elicits a salivary response (Lagerlof & Dawes, 1985) to buffer the fall in pH. The requirement for a salivary input to this system formed the basis of the next series of experiments. These experiments attempted to assess the effect of saliva on plaque pH and the nature of this effect.

Measurement of plaque pH reduction by exposure to 10% sucrose solution and subsequent effect of added stimulated saliva

A 10 % sucrose solution was used to induce the fall in plaque pH as described previously. This was monitored, in the same manner as before, using the combination microelectrode. Whilst the plaque pH was falling, the subject was asked to produce a sample of stimulated saliva by chewing a piece of sorbitol-containing chewing gum. Initial chewing for 2 min. cleared the salivary ducts of resting saliva before subsequent collection of stimulated saliva under 2 ml of paraffin oil. Saliva was collected under paraffin oil to prevent loss of CO_2 to the atmosphere which affected the buffering capacity (figure 6.31).

A sterile polythene tube (internal diameter of 1.15 mm) was passed from the saliva below the paraffin oil, through a peristaltic pump (LKB Perpex peristaltic pump 10200, Stockholm, Sweden), so the saliva would flow continually over the plaque/electrode interface (figure 6.32). The flow of saliva started 10 min after the addition of the sucrose solution and was assisted by the slight incline of the appliance trough. The 10 min starting time for salivary flow was the result of pilot studies which suggested that after 10 min the pH had fallen to the same value on all three occasions and any subsequent fall in pH occurred at a much reduced rate. The rate of flow of saliva through the tube was found to be 0.2 ml per min. The pH was recorded at one minute intervals for a further 10 min. The appliance was immediately replaced in the mouth of the volunteer whilst tests were undertaken on the collected saliva.

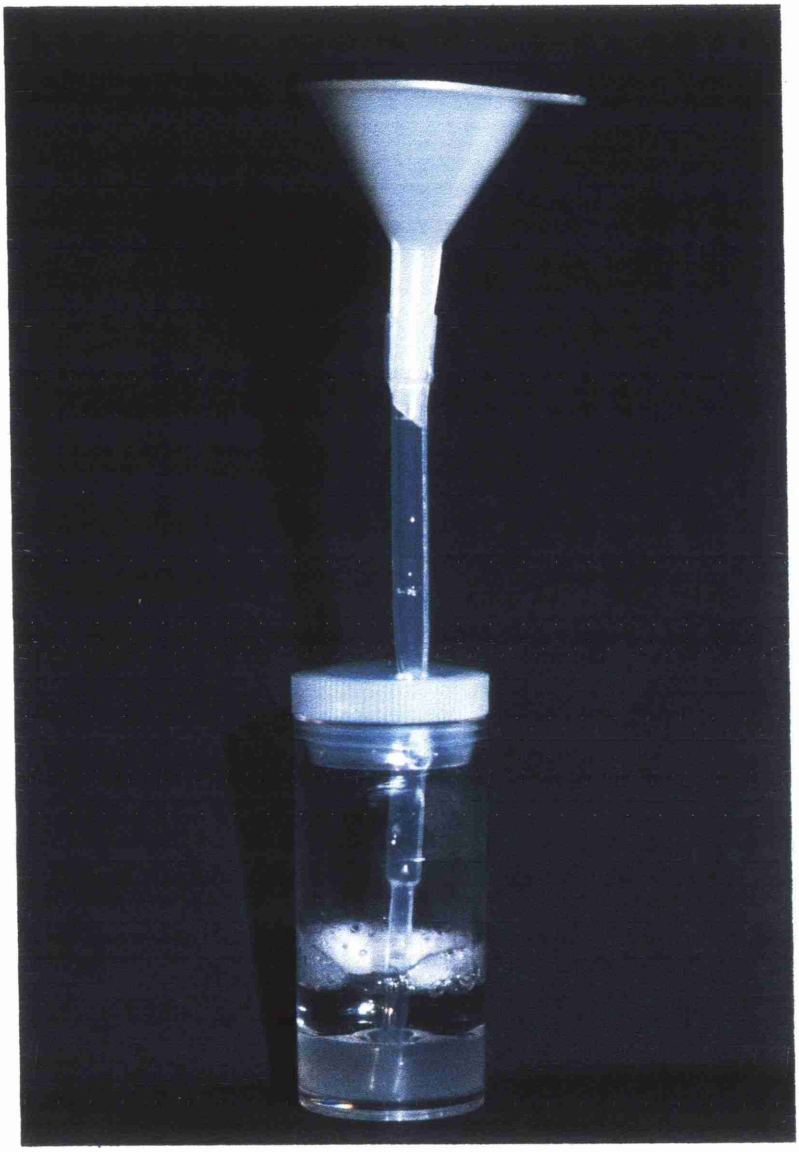


Figure 6.31 Collection of saliva under paraffin oil

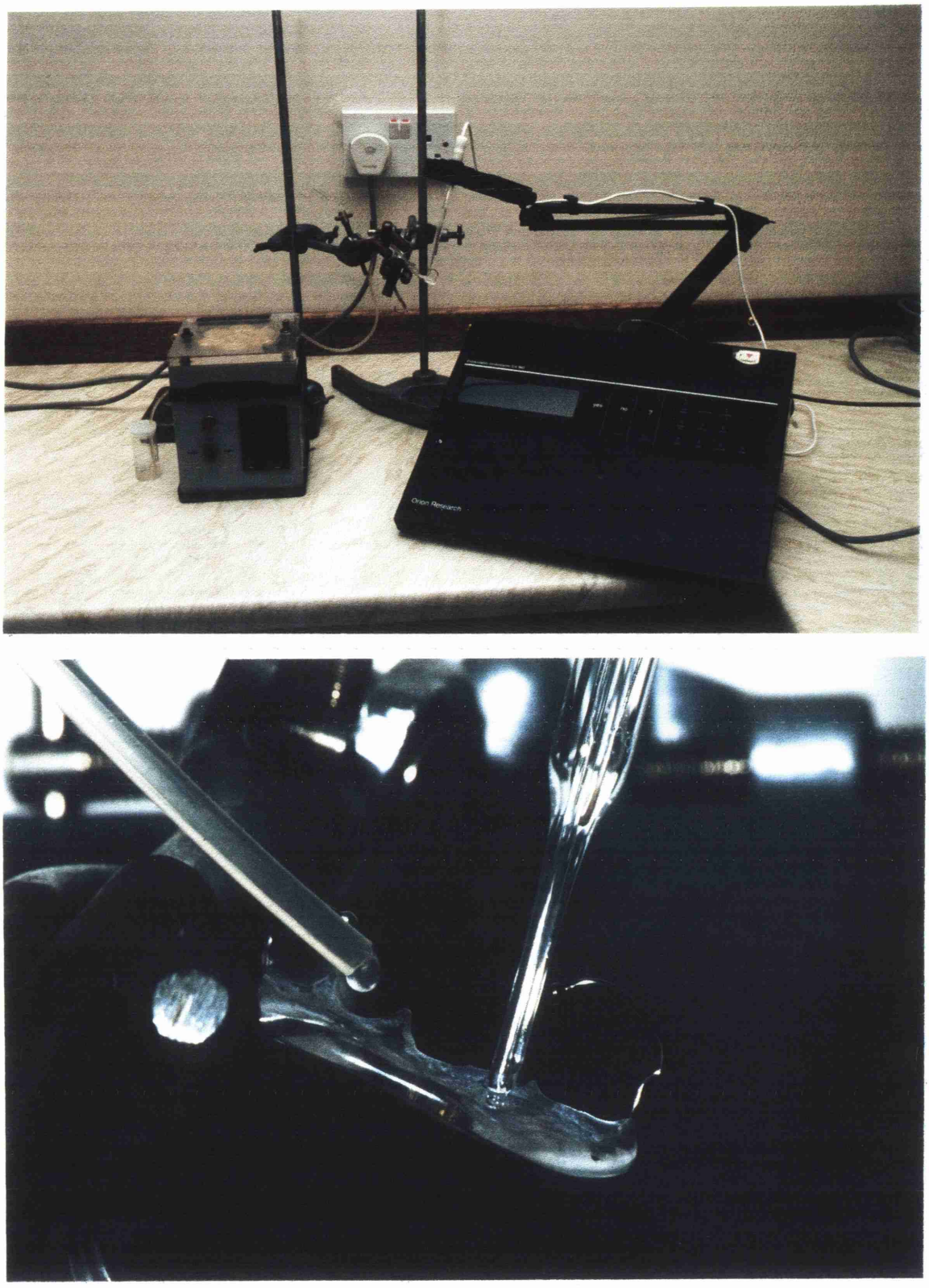


Figure 6.32 Photographs of apparatus to determine the change in plaque pH on exposure to a steady flow of stimulated saliva.

Determination of the change in pH of the saliva as it flows through the experimental system

The passage of saliva from the collection vessel, through the pump to the appliance trough may have taken sufficient time for the saliva to lose a considerable amount of CO_2. The loss of CO_2 to the atmosphere is known as phase buffering (Nikiforuk, 1985) and would have affected the buffering capacity and any subsequent pH change recorded by the pH electrode.

The pH of the saliva in the collection vessel was recorded as time = 0 min. Saliva was then pumped through the polythene tubing to flow over the base of the trough of an unused appliance. The combination microelectrode was placed in the path of the saliva and the pH recorded at one minute intervals for 20 minutes. Finally the pH of the saliva in the collection vessel was recorded, once again, at the end of the experiment.

Methodology to determine if the change in pH induced by saliva was as a result of salivary buffering capacity or simple dilution.

To determine the effect of dilution on plaque pH, a further series of experiments was performed. In keeping with the previous experimental protocol, this work was also repeated on three consecutive visits for each volunteer. The appliance was removed from the mouth and the plaque pH, once again, reduced by the addition of a 10% sucrose solution. Attempts were

made wherever possible to place the electrode in a similar position as that used to record the previous plaque pH response with sucrose. At the 10 min mark, deionised water, instead of stimulated saliva, flowed over the plaque/electrode interface at 0.2 ml per min. The resulting pH was recorded at one minute intervals for a further 10 min.

Methodology for production of sorbitol gum extract

Chewing peppermint flavoured, sorbitol-containing gum formed a considerable part of the three-way crossover trial detailed in Chapter 5. This gum was employed as it provided an excellent salivary stimulus (Dawes & Macpherson, 1992). Furthermore, the reduction in plaque pH with sorbitol is much less than that seen with sucrose (Birkhed *et al.*, 1978). The effect of this gum on the plaque within the appliance trough is unknown and it was decided that this should form the basis for the final investigation of plaque pH response.

A method had to be devised to produce a gum extract solution of appropriate concentration to expose to the plaque in the appliance trough. The act of gum chewing releases several chemical constituents which, in combination, are responsible for the gum flavour. The concentration of these constituents within saliva is variable depending on the individual chewing the gum. Dawes & Macpherson (1992) measured a mean total volume of 38.7 ml of stimulated saliva produced by each of 22 subjects in response to peppermint flavoured

sorbitol-containing chewing gum. To prepare the gum extract, five sticks of pre-weighed gum were placed, individually, into pre-weighed polythene bags. forty millilitres of deionised water was added to each bag. The bags were then placed into a stomacher laboratory blender (Model 6020, Colworth Laboratory Equipment, Bury St. Edmunds) for 1 min (figure 6.33). This apparatus has two paddles which pummel the bag, containing the chewing gum and deionised water, against a solid surface. A processing time of 1 min. was recommended by the manufacturer.

The extract was poured out of the bags and into a communal container. The bags were then placed in a drying oven for 48 hrs prior to re-weighing. Gloves were worn at all times to prevent contamination of the bags and distortion of the recorded mass.

It was calculated that the average mass of the gum had decreased by 50% during this process and it was assumed that this was now contained in the gum extract. The probable composition of the gum extract was kindly verified by the Wm. Wrigley Jr., Company offices in Portsmouth, England. They suggested that the extract would be mainly sorbitol as this formed 50%, by mass, of the gum. In addition the extract would probably contain small amounts of mannitol, glycerine, peppermint oil, aspartame and lecithin.

Figure 6.33 Photograph of stomacher laboratory blender used to create gum extract.

Methodology to determine the effect of a sorbitol gum extract on plaque pH and the subsequent of the addition of stimulated saliva

The gum extract was added to the plaque in the same manner as the 10 % sucrose solution and the pH recorded as before. In addition, gum stimulated saliva flowed over the plaque after 10 minutes exposure to the gum extract. The pH was recorded for a further 10 minutes. This procedure was repeated three times on three separate days for each of the six **volunteers**,

6.4.3 Results

Measurement of plaque pH reduction on exposure to 10% sucrose solution

This experiment was undertaken for 6 volunteers and the entire data set for volunteer 18 is shown in figure 6.34. The reproducibility of this experimental protocol justified the use of an arithmetical mean value of the three experimental runs undertaken for each volunteer. This data is shown in figure 6.35.

Measurement of other plaque and salivary pH changes

The effects of the addition of stimulated saliva and deionised water on *in situ* appliance trough plaque pH reduced by exposure to 10% sucrose solution are shown in figures 6.36 to 6.38. These figures also show data for the effect of sorbitol gum extract on plaque pH. All data shown is for the arithmetical mean values of three experimental runs.

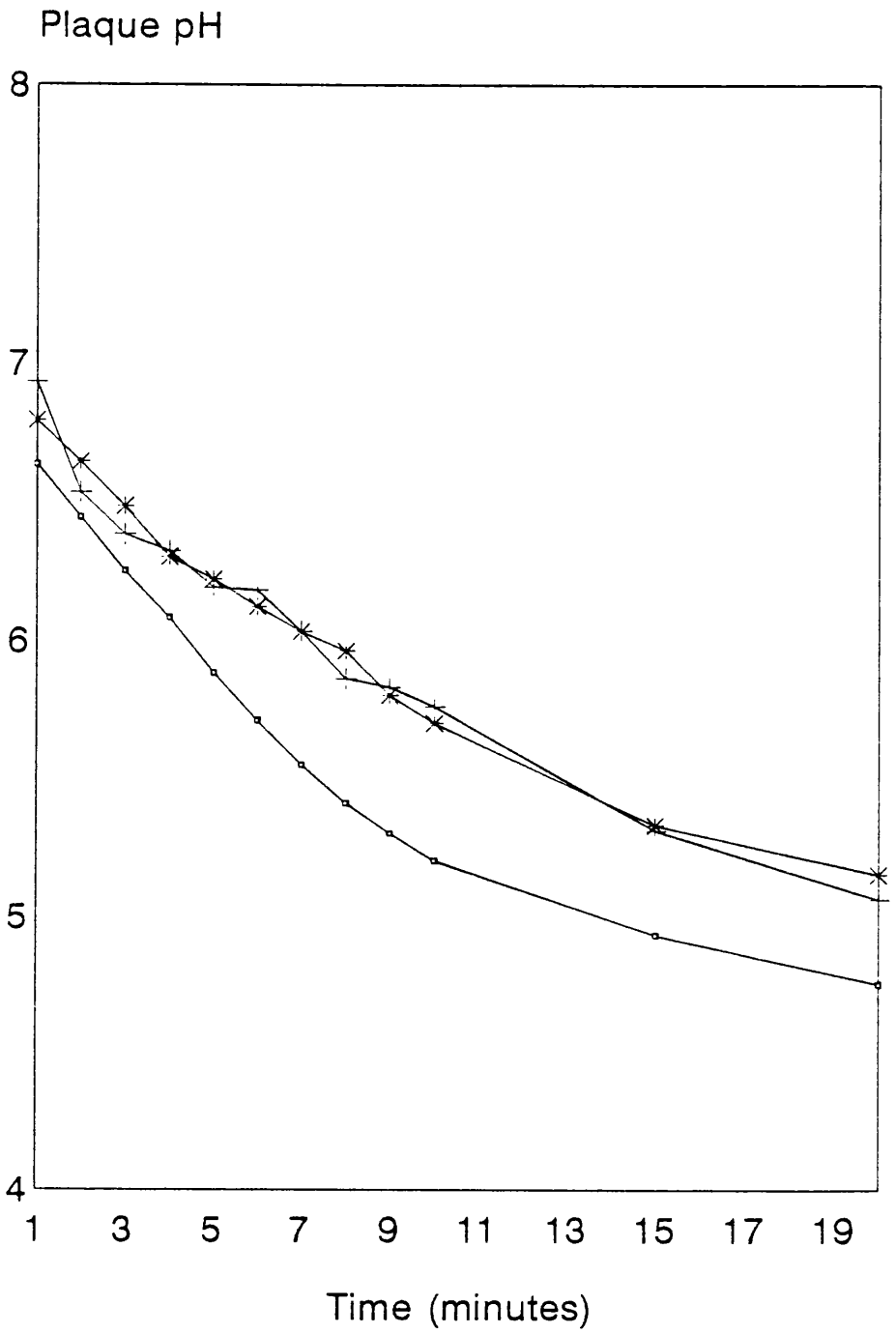


Figure 6.34 Graph to demonstrate 3 repeated measurements of a change in plaque pH within the appliance trough over a period of 20 minutes with exposure to 100 µl of 10% sucrose solution.

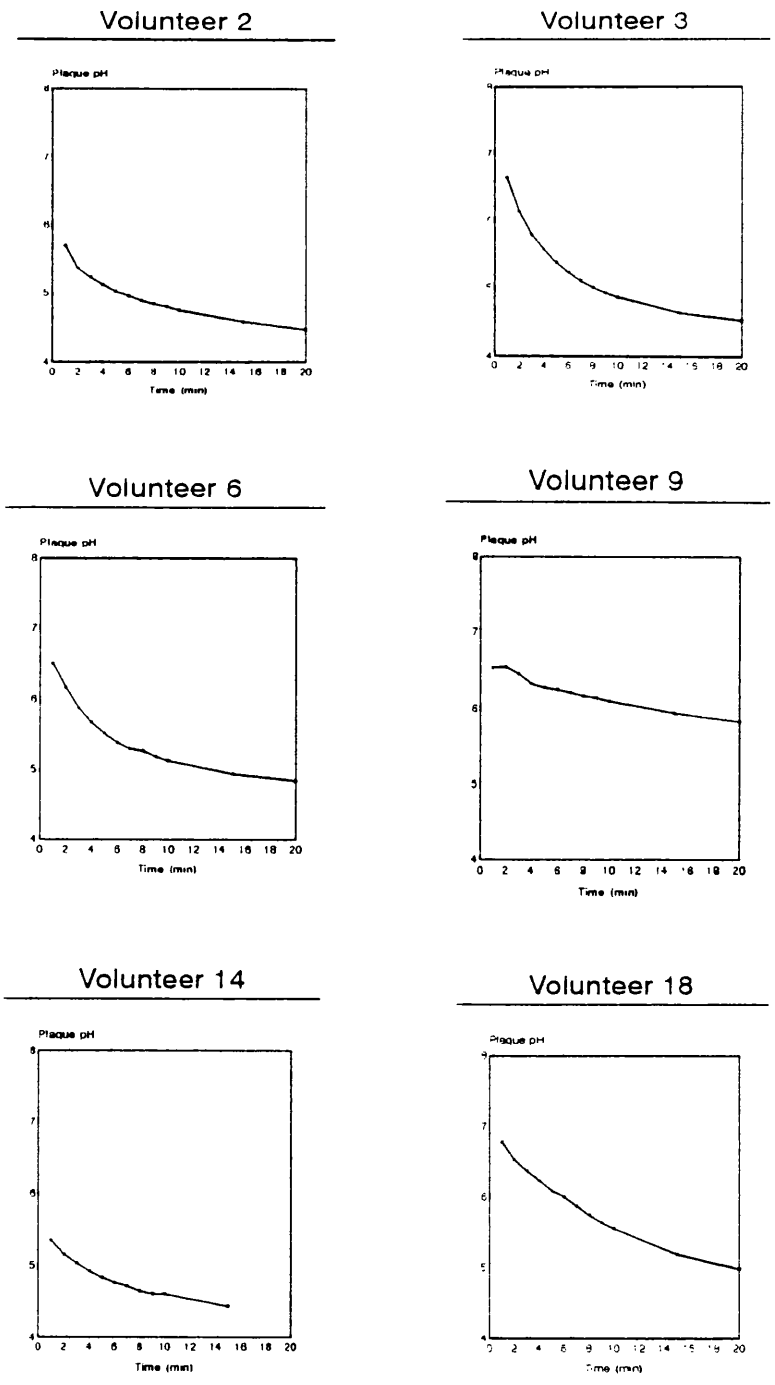
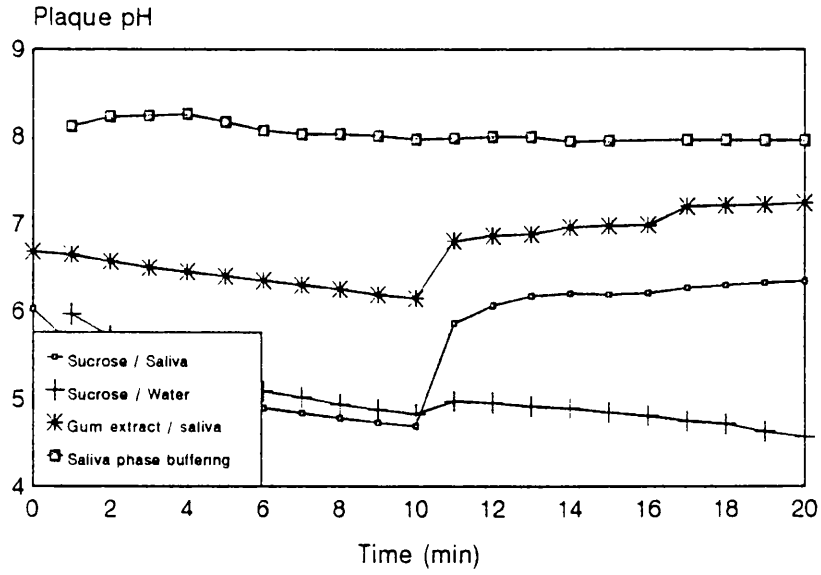


Figure 6.35 Graphs to demonstrate mean changes in plaque pH within the appliance trough over a period of 20 minutes with exposure to 100 µl of 10% sucrose solution. Each point on each graph is an arithmetical mean of 3 repeat experiments.

Volunteer 2



Volunteer 3

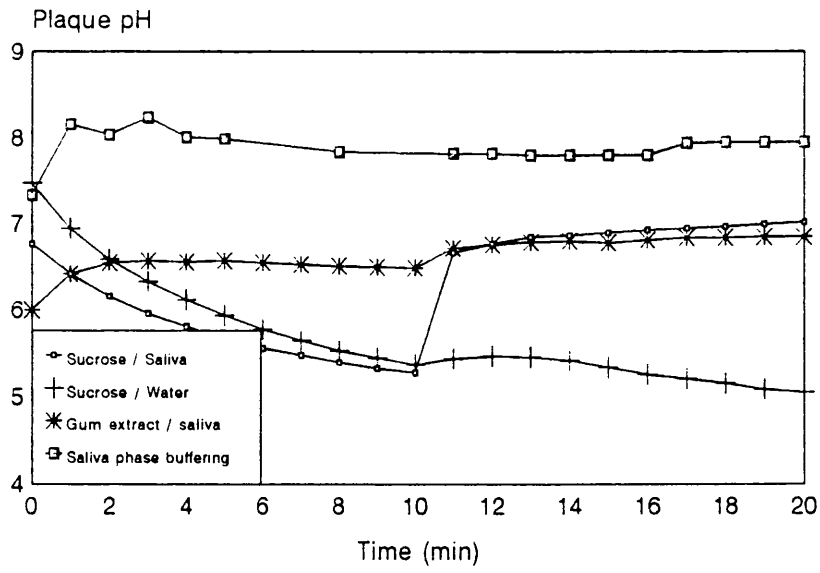
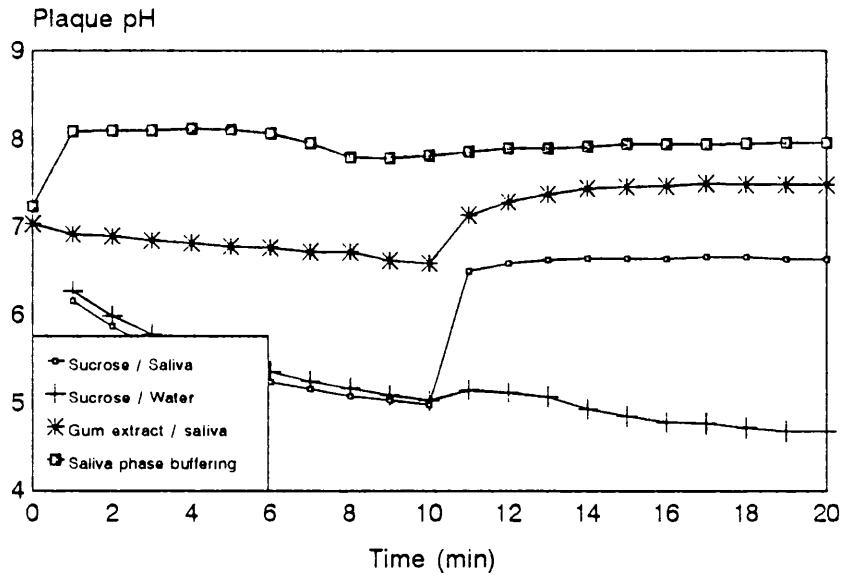


Figure 6.36 Graphs for volunteers 2 and 3 to show the change in plaque pH within the appliance trough under various protocols. Each point is an arithmetical mean of three repeat experiments.

Volunteer 6



Volunteer 9

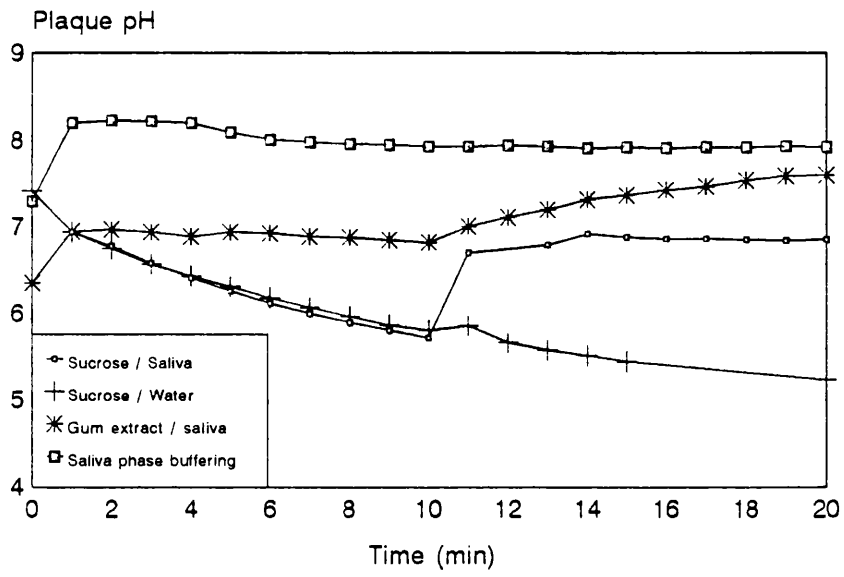


Figure 6.37 Graphs for volunteers 6 and 9 to show the change in plaque pH within the appliance trough under various protocols. Each point is an arithmetical mean of three repeat experiments.

Comparison of plaque and salivary pH changes

Initial observations of individual and combined data supported the conclusions that:-

1 The first 10 min. sucrose curves were very similar for the "sucrose only", "sucrose and saliva" and "sucrose and water" protocols.

2 Exposure to stimulated saliva rapidly increased the pH.

3 Exposure to the same flow rate of deionised water had little effect on the fall in plaque pH.

4 Exposure of plaque to gum extract induces a fall in plaque pH but not to the same extent as sucrose solution.

Further analysis of this data was undertaken with care. The initial difficulty in recording reproducible changes in plaque pH, over the first five minutes, with the addition of 10% sucrose solution, or gum extract, meant that this data was not suitable for further analysis. In addition the rate of fall in pH over the first 10 min period could also not be determined accurately. The pH at 10 min was chosen as an "artificial" plaque pH minimum. The pH value at 12 min was recorded to determine the "rapid" pH change during the first 2 min after exposure to saliva or deionised water and the pH value at 20 min was recorded to determine the "overall" pH change in a 10 min. period after exposure to stimulated saliva or deionised water. This data is presented in Table 6.7 and statistical analysis in the form of paired t-tests in Table 6.8.

Table 6.7

Mean pH data for 6 volunteers for 5 different plaque pH experiments

Subject	Sucrose only		Sucrose then saliva					Sucrose then water					Gum extract then saliva					Salivary Phase Buffering
	10 min	20 min	10 min	12 min	12-10 min	20 min	20-10 min	10 min	12 min	12-10 min	20 min	20-10 min	10 min	12 min	12-10 min	20 min	20-10 min	
2	4.75	4.48	4.69	6.07	1.38	6.31	1.62	4.88	4.98	0.1	4.72	-0.16	6.15	6.87	0.72	7.21	1.06	8.05
3	4.87	4.53	5.28	6.77	1.49	7.02	1.74	5.37	5.47	0.1	5.06	-0.31	6.50	6.72	0.22	6.85	0.35	7.87
6	5.13	4.89	4.98	6.59	1.61	6.67	1.69	5.03	5.12	0.09	4.68	-0.35	6.59	7.29	0.7	7.50	0.91	7.93
9	6.26	5.87	5.72	6.59	0.87	6.85	1.13	5.81	5.67	-0.14	5.24	-0.57	6.82	7.01	0.19	7.60	0.78	7.96
14	4.60	-	4.83	5.49	0.66	6.19	1.36	5.07	4.99	-0.08	4.57	-0.50	6.14	7.03	0.89	7.29	1.15	7.83
18	5.55	4.99	6.00	7.40	1.40	7.34	1.34	6.17	6.21	0.04	5.95	-0.23	6.78	7.39	0.61	7.48	0.7	7.95

Table 6.8

P values for paired t-test analysis of the plaque pH at 10 min under four different protocols

	Saliva 10 min.	Deionised water 10 min.	Gum extract 10 min.
Sucrose 10 min.	0.73	0.30	0.001
Saliva 10 min.		0.056	0.0001
Deionised water 10 min.			0.003

Table 6.8 compares the pH at 10 min for the sucrose alone, sucrose and saliva, sucrose and water and sorbitol gum extract and saliva experiments.

There were no significant differences between the 10 min plaque pH values for the sucrose alone protocol and either the sucrose and saliva or sucrose and deionised water protocols ($p = 0.73$ and 0.3 respectively). There was a difference of borderline significance ($p = 0.056$) between the 10 min plaque pH values for the sucrose and saliva and the sucrose and deionised water protocols. The 10 min plaque pH measurements for the sorbitol gum extract and saliva protocol were highly statistically significantly different from the each of the three sucrose protocols ($p = 0.001$ for sucrose alone, $p = 0.0001$ for sucrose and saliva and $p = 0.003$ for sucrose and deionised water).

Comparison of plaque pH changes with predicted mineral changes

Comparison of plaque pH changes with predicted mineral changes was undertaken initially using linear regression analysis. The results of this analysis are presented in Table 6.9. In view of the small amount of data for each regression, linear regression analysis was considered to be a reasonable initial approach. The data for predicted lesion changes has previously been presented in Table 6.2.

The plaque pH measurements at 10 min were included as an indication of the acidogenicity of the plaque which may have been an important factor in the explanation of lesion dynamics. In addition, the change in plaque pH between

Table 6.9

P-values for regression analysis of plaque pH measurements and predicted lesion change from *in situ* trials reported in Chapters 3 and 5.

	Sugar Gum Trial (Chapter 3)						Three Way Crossover Trial (Chapter 5)								
	F paste only			F paste and sucrose gum			F paste only			Non-F paste and sorbitol gum			Non-F paste only		
	Δz	I.B	SZ	Δz	I.B	SZ	Δz	I.B	SZ	Δz	I.B	SZ	Δz	I.B	SZ
Sucrose 10 min	0.507	0.529	0.537	0.254	0.610	0.425	0.462	0.297	0.213	0.344	0.807	0.958	0.291	0.426	0.896
Saliva 10 min	0.377	0.217	0.185	**0.022**	0.236	0.069	0.417	0.843	0.858	0.217	0.495	0.417	0.069	0.940	0.473
Deionised water 10 min.	0.430	0.168	0.181	**0.026**	0.229	**0.030**	0.522	0.779	0.964	0.268	0.388	0.359	0.090	0.932	0.547
Gum extract 10 min.	0.321	0.442	0.271	0.084	0.345	0.389	0.252	0.807	0.436	0.172	0.954	0.639	0.102	0.794	0.386
Saliva 12-10 min.	0.077	0.667	0.235	0.413	0.201	0.649	0.058	0.436	0.466	0.112	0.672	0.696	0.228	0.278	**0.009**
Gum 12-10 min.	0.519	0.720	0.976	0.322	0.958	0.951	0.229	0.625	0.552	0.359	0.468	0.373	0.302	0.141	0.655
Saliva 20-10 min.	0.681	0.536	0.963	0.727	0.949	0.177	0.530	0.395	0.388	0.826	0.914	0.955	0.958	0.103	0.306
Gum 20-10 min.	0.362	0.989	0.563	0.144	0.549	0.762	0.160	0.498	0.774	0.241	0.973	0.792	0.138	0.407	0.193

10 and 12 min, and 10 and 20 min, for the saliva and gum protocols are also included. In both cases, the ability of saliva to counteract a change in plaque pH constitutes an *in vitro* measurement of salivary buffering capacity. Results of linear regression analysis of plaque pH measurements and lesion predicted changes are, for the most part, non-significant. However, some statistically significant values were obtained.

Graphs of these relationships are presented in figure 6.39. In each case it would appear that a high pH after 10 min exposure to a 10% sucrose solution or a large plaque pH increase induced by exposure to stimulated saliva is associated with increased mineral deposition within a lesion.

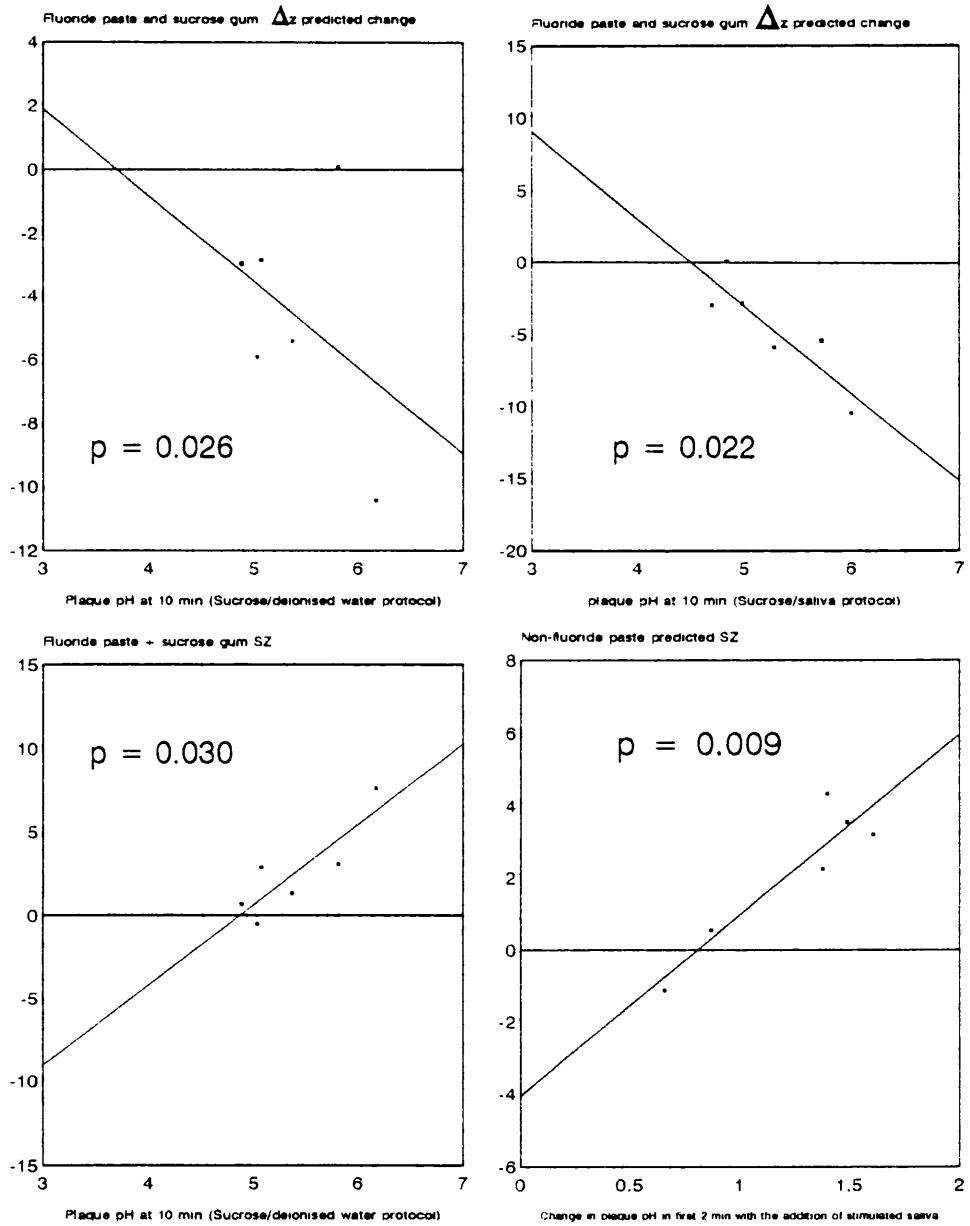


Figure 6.39 Graphs to show the results of linear regression analysis of plaque pH under different protocols and predicted mineral changes from intra-oral trials.

Δz - (% vol.mineralxμm)/100
SZ - %vol.mineral

Note: A negative change in predicted Δz indicates remineralisation
A negative change in predicted SZ indicates demineralisation

6.4.4 Discussion

The plaque grown in the appliance trough is clearly capable of reacting to sucrose stimuli in the same manner as natural plaque growing on tooth surfaces. The response by plaque within the trough of the appliance is also reproducible when exposed to a standardized stimulus and measured under standardised conditions. Evidence of this is seen in the reproducibility of the curves shown in figures 6.33 and 6.35 to 6.37. The use of a plaque stimulus containing sorbitol rather than sucrose resulted in less acid production which is in agreement with the results of Birkhed *et al.* (1978). In addition, this model has demonstrated the ability of saliva to counteract a fall in plaque pH and these results are in agreement with those of Englander *et al.* (1959).

The question of a chemical or physical interaction between saliva and plaque is answered, to some extent, by the substitution of stimulated saliva by deionised water. The flow of stimulated saliva over the sucrose-stimulated plaque resulted in a dramatic increase in pH. The flow of deionised water at the same rate over sucrose stimulated plaque resulted in a continued fall in plaque pH. The possibility that saliva counteracts the fall in pH by washing away H^+ ions would seem to be unlikely. A more probable method of pH regulation is the interaction between H^+ ions in dental plaque and bicarbonate ions in saliva.

The ability of saliva to counteract a change in plaque pH is a definition of

salivary buffering capacity. The increase in plaque pH within the appliance trough upon exposure to stimulated saliva may also be interpreted as a measurement of salivary buffering capacity. From the results obtained by regression analysis in Table 6.15 it would appear that this measurement may bear some relation to mineral deposition within the lesion. In addition, the depth to which pH falls after 10 min exposure to sucrose may also be related to mineral deposition within lesions.

The method of pH assessment does seem to be reproducible and worthy of further investigation. For example, the flow rate of saliva is associated with a variable bicarbonate concentration (Dawes, 1974); is this variation detectable with this model system? In addition, does the rate of flow of saliva, rather than the bicarbonate content, affect its ability to alter plaque pH?

6.5 SUMMARY

Supplementary investigations have been performed on a selection of volunteers who have completed two *in situ* trials. These investigations have yielded the following results:-

Salivary fluoride levels were found to be much lower than those in dental plaque. This result may be more easily observed by comparison of measurements averaged over a longer time period rather than single sample estimations. Measurements of salivary fluoride levels were able

to more easily distinguish between two different fluoride protocols compared to plaque fluoride levels. This may be due to the greater inherent variability of plaque as a medium for the measurement of fluoride.

A modified *in situ* appliance was able to determine the rate of clearance of potassium chloride by saliva from the appliance trough. In addition the model was capable of distinguishing between unstimulated and stimulated salivary conditions. Comparison of results of this experimental work with a study determining salivary clearance in the lingual sulcus would indicate very little reduction in salivary flow afforded by the appliance trough when compared to data from the lingual sulcus collected by Dawes & Macpherson (1993).

The use of plaque within the appliance trough has provided a convenient, easily accessible source of plaque capable of responding in a reproducible manner to various stimuli. Moreover, this model was able to demonstrate the effects of plaque exposure to saliva and deionised water.

An additional purpose for undertaking these investigations was to determine their ability to account for volunteer behaviour during an *in situ* trial. Data for predicted lesion changes have been described in Chapters 3 and 5. Several difficulties were encountered in comparing these data sets. These included:-

Data for the supplementary experiments described in this Chapter were undertaken using small numbers of volunteers. In some cases volunteers were chosen according to their ability to demineralise or remineralise as evidenced by results from previous trials although it is very difficult to determine whether or not this has been of benefit.

The nature of these supplementary investigations has been to repeat experiments and to take arithmetical mean values in an effort to produce data with very little individual variation.

The nature of the *in situ* trials is that the results for individuals were, to some extent, a single estimate. The repeatability of such estimates is difficult to demonstrate; hence, a greater degree of variability in predicted lesion changes for any one individual.

Therefore, the variable nature of the data and the small sample sizes has made identification of significant relationships very difficult. Indeed, the word "association" is probably more applicable than relationship. In addition, any associations that have been observed have sometimes been difficult to justify and may be by chance. The following associations were observed and may form the basis for a series of recommendations for further work:-

1 It would appear that a low clearance rate by saliva from the appliance trough is associated with increased remineralisation for

predicted Δz and SZ values.

2 It would appear that the ability of saliva to counteract a change in plaque pH using an *in vitro* model may be associated with predicted lesion changes although the nature of this association is not clear.

This work has potentially increased the application of this *in situ* model but additional work is required to determine how repeatability of results can be obtained or, indeed, why volunteers can react in such a variable manner under similar protocols.

CHAPTER 7 CONCLUSIONS AND DISCUSSION

The aim of this work was to determine the relative effects of both fluoride and stimulated salivary flow on mineral changes within artificial enamel lesions using an intra-oral model. In addition, attempts were made to determine some of the ways in which saliva may influence such changes.

Initial work involved the determination of the effects of both stimulated salivary flow, and fluoride, on lesion remineralisation using the Glasgow model. Preliminary trials established the ability of salivary stimulation, within a fluoridated environment, to achieve increased remineralisation compared to remineralisation of lesions in a fluoridated environment with no salivary stimulation. In addition, pilot studies and subsequent published studies by other authors have established the ability of the Glasgow model to detect differences between fluoridated and non-fluoridated environments.

In light of this work, a large *in situ* trial, using the Glasgow model, was designed to determine the relative effects fluoride and stimulated salivary flow on artificial lesion remineralisation. The results of this study were not as expected, despite rigid control of methodological procedures in an effort to reduce the impact of experimental variables, such as the size and origin of the artificial lesions and the relative effects of individual volunteers, on the outcome of the trial.

The unexpected result suggested an in-depth analysis of experimental data was required to determine the reasons for such an outcome. Analysis of data concentrated on the relative measurements themselves, the range of lesion sizes in addition to actual, rather than predicted, lesion behaviour.

In all *in situ* trials, there have been three measurements analyzed for each lesion. These were Δz and surface zone and lesion body mineral contents. In all intra-oral trial work presented in this thesis, lesion body measurements have consistently achieved statistical significance, or have been the measurement closest to achieving statistical significance in the case of the three-way crossover trial reported in Chapter 5. The surface zone mineral content measurement has consistently failed to achieve statistical significance, and in some cases, has demonstrated trends opposite to other lesion measurements. Δz has been of equivocal success in demonstrating significant differences between the protocols using an intra-oral model.

The relative success, or lack of success, of these lesion measurements in demonstrating differences between protocols may be inherent in the nature of each measurement. The position of the surface zone means there is intimate contact between the mineral component of this zone and the overlying plaque and saliva. Mineral transfer between plaque and saliva covering the tooth and the different parts of the carious lesion is likely to occur most rapidly in the surface zone. This part of the lesion is therefore most likely to undergo the most rapid mineral changes in response to the overlying plaque and salivary

conditions. The nature of this response may vary on an hourly or even minute basis. Certainly the mineral content of the surface zone is likely to be much more variable than the mineral content of the lesion body. Measurement of surface zone mineral content may constitute a "snapshot" at one moment in time of a highly variable part of a carious lesion.

Mineral determination of lesion body is less likely to demonstrate such rapid fluctuations. Potentially buffered against rapid changes in plaque and salivary biochemistry by the surface zone, the lesion body may only respond to longer-term changes in the local environment.

The nature of the Δz measurement is dependent on the mineral content of both the surface zone and the lesion body as well as the depth of the lesion. A large and unpredictable variation in the surface zone mineral content may account for difficulties in demonstrating differences between experimental protocols using this measurement.

Perhaps further consideration should be given to the type of measurement recorded for future trials. However, this would, in part, be dependent on how lesion remineralisation is perceived.

High dose fluoride treatment results in rapid remineralisation of the surface zone of a lesion without much effect on the underlying areas of mineral loss. The newly remineralised surface layer is now more resistant than the

surrounding, previously unchanged enamel, to further demineralisation. In effect, the lesion is arrested and has increased resistance to further demineralisation. However, it is still histologically a carious lesion. In this case the surface zone measurement would probably be the best parameter to measure. If lesion remineralisation is perceived as a complete replacement of mineral loss from the base of the lesion to the surface then Δz or lesion body may be better measurements to record.

The lesions used for the three way crossover trial described in Chapter 5 had a Δz of between 30 - 60 (% vol.mineral x μm)/100. Furthermore, examination of individual lesion behaviour demonstrated that lesions with a larger Δz exhibited the greatest degree of remineralisation. The relationship between baseline lesion size and mineral change appeared to be linear, which has also been shown by other authors. Extrapolation along a regression line showed that larger lesions could potentially have demonstrated significant difference between the protocols of the experiment described in Chapter 5. However, that does not resolve the question of a failure to observe statistically significant differences between fluoride and non-fluoride protocols in this study when other studies have demonstrated this difference.

Presently measurements have been made and changes compared for single lesion parameters. For example, lesion body measurements are made with little or no concept of how surface zone measurements may affect the ability of the environment to change the lesion body. Does a well mineralised surface zone

inhibit lesion body remineralisation to a greater or lesser extent than a poorly mineralised surface zone? This interaction between the different parts of the lesion may provide answers to questions about unexpected results from studies. This data has not been examined to determine how lesions with multiple characteristics behave and this is perhaps a direction in which future work which could be undertaken. Better lesion characterisation may provide a more valid interpretation of results from intra-oral studies.

Throughout this work volunteers have been characterised by their ability to remineralise artificial carious lesions. Some volunteers seem to produce consistently high levels of remineralisation whereas some volunteers have consistently demineralised lesions under almost all protocols. Whether this is an indication of volunteer compliance or individual volunteer characteristics is uncertain. The consistency of such outcomes, however, would indicate that perhaps the individual volunteer characteristics are more responsible.

Determination of volunteer compliance is difficult and the extent to which compliance will affect outcome is unknown. Presently, it is easier to begin from the premise that such differences in volunteer results reflect individual volunteer characteristics and try to identify which of these characteristics may be responsible for each volunteers performance in an intra-oral trial.

The comparison of mineral changes recorded for lesions mounted in the intra-oral appliance trough and plaque and salivary measurements in the appliance

trough may provide some indication as to why some volunteers behaved in different ways. The stratification of volunteers as good, bad or indifferent remineralisers sought to magnify such differences which may exist in plaque and salivary physiology.

Data for plaque and salivary fluoride levels recorded during the 4 week washout period did not demonstrate any relationship to lesion mineral changes recorded during the subsequent appliance wearing period. However, salivary fluoride levels did exhibit a significant fall over the 4 week washout period when subjects changed from a fluoridated to a non-fluoridated dentifrice. In addition, plaque fluoride levels also exhibited a downward trend over the 4 week washout period, but this was not significant. The impact of this change on lesion remineralisation is unknown and should be investigated further.

Determination of the ability of saliva to clear potassium from the appliance trough has shown the limited reduction in salivary flow over the sections afforded by the appliance trough. The placement of the enamel lesions in an area of relatively rapid salivary clearance would suggest the model was biased towards remineralisation. The effect which saliva may have cannot, however, be very marked. Volunteers still exhibit a wide variety of responses under all protocols and the removal of fluoride from a model protocol often overcomes the remineralising abilities of saliva.

Data to compare lesion remineralisation with salivary clearance would indicate a low salivary clearance is related to remineralisation. This is a controversial result, if indeed it is a true result rather than statistical artifact due to the small sample size. Low salivary clearance is associated with a reduced salivary flow rate and decreased calcium and phosphate saturation. Therefore, local undersaturation with respect to calcium and phosphate at the saliva/plaque fluid interface could be expected. The presence of fluoride has a dramatic effect on calcium and phosphate solubilities. An explanation for this result could lie in the ability of plaque to maintain a fluoride concentration gradient against saliva under conditions of low salivary flow. This gradient may be erroded under conditions of rapid salivary flow with a subsequent reduction in calcium and phosphate saturation at the enamel/plaque fluid interface despite stimulated saliva being supersaturated with respect to both species.

The effect of saliva on plaque pH and the ability of plaque in the appliance trough to respond to sucrose stimuli has also provided interesting results. Once again, the small sample size introduces a note of caution to the interpretation of the analysis. Low plaque pH and low salivary buffering capacity would appear to be associated with a reduced ability of volunteers to remineralise artificial enamel lesions. This finding is in agreement with the results of other authors.

A fundamental point of all measurements of lesion mineral change, salivary fluoride levels and plaque and salivary physiology is that these measurements

are representative of one moment in time. The longitudinal stability of these parameters is unknown, especially for the plaque and salivary measurements. Further collection of this data at regular intervals may assist in better characterising volunteer behaviour with respect to performance in an intra-oral trial.

These studies have attempted to relate plaque and salivary measurements, perceived to be important clinically, to mineral changes recorded using an intra-oral model. Further investigation of such plaque and salivary measurements in relation to mineral changes within lesions may establish a better understanding of factors that control changes within early carious lesions. From this basic understanding can develop the capacity to predict caries or establish a risk of developing caries through the measurement of different facets of oral physiology. This may further lead to the development of the ability to target preventative treatment to the minority of patients who are affected by the majority of caries.

In summary, a better understanding of the relative interactions between different parts of an early enamel lesion is required to characterise the ability of lesions to respond to a given protocol. In addition, longitudinal plaque and salivary data collected from volunteers would further assist the understanding of the nature of lesion mineral changes for individual volunteers. the production of lesions with predictable behaviour would assist the determination of experimental outcome but the validity of the findings of

intra-oral models can only ever be truly verified by the use of a clinical trial. However, they do provide a valuable adjunct to clinical trials and a potential screening method to reject protocols which are not viable.

Appendix I

Approval for *in situ* model work by the Area Dental Ethics Committee

GREATER GLASGOW HEALTH BOARD

225 BATH STREET
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Telephone: 041-204 2755

Ref.: RMcK/EMcG

ur Ref.:

honung for:— Mr R. McKechnie

All communications must be addressed to

.....

9 August 1984

Dr. K.W. Stephen
Reader in Oral Medicine and Pathology
Glasgow Dental Hospital and School
378 Sauchiehall Street
GLASGOW
G2 3JZ

Dear Dr. Stephen

AREA DENTAL ETHICS COMMITTEE

I write to inform you that your application of 6 August 1984 for clinical research has now been discussed by the Chairman of the Area Dental Ethics Committee, the aim of the project being:

"Investigations Relating to Clinical Topical Fluoride Therapies"

This project has now been approved and you may proceed with your research. The decision will not be minuted until the next formal meeting of the Area Dental Ethics Committee.

The Committee would be grateful if you would inform them of the results of your project and any ethical problems encountered whenever the project is completed.

Yours sincerely

R. McKECHNIE
Chief Administrative Dental Officer

Appendix II

Derivation of the equation by Angmar *et al.* (1963)

this equation is used to determine the mineral content of dental hard tissues from a microradiographic image of the hard tissue sample and an aluminium step wedge of known thickness and absorbtion coefficient. For the purposes of the calculation certain assumptions had to be made. These included:-

1 The mineral salts were assumed to have a density of 3.15.

2 The average composition of the mineral was assumed to be 37.1% Ca, 0.5% Mg, 18.1% P, 43.3% O, 0.7% C and 0.3% H.

3 The resulting Ca:P ratio was 2.05.

4 The volume not occupied by mineral salts was assumed to be filled with organic matter and water.

The calculation for the amount of mineral from densitometric data is given by the following equation:-

$$V = \frac{100[(\mu.\varrho)_A \cdot t_A - (\mu.\varrho)_o \cdot t_S)}{[(\mu.\varrho)_M - (\mu.\varrho)_o] \cdot t_S}$$

Where $(\mu.\varrho)_A$ is the linear absorbtion coefficient for aluminium and has a value of 131.5.

t_A is the thickness of the aluminium having the same photographic density as the section at any measured point This is interpolated from the density curve of the step

wedge.

$(\mu.\varrho)_o$ is the linear absorbtion coefficient for organic matter and water and has a value of 11.3.

t_s is the thickness of the individual section and is determined using a micrometer.

$(\mu.\varrho)_M$ is the linear absorbtion coefficient for the mineral salts and has a value of 260.5.

Substituting these values, the equation then becomes:-

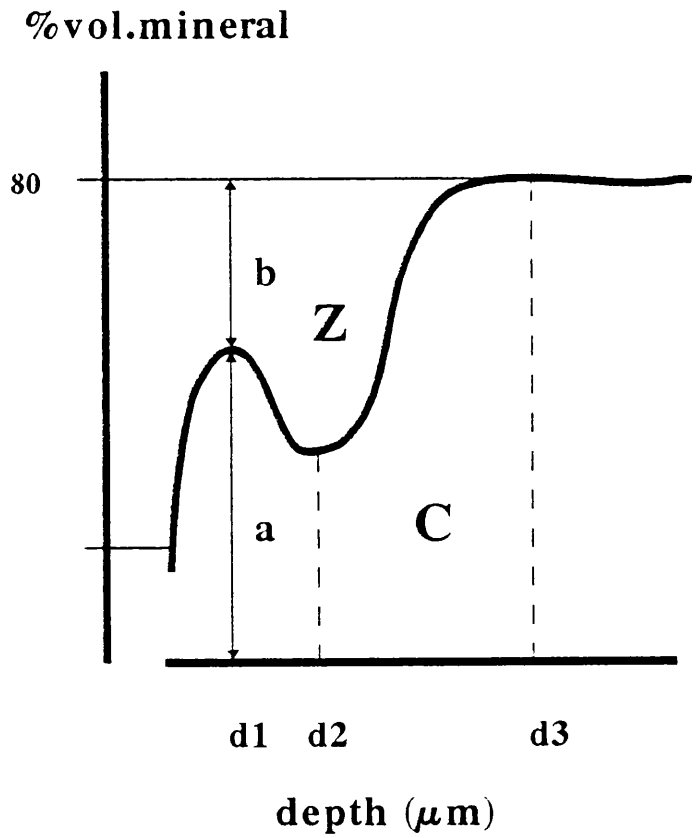
$$V = \frac{52.77 \,.\, t_A}{t_S} - 4.54$$

The only unknowns are now the thickness of the aluminium and the section thickness. The aluminium thickness was 52.5 μm and the thickness of each section was determined individually using a micrometer.

Appendix III

Measured parameters on a densitometric tracing of an artificial carious lesion in enamel

A microdensitometric scan through an artificial white spot lesion showing the variation in mineral content (% vol. mineral) with the depth from the surface of the lesion. On this occasion the mineral content for normal enamel has been arbitrarily assigned a value of 80 % volume mineral which is within the range reported by Groeneveld (1978).



Mineral Content at a Single Point

Mineral content at a single point may be expressed as mineral loss from normal enamel (*b*) or as a value in its own right (*a*). This parameter is useful when assessing the change in mineral distribution at different points within a lesion over time. In this case the maximum mineral content of the surface zone (*a*) is shown. The units for mineral content at a single point are either kg/m^3 or % volume mineral.

The Integral of the Change in Mineral Content Over a Known Distance

This parameter may be similarly expressed as a value in its own right (area C - below the curve) or as the mineral loss compared with normal enamel (area Z - above the curve). Both values are of use but the area above the mineral profile (Z) is most often quoted. This area has been designated the symbol Δz (Gelhard & Arends, 1984) and has units of kg/m^2 or % vol.mineral x μm. It should be noted that there are several ways in which Δz can be calculated. These have been discussed in section 2.4.1. For the purposes of this thesis, the units of Δz are (%vol.mineral x μm)/100.

Lesion Depth

The depth of different parts of the lesion scan are often important. On the above diagram, d_1 represents the depth from the surface of the maximum mineral content of the surface zone, d_2 represents the depth from the surface of the minimum mineral content of the lesion body and d_3 represents the lesion depth defined as the depth where normal enamel mineral content is encountered.

Appendix IV

Chemical formula for low level total ionic strength adjustment buffer (TISAB)

distilled water
57 ml glacial acetic acid
58 g sodium chloride (NaCl)
5 M sodium hydroxide solution (NaOH)

Place 500 ml of distilled water in a 1 litre beaker. Add 57 ml of glacial acetic acid and 58 g of NaCl. Stir to dissolve. Place the beaker in a water bath for cooling. Immerse a calibrated pH electrode into the solution and slowly add 5 M NaOH until the pH of the solution is between 5.0 and 5.5. Cool to room temperature. Pour into a 1 litre flask and dilute to the mark with distilled water.

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