INVESTIGATIONS INTO THE GENERATION AND CONTROL OF ENDOGENOUS EROSION, USING "IN VITRO" MODELS

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DECLARATION

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For Mum and Dad,

to whom I am indebted,

for their many sacrifices, support and inspiration.

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SUMMARY

In recent times, dental erosion has been recognised as a significant cause of tooth destruction. It would appear that the proportion of the general population whose dentitions are at risk of developing erosion is increasing, and this may in part be explained by the current trend for people to retain their teeth into old age. To effectively prevent and manage the clinical problems arising from dental erosion, the profession urgently requires more information concerning the kinetics of erosive demineralisation.

Dental erosion of enamel occurs through the contact of a variety of strong acids of endogenous and exogenous source with the tooth surface. Endogenous sources of acid are usually from refluxed or otherwise orally emitted stomach acid.

The mechanisms and effects of dental erosion of enamel are difficult to investigate clinically. A simple "in vitro" continuous erosion model was developed by Hunt and McIntyre (1992). These authors have shown that 0.06M hydrochloric acid (HCl), with the addition of 2.2mM calcium hydrogen phosphate ($CaHPO_4$), to represent salivary calcium and phosphate ions, erodes dental enamel "in vitro" to result in an effect somewhat similar to that seen clinically from regurgitated stomach acid contact with teeth, over a practically observable time period. Lekkas, Hunt and McIntyre (1992) have also developed an "in vivo/in vitro" model system to simulate more closely the clinical aspects of erosion, and to investigate potential control mechanism.

In this present study, the "in vitro" model test system was used to investigate the generation and control of endogenous enamel erosion, using the 0.06M HCl and 2.2mM $CaHPO_4$ erosion system. At the same time, refinements of the model were investigated, in terms of differences between an intermittent and continuous challenge sequence.

A variety of methods were tested for their usefulness in measuring both the amount of mineral loss during the erosive challenge, and any resulting changes in the physical

characteristics of the remaining surface enamel. Methods include the use of scanning electron microscopy, microhardness testing, and polarised light microscopy to assess any changes in enamel as a result of erosive demineralisation. Polarised light microscopy proved to be of questionable value in acquiring information. Nevertheless, the other techniques may be useful and suitable means of gaining data on the resulting changes in the physical characteristics of the remaining surface of enamel, often with certain modifications.

Topical fluorides are widely used clinically to help combat the effects of dental erosion. However, their effectiveness has not been scientifically evaluated in relation to erosion. In this study, a modified model system was developed, to permit pilot investigations of the ability of topical fluorides to inhibit erosive demineralisation, and to detect any changes resulting in the remaining enamel.

A "cyclic" modification of the basic in vitro "continuous" erosion model was made as a consequence of early investigations in this present study. This model allowed various situations to be tested, such as, the time period of incremental exposure of the specimens to the erosive solution, and the sequence and frequency of topical fluoride exposure. It was demonstrated that the frequent and regular application of fluoride, and not necessarily prior to every cycle of acid exposure, was sufficient to impart a considerable reduction in the depth of erosive loss.

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CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

The subject of dental erosion has been discussed in the dental literature for many years. As early as 1892, Darby reported on the erosive action of fruit juices. Miller (1907) later investigated the phenomenon of "wasting" of tooth tissue and arrived at a list of causative agents. He inferred that the wasting of tooth tissue was a modern day phenomenon. In 1884, Miller examined all the skulls in the Berlin anatomical museum, and he did not find a single case of "undisputable wasting". A colleague of Miller's, Dr Grevers of Amsterdam, also examined no less than 6000 old skulls, and did not find a single case of wasting among the entire number.

1.2 DEFINITION

Erosion has been defined by Pindborg (1970) as a mainly superficial loss of dental hard tissue by a chemical process which does not involve bacteria. Tooth surface erosion is frequently associated with abrasion and attrition, and these conditions may be exacerbated in the presence of erosive factors. It is likely that none of these conditions ever occurs in complete isolation (Smith, 1991). As a result, the term "tooth surface loss" was proposed by Eccles (1982) to describe the conditions of abrasion, attrition, and erosion. Smith and Knight (1984) also suggested the term "tooth wear".

1.3 INCIDENCE AND PREVALENCE

Research in the prevalence and incidence of dental erosion is lacking. The extent of the problem is not known and there is a lack of studies defining this. There have been many individual case reports written and published, although data on the prevalence,

incidence, nature and severity of erosion in populations are scarce. The task of studying the epidemiology of dental erosion is not an easy one, merely because there is the difficulty of making a differential diagnosis between the various causes of tooth wear.

The incidence of dental erosion has been investigated, but these studies do not include reviews of patient history or the parameters of saliva, such as pH, flow rate, and buffering capacity, for possible causes.

Shulman (1948) reported the incidence of dental erosion to be 12% in 1345 teeth examined (excluding cervical lesions). A year later, Zipkin and McClure studied a smaller sample of 83 teeth where an incidence of 20% was reported in which cervical lesions were included. In a study by Xhonga and Sognnaes (1972), erosion lesions were seen in 18% of 10,000 extracted teeth with cervical lesions included. The authors found the cervical areas of incisors to be the most common site of occurrence. Brady and Woody (1977) reported an incidence of 5.3% in 900 teeth. An incidence of 25% in some hundreds of teeth was recorded by Xhonga and Valdmanis (1986).

In a study by Xhonga and Van Herle (1973), a greater incidence of erosion was demonstrated in patients with hyperthyroidism. The authors suggested that this finding may be attributed to the elevated thyroid hormone levels or the resulting increase in the metabolic rate.

Finch (1957) reported on an erosion case associated with diabetes insipidus. In the case presented, there was association of a high intake of citrus fruit drinks with the medical condition and very marked and rapidly developing erosion lesions.

The incidence of erosion has also been studied in special groups and categories. Centerwell et al. (1986) reported the occurrence of dental erosion in competitive swimmers at a gas-chlorinated pool. Lactovegetarians were studied by Linkosalo and Markkanen (1985) in which they found an incidence of 77% in 26 subjects. Jones et al. (1989) recorded an incidence of 69% in 11 bulimic women. An incidence of 40% in 3 patients with chronic alcoholism was stated by Robb and Smith (1990).

Prevalence studies in various groups of industrial and non-industrial origin have been reported. Many of these have been in the form of anecdotal and case reports. Pindborg (1970) found a prevalence of erosion in only 2.1% of 1345 American male students surveyed. Dental erosion is a common finding among sufferers of eating disorders, namely, anorexia nervosa and bulimia nervosa as reported by Roberts and Tylenda (1989). Lussi et al. (1991) performed a study with the purpose of determining the prevalence of dental erosion in an adult population in Switzerland. The sample group consisted of 391 randomly selected persons from two age groups (26-30 and 46-50 years), and information was gathered on lifestyle, dietary and oral health habits. For facial surfaces, 7.7% of the younger age group and 13.2% of the older age group showed at least one tooth with Class II erosion (involving dentine for less than one third of the surface). Occlusally, at least one tooth showing severe erosion was observed in 29.9% of the younger sample and 42.6% of the older sample. Slight lingual erosion was seen in 3.6% of the younger age group and 6.1% of the older age group. Severe lingual erosion lesions were found to be scarce.

Studies on dental erosion of non-industrial origin are mainly related to dietary factors as reported by Eccles (1979). Prevalence studies have otherwise been conducted more on erosion of industrial origin. Dental erosion in industry has been recognised by ten Bruggen Cate (1968). A pilot study was performed by Skogedal et al. (1977) among the workers in a Norwegian electrolytic zinc factory to investigate the prevalence of dental erosion. Petersen and Gormsen (1991) reported the prevalence of erosion among German battery factory workers.

1.4 MECHANISMS OF ENAMEL DEMINERALISATION

1.4.1 Enamel Composition and Structure

Dental enamel provides an acellular, hard protective coating 1-2mm thick over the coronal dentine. It consists mainly of a dense mineral containing calcium, phosphate and other ions in an hydroxyapatite-like structure. Dental enamel is porous and contains about 96% mineral by weight which is equivalent to 85% by volume. The remaining volume is occupied by water, protein, and lipid, which form the diffusion channels through which acids and minerals can travel in and out of the tooth.

Before examining mechanisms of erosive demineralisation of teeth, it is useful to analyse how our understanding of the carious demineralisation process developed.

1.4.2 Mechanism of Enamel Caries

The basic mechaninsm of dental caries was described one hundred years ago by W.D.Miller.

Increase in the production of hydrogen ions in the bacterial plaque on the enamel surface is the first step in the formation of the carious lesion. This results in a pH decrease in the oral environment. As pH falls, the solubility of the enamel apatites will increase dramatically. Thylstrup and Fejerskov (1986) report that a pH drop of one unit within the range of pH 7-4 gives rise to a seven-fold increase in the solubility of hydroxyapatite.

These hydrogen ions are produced as a result of glycolysis, the process by which simple sugars, mono- and disaccharides, and fermentable carbohydrates from our diet are metabolised until inorganic acids are produced. The process begins essentially at the crystal peripheries right at the enamel surface. The acids produced are primarily lactic, acetic, and formic. These are weak acids but they readily penetrate the porous enamel. All this occurs in the oral environment where the enamel surface is protected by proteins and lipids

derived from the saliva.

The concentrations of calcium and phosphate already present in the oral fluids determine the pH at which the aqueous phase is exactly saturated with the enamel apatites. This pH is referred to as the "critical pH". Clinical assessments of the critical pH show a variation between 5.2 and 5.5 (Thylstrup and Fejerskov, 1986).

When saliva becomes undersaturated with respect to hydroxyapatite, it still remains supersaturated with respect to fluorapatite. The pH at which salivary fluorapatite begins to reach saturated levels has been determined at about 4.5. When the enamel is exposed to a buffer unsaturated with respect to hydroxyapatite but supersaturated with respect to fluorapatite, a carious lesion is formed, with a relatively unaffected surface superficial to a subsurface demineralisation. Subsurface demineralisation occurs as the edges of the crystals are partially dissolved (Thylstrup and Fejerskov, 1986).

The same authors state that, "Caries progression from ultrastructural changes to visible decay should be regarded as the cumulating effect of a long series of alternate dissolution at low pH and a partial precipitation when the pH rises". With further progress after months or years, depending on the cariogenic challenges, a "white spot" lesion results. Clinically, this is seen as a white opacity which will progress to eventual cavitation if it is not reversed or arrested by the process of remineralisation. There is a major distinction in the formation of enamel caries compared with that of enamel erosion or surface etching of the enamel.

1.4.3 Enamel Caries and Enamel Erosion

For more than eighty years, acids have been recognised as the principle agent of enamel dissolution arising either from bacterial metabolism in the case of dental caries, or from extraneous sources such as acidic foods, gastric acids, and airborne acid dusts in the case of dental erosion.

There is a distinct difference in the macroscopic and microscopic appearances of the two types of enamel dissolution lesions. The initial carious lesion is the result of a subsurface loss of material. On the other hand, dental erosion is largely the result of a surface dissolution. The two types of enamel dissolution are chemically distinct (see Figure 1).

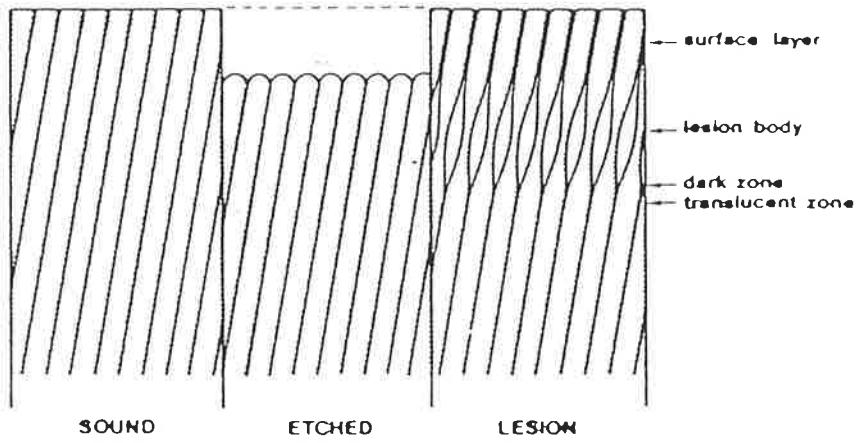


Figure 1. Schematic representation of the formation of eroded enamel and subsurface enamel lesions (Featherstone, 1989).

1.4.4 Mechanism of Enamel Erosion

The process of dental erosion is initiated by a stronger acid attack than that described for dental caries. In a proposed hypothetical model of demineralisation, the actual pH of the acid at the tooth surface, and time, are believed to be the major variables affecting the extent of erosion. There is usually a considerable chelating effect by acid anions, such as lactate and citrate, in dental erosion. The acid attacks the ends of the crystal structure at the tooth surface to dissolve them directly and completely. The layers of mineral are removed directly.

The hydrogen ion of any acid will eventually dissolve the calcium phosphate mineral component. However, even at a neutral pH, strong chelators such as citrate and EDTA (ethylene diamine tetra-acetic acid) can also readily complex with the calcium in the apatite structure and cause dissolution. Chelation is in general, not acid-dependent, and even if

the acid is neutralised, the chelating process can proceed. Bevenius et al. (1988) state, "pH itself does not provide an indication of erosive potential".

The process of enamel erosion first begins with the contact of strong acids such as hydrochloric, citric, and phosphoric acids at the tooth surface. This often occurs when the oral environment is at a resting pH (6.8). These acids then overwhelm the natural protective mechanisms of the salivary hydrogen phosphate buffer system, and the presence of a thin layer of plaque in many cases, before the bicarbonate buffer system generated by salivary stimulation can come into play. Even if the bicarbonate system is generated, it is quickly overwhelmed. At a pH of less than 5.5, the acid ions quickly react with the phosphate groups of hydroxyapatite, and even with the fluorapatite if the pH is less than 4.5. If the pH is below 4.5 (the critical pH at which dissolution of fluorapatite becomes predominant), any further immediate buffering or remineralisation to protect the tooth surface is significantly reduced, and total mineral dissolution proceeds. Surface enamel dissolution continues until there is a sudden and rapid reduction in the concentration of hydrogen ions.

In theory, if the supply of hydrogen ions is exhausted or neutralised, then, as long as there is sufficient calcium and phosphate ions retained at the tooth surface, the process of remineralisation can occur. However, this is rarely so in practice. If there has been sufficient protection of the surface by the presence of pellicle or plaque, and the pH is above 4.5 for a brief period, sufficient retention of calcium and phosphate ions may have been generated to allow remineralisation of the surface layer, and partial subsurface remineralisation. However, if the pH reaches 4.5 or below, and little or no surface protection is present, then the ideal environment is present for dental erosion to proceed (see Figure 2).

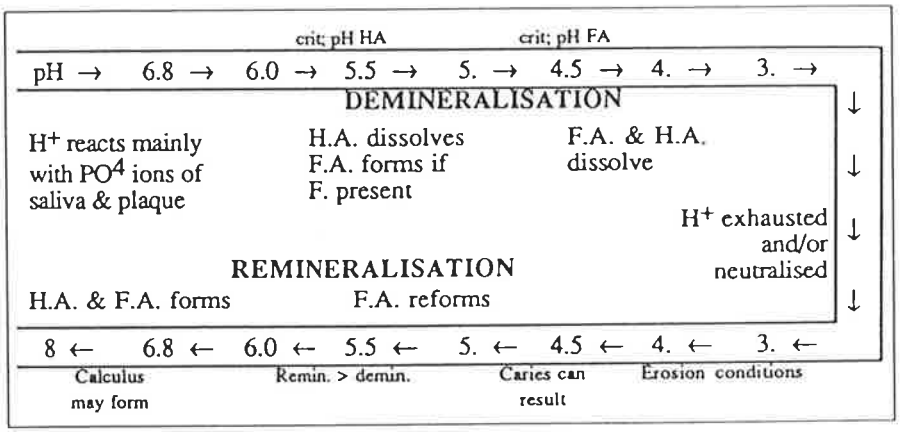


Figure 2. Nature of hypothetical pH cycle at the tooth surface (McIntyre, 1992).

1.5 NATURAL DEFENCE MECHANISMS

There are naturally occurring factors in the oral environment which operate to counteract erosion. These are found in the saliva (its flow rate and buffering capacity), the pellicle (protective glycoproteins attached to the enamel prisms), and the layer of plaque.

1.5.1 The Nature of Saliva

Saliva is a very complex and comprehensive system providing protection for the mouth against many challenges. In most persons, saliva is continually in the mouth and is continually secreted from the salivary glands. The salivary flow rate in the resting state is approximately 0.2ml/minute, and approximately 1-2ml/minute when stimulated. The resting and stimulated saliva provides a flushing action.

Saliva contains calcium and phosphate in a super-saturated state which is maintained by salivary proteins which hold calcium in a complexed but reversible state. This means that there is excess calcium and phosphate ready to precipitate and remineralise hydroxyapatite crystals that have been damaged by acid or chelators.

1.6 POSSIBLE CAUSES OF CLINICAL EROSION

The possible causes of dental erosion can be classed as exogenous (extrinsic) due to influences outside the mouth, and endogenous (intrinsic) due to sources within the mouth. The causative agent is usually an acid, that is, there is an extrinsic or intrinsic supply of acid (Eccles, 1979).

1.6.1 Exogenous Causes of Erosion

There are two types of exogenous erosion. These are industrial, due to exposure of the teeth to atmospheric acids, and dietary, due to demineralising foods such as citrus fruits and acidic beverages. Extrinsic erosion has been attributed largely to excessive consumption of acidic substances with low pH and high citric acid content (certain cola type drinks). The method by which the erosive product is consumed will no doubt be a modifying factor in the erosion seen.

The industrial type of erosion was not uncommon in the days before effective occupational health measures were adopted. Exposure to acids was common in chemical and metal industries. A prevalence study in such a group was recently reported by Petersen and Gormsen (1991) among German battery factory workers being exposed to sulphuric acid mists. In a cross-sectional study by Tuominen and Tuominen (1991), the authors explored the occurence of dental erosion and the relative importance of some related factors among factory workers exposed to inorganic acid fumes. Results showed that the anterior teeth were affected more often than the posterior teeth in the sample of 76 workers. Factors such as the exposure to acid fumes, increasing age, and frequency of intake of fruits increased the probability of dental erosion. Other reports have been made by Miller (1907), Lynch and Bell (1947), Malcolm and Paul (1961), ten Bruggen Cate (1968), and Skogedal et al. (1977).

Exposure to extrinsic sources of acid can also be associated with various activities such as frequent swimming in chlorinated pool (Centerwell et al. 1986). In a study by Robb and Smith (1990) on the prevalence of toothwear with chronic alcoholism, the

authors found that dental erosion was present, "Being most marked in males, and those whose consumption was continuous rather than in the form of episodic binges".

The range of potentially erosive substances in the diet is large. The dissolution of tooth mineral by such substances were recognised many years ago by Stafne and Lovestedt (1947). It is believed that the consumption of any food or drink with a low pH does not produce any significant tooth damage unless the food or drink is consumed in greater quantities than normal, or ingested in an unusual way so as to prolong the contact between the food or drink and specific tooth surfaces. Common dietary sources of erosion are listed in the table below.

1. Citrus fruits (Eccles & Jenkins, 1974; Fuller & Johnson, 1977)
2. Acidic beverages
   - (a) Acidic juices (Levine, 1973; Eccles & Jenkins, 1974; Touyz & Glassman,1981)
   - (b) Baby fruit juices (Smith & Shaw, 1987; Grenby, 1990)
   - (c) Wines
3. Carbonated beverages (High, 1977; Mueninghoff & Johnson, 1982; Asher & Read, 1987)
4. Vinegar and pickles
5. Herbal teas (Angmar-Mansson et al., 1980)
6. Medicines
   - (a) Effervescent vitamin C preparations, chewable vitamin C tablets (Giunta, 1983; Erikson & Angmar-Mansson, 1986; Meurman & Murtomaa, 1986)
   - (b) Iron tonics (James & Parfitt, 1953)
   - (c) Acetylsalicylic acid
   - (d) Hydrochloric acid for replacement therapy (Turner & Missirlian, 1984)
7. Lactovegetarian diet (Linkosalo & Markkanen, 1985)

Although it has been recognised that acids can cause dental erosion, the frequency of exposure and the magnitude of the risks have not been fully realised. Jarvinen et al. (1991) in a study found that when citrus fruits were consumed more than twice a day, an erosion risk thirty-seven times greater than that in those who consumed citrus fruits less often was seen. For citrus fruit, it could be suggested that the critical frequency of consumption is more than twice a day. This could be even less in some patients. Elsbury (1952) found in an in vitro study, that citric acid erodes tooth structure at a rate more than double that of hydrochloric or nitric acid at the same concentration. This may be because its chelating action on enamel calcium continues even after the pH increases at the tooth surface (McClure and Ruzicka, 1946). In the case of soft drinks, once a day or more may be the critical frequency of consumption. Further research in this area would be of great benefit in the prevention and control of dental erosion.

1.6.2 Endogenous Causes of Erosion

Endogenous erosion is the result of habitual regurgitation or reflux of acidic gastric contents. This has been documented in patients with the psychological eating disorders of anorexia nervosa and bulimia nervosa who induce vomiting (Hellstrom, 1977; Stege et al. 1982; Clark, 1985; Harrison et al. 1985; Spigset, 1987; Roberts and Li, 1987; Knewitz and Drisko, 1988; Roberts and Tylenda, 1989 and Altshuler, 1990).

Associations have also been made between erosion and recurrent vomiting due to systemic disorders or abnormality of the gastro-intestinal tract which result in the regurgitation of stomach contents. Those disorders that have been implicated in causing erosive dental lesions are hiatus hernia (Howden, 1971), gastric ulcer (peptic and duodenal), renal dysfunction, and gastritis as seen in chronic alcoholism. The dental status of 109 patients with gastro-intestinal symptoms was examined by Jarvinen et al. (1988). It was concluded that gastro- intestinal disorders with increased output of gastric acid may be linked to dental erosion. Cases have been reported by Ismail-Beige et al. 1970; Eccles, 1978; Pope, 1982 and Myllarniemi and Saario, 1985. Taylor et al. (1992) reported a case of dental erosion associated with asymptomatic gastroesophageal reflux in an eight year old girl, whose

diagnosis could not be elicited from her symptoms, or medical or dental history. Regurgitation erosion has also resulted from persistent morning sickness during pregnancy.

Intrinsic salivary factors have also been linked to the erosive process (Wöltgens et al, 1985 and Järvinen et al. 1991). A low salivary flow produces an inadequate flushing action and buffering of demineralising acids in contact with the tooth surfaces. In a study by Wöltgens et al.(1985), salivary tests from erosion-susceptible patients were compared with controls. The results of the tests indicated that the erosion-susceptible patients showed a very low unstimulated flow of saliva and a significantly higher glucose clearance time. The inherent factors of saliva such as flow rate, viscosity, and buffering capacity have perhaps been underestimated in the erosion of enamel.

The term "iatrogenic" or "idiopathic" erosion has been applied in patients in whom no apparent aetiology can be elicited. It has been put forward that perhaps these patients suffer from impaired and ineffective salivary defence factors. Two distinct types of cervical lesions have been noted: shallow, saucer-shaped lesions with poorly defined margins, or deep notches with sharply-defined borders, particularly in enamel. These two different lesions may have quite different aetiologies,, or may represent different stages of the same lesion (Bevenius, L'Estrange, Karlsson and Carlsson, 1993). Occlusal stresses caused by mastication, malocclusion or lack of canine disclusion in lateral excursive movements have all been proposed as aetiologic factors (Yettram et al., 1976; Brady & Wood, 1977 and Lee & Eakle, 1984).

The range of of potentially erosive materials is large, (McIntyre, 1990) and hence, the clinician needs to be fully aware of the possible causes of dental erosion.

1.7 QUANTITATIVE AND QUALITATIVE STUDIES OF EROSIVE DEMINERALISATION OF TEETH

1.7.1 The Need for Models of Erosion to be Developed

In order to fully understand the nature and kinetics of the erosive demineralisation process, and the erosive potential of various products, it is necessary for models of the process to be developed which do not result in damage to the teeth of the human subjects. Such knowledge is necessary before major advances in developing methods of prevention or control can take place.

The development of such models of dental caries provided the oportunity for much of our current knowledge of its mechanism of action, and methods for its control.

1.7.2 Potential Models to Study Erosion

The major models used to study dental caries have been:

(a) Animal models

(b) Chemical "in vitro" models

(c) Biological "in vitro" models

(d) "In vitro-in vivo" models

Similar models have been used in the study of the mechanisms of erosion, and of the erosive potential of a variety of products.

1.7.2 (a) Animal Models

Numerous animal studies have been done for many years in an attempt to explain the aetiology of dental erosion and methods of control. However, the animal models used for erosion studies are still not uniformly accepted (Featherstone, 1989).

In 1943, McClure studied erosion in rat molar teeth. He concluded that the erosion occurred as a result of their drinking acid beverages in place of water. Since then, his results have been confirmed by other researchers (Restarski, Gortner and McCay, 1945; McCay and Will, 1949). Dogs and rats were used by Holloway et al. (1958) to perform experiments in which they demonstrated that fruit juices also produced similar erosion effects. These results supported the work of Wynn and Haldi (1948) and Miller (1950, 1951).

Workers such as Fuks (1973) and Gedalia (1975) performed studies to test the effect of fluoride on erosion lesions in hamsters. Fuks used grapefruit juice containing 1.9ppm fluoride and found that the rate of erosion in the molars of the hamsters fed with the fluoridated juice was significantly lower than the group with no fluoride supplementation. Gedalia and his workers studied more the effect of fluoride on caries. Their results showed that erosion caused by citrus beverages predisposed the teeth of the hamsters to caries. Similarly, 1.9ppm fluoride added in the beverages exerted a protective effect against caries.

Rats have also been a popular model for experiments to be carried out on. McDonald and Stookey (1973) demonstrated in rats that the addition of a sucrose-containing soft drink to the diet significantly increased erosion. This effect was shown to be reduced by the addition of phosphate to the mix. Reussner et al. (1975) obtained similar results in their investigation on the effects of phosphates in acid-containing beverages on tooth erosion.

Regolati et al. (1975) tested the effect of sodium fluoride or monofluorophosphate against erosion in the rat's dentition. These agents demonstrated a favourable effect against such lesions.

A recent study by Sorvari et al. (1988) tested sport drink mixtures with a pH of 3.2, supplemented with either 15ppm fluoride or 39ppm magnesium, or both. Marked erosion due to the acidic sport drink was seen in the rats. The addition of fluoride was found to produce less severe erosive lesions.

The interpretation of data resulting from studies performed on animals should always be made with caution. The oral environment in animals is quite different to that in humans. One major difference is the composition of the saliva. Another point that biases the model when performing such studies is the consumption of the drinks, continually. This does not allow the normal repair processes to work that naturally occur when the challenge is removed.

However, these studies do show unequivocally that there is a potential for erosion to occur with excessive consumption of acidic food and beverages. An important conclusion was arrived at from these investigations. This was that neither the total acidity of the beverages nor their pH values could be used to predict erosion.

1.7.2 (b) "In Vitro" Models

Many "in vitro" studies have been carried out to test the many various foods and drinks that will dissolve tooth structure.

Grobler and van der Horst (1982) tested twenty-three "cooldrinks" and showed clearly their erosive potential. The degree of enamel erosion caused by five kinds of fruit was also investigated by Grobler (1989). Grobler et al. (1990) reported that the degree of enamel erosion initiated by a fruit juice was about five to eight times higher than that of the fruit. They also found that the degree of enamel erosion by different fruits depended on a combination of factors, such as the pH, amounts, and ratios of the types of organic acids and other chemical components in the fruits.

Grobler and co-workers in the above studies performed tests "in vitro" on rectangular blocks of intact human enamel coated with varnish except for a window measuring 3mm x 3mm. In the 1990 study, the blocks were then exposed to various juices and cola beverages for 2, 4, 5, 6 or 40 minutes. The results showed that Pepsi Cola and orange juice produced the same degree of enamel demineralisation, followed by apple juice and Diet Pepsi Cola. Diet colas appear to demonstrate less demineralisation than other acid drinks.

In a study by Kelly and Smith (1988), three solutions, namely, human saliva, an artificial calcifying solution and a sodium fluoride mouthrinse (0.05%), were tested to determine their effect on erosion lesions on extracted human maxillary incisor teeth. The three solutions had small but statistically insignificant effects on reducing the amount of erosion under experimental conditions.

These studies are however, difficult to interpret as they are not performed in the oral environment where the natural defence mechanisms and protective factors in the saliva, as well as the flushing action of the saliva flowing over the tooth surface, can operate.

Another consideration when interpreting in vitro studies must be kept clearly in mind. When a tooth crown is placed in an acidic solution, the mineral will naturally dissolve. However, in time, the dissolution process becomes diffusion-controlled as the acid has to penetrate deeper into the tooth structure. Furthermore, the volume of the test solution is restricted. As a result, the level of calcium and phosphate in the solution may increase, eventually retarding or stopping the process of dissolution.

There is a lack of models which successfully combine the alternating periods of erosion and remineralisation as it normally occurs in the oral environment. The fact remains that, "erosion activity has proved difficult to quantify in vivo" (Lekkas, Hunt, and McIntyre, 1992). Rytömaa et al. (1988) reported studies in which they bathed bovine enamel in saliva between exposures to the test acidic drinks and various foodstuffs. Interestingly, they found that the presence of saliva did not have any influence on the severity of the lesion. This may have been due to the fact that they did not have a realistic and equilibrated model.

Lekkas, Hunt, and McIntyre (1992) developed an "in vitro-in vivo" model of dental erosion. The concept of the model was to simulate the interaction between the two components involved in dental erosion, namely, demineralisation and remineralisation. It aimed to "simulate the alternating cycles of erosive demineralisation and the protective mechanisms of saliva".

1.7.3 Review of Methods Which May be Used in "in vitro" Studies of Erosive Demineralisation, to Analyse the Nature and Kinetics of the Process

Erosion results in the total loss of surface layers of enamel from specific exposed surfaces of tooth, leaving behind a layer which may exhibit physical characteristics which are different from those of normal enamel. Thus, an understanding of the nature and kinetics of the process requires accurate estimation of the volume and rate of mineral loss under various erosive conditions.

Quantitation of volume of enamel loss has proven to be a very difficult task, not only clinically, but also in in vitro studies. Estimates of grades of erosion, as described by Eccles (1979) are based on clinical observation, and are of a qualitative rather than a quantitative nature.

The measurement of depth loss has been attempted in abrasion studies by means of a surface profilometer (Ashmore et al., 1972; Davis & Winter, 1976, 1977, 1980). In these abrasion studies, depth losses of less than 0.1μm were measured. This technique has now been further developed for measuring in vitro, the effects of erosion, and also the protective effects of treatments such as, the use of fluoride. Two-millimetre windows across the width of the teeth were exposed to acid erosion. The amounts of tissue lost were then quantified by measuring the average depth of the abraded groove below the original surface line (dividing the cross-sectional area of the groove by its width).

Rytömaa et al. (1988) measured the loss of tooth material after erosive exposure, using a profilometer. The technique then involved digitisation and computerisation of the analogue reading in order to produce graphs which could be zoomed and scaled as required.

Another method tried for measuring the level of mineral loss was attempted by Macpherson et al. (1991). A radiographic image of the enamel specimens was obtained and divided into five equal zones from occlusal to cervical margin. Microdensitometric analysis

was then performed at each site, and the total mineral loss of each site was calculated.

Strang et al. (1987) combined the techniques of microradiography and microdensitometry to measure percent volume mineral content of the surface of the erosion lesion, and to calculate the total mineral loss. The microradiograph of the lesion was digitised, and this digitised image was transferred to a microcomputer, on which the average mineral content profiles were calculated. The area above the microdensitometric profile was calculated to give the total mineral loss.

The surface area of the tooth surface lost through an erosive process caused by a sports-drink mixture was measured by a semquantitative method by Sorvari and Kiviranta (1988) on rats. The measurements were done with the aid of a drawing tube mounted on a stereomicroscope, and drawings of the intact and eroded areas were made. A computer-coupled graphics analyser was used to measure the areas of the intact surface and eroded enamel in square millimetres. A second aspect of analysis is the macroscopic and microscopic analysis of the remaining enamel, both in terms of surface damage caused, and in terms of histological changes resulting. Combined with this is an analysis of the hardness of the remaining surface material, to determine any changes in the physical characteristics, such as mineral density or porosity.

1.7.3(a) Quantification Techniques of the Effects of Enamel Demineralisation

Arends and ten Bosch (1992) wrote a good review article on the techniques suitable for direct and indirect mineral quantification. These techniques have been largely used for the study of carious lesions. The various techniques were discussed and they were compared with regards to their suitability for the determination of mineral content in volume percent, mineral changes and mineral distributions. In the last twenty-five years, several methods have been used for the assessment of demineralisation and remineralisation, either quantitatively or qualitatively. The techniques available included:

(a) Microradiography

(b) Iodine absorptiometry

(c) Various microhardness tests

(d) Polarised light microscopy

(e) Light-scattering

(f) Iodide permeability

(g) Wet chemical analysis

The principles of these techniques will be explained briefly.

(a) Microradiography

Microradiography is a technique which utilises X-ray absorption. The absorption of the X-rays is directly reflected in the optical density of the developed film. Densitometry can then be used to calculate the mineral content. There are three different microradiographical techniques - transverse microradiography (TMR), longitudinal microradiography (LMR), and wavelength independent microradiography (WIM).

The TMR technique allows reasonably accurate measurements. However, the disadvantages are that the sample is destroyed, phenomena less than 10μm in depth are not measured due to the finite densitometer slit width and specimen curvature, and the presence of ions with a very high absorption coefficient for X-rays can cause misinterpretation of the data. This technique has been demonstrated by various researchers, such as, Groeneveld & Arends, 1975 and Arends & ten Bosch, 1985).

The LMR and WIM are both non-destructive techniques. WIM permits the determination of the mineral content in whole teeth. Herkströter et al. (1990) have employed these two methods of measurement.

(b) Iodine absorptiometry

Photons with an energy of 27.4 keV resulting from the decay of a I^{125} source are used to irradiate longitudinal tooth sections. The amount of absorbed photon radiation is a measure of the amount of mineral per unit area. This method provides a sensitivity of measurement similar to that of LMR and has been demonstrated by Almqvist et al., 1988.

(c) Microhardness

Microhardness techniques have been used as far back as 1966 by Koulourides. A Knoop or Vickers diamond is lowered onto the sample with a given load for a given time and the indentation length produced by the diamond is determined microscopically. There are two types of hardness measurements:

(i) Surface microhardness (SMH): where the indenter load is perpendicular to the surface and encounters a non- homogeneous surface, and

(ii) Cross-section microhardness (CSMH): where the indenter load is parallel to the tissue's anatomical surface and encounters a homogeneous surface.

Arends et al. (1980) has shown with the SMH that if the indenter load is perpendicular to the flat surface, a linear relation between the Knoop indentation length and lesion depth can be found with a correlation coefficient of 0.95. In CSMH, Featherstone et al. (1983) found that the volume percentage of mineral as determined from microradiography was directly proportional to the square root of the Knoop hardness number.

Featherstone et al. (1983) reviewed in their article that Arends et al. (1979) showed a correlation between the indentation length and the lesion depth for lesions induced with acidified HEC (6% hydroxyethylene-cellulose solution in a sodium lactate-lactic acid buffer) solutions or gelatin gels. An unpublished study by Gelhard and Arends later showed that this linear relationship between indentation length and lesion depth no longer held true following remineralisation. However, the indentations in these studies were made on the outer enamel surface and therefore cannot be used to give details of the hardness changes below the

surface of the lesion. Featherstone et al. concluded that techniques which yield mineral profiles through the lesion and into the underlying sound enamel are needed.

Experiments on cross-sections were performed by Davidson et al. (1974) and these demonstrated that "the microhardness was a linear function of the local calcium content". Purdell-Lewis et al. (1976) employed the same technique and concluded that it was a very reliable technique with a standard deviation of less than 5% for enamel specimens.

Featherstone et al. (1981, 1982) also produced microhardness profiles in the same way through cross-sectioned specimens to compare changes during demineralisation and remineralisation. However, they did not realise at that time, what these changes implied quantitatively in terms of mineral loss. The details of this method of correlating microhardness data on cross-sections of the lesions with the mineral content was described by Featherstone et al. (1983) in their study and will be explained later in the discussion.

(d) Polarised light microscopy

This is a very sensitive technique for showing changes in hard tissues. Birefringence experiments can qualitatively show mineral loss and gain. Polarised light measurements can provide quantitative information on the pore volume or porosity in demineralised and remineralised enamel, and on lesion characteristics (Arends & ten Bosch, 1992).

Quantitative Polarised Light Microscopy

Changes in the optical properties of dental hard tissue are commonly measured by polarised light microscopy. Polarised light microscopy involves the passing of plane polarised light through an object which has birefringent properties. Tooth enamel, dentine and cementum have such properties, and sections of teeth split the single plane of light into two components, the phase of one being retarded in relation to the other. Quantitatively, the retardation is seen as a series of interference colours. It may also be measured quantitatively

by use of a compensator.

Hydroxyapatite, the major component in enamel, is birefringent, and extensive studies by Darling (1958), Poole et al. (1981) and Silverstone (1968) have determined the broad structure through normal enamel, and enamel in which incipient carious lesions have developed, using qualitative and quantitative polarised light microscopy in conjunction with other methods. Silverstone has used quantitative polarised light microscopy to show that the porosity volume through an incipient lesion is 1-5% at the surface zone remaining relatively mineralised until late in the carious process, up to 25% in the body of the lesion with varying numbers of centres of demineralisation so that in fact, a lesion may be rather heterogeneous in terms of mineral quantity, 2-5% in the dark zone, and 1% in the translucent zone. This compares with 0.1- 0.2% in normal enamel. However, there remains a great deal of controversy surrounding the interpretation of the quantitative method and resulting data. Theuns et al. (1993) believe Silverstone's assessment of porosity of the carious lesion substantially underestimates the real values, and found by their calculations, that the porosity in the body of an incipient enamel lesion was likely to be more in the order of 50-55%.

The only report of an attempt to estimate porosity changes in enamel through an erosive acid attack was by Silverstone (1975). He reported changes in porosity to a depth of 50μm following the treatment of enamel with 37% phosphoric acid for 60 seconds, as estimated using quantitative polarised light microscopy. Imbibition studies with the polarised light microscopy have shown that etched sound enamel is affected at three levels. There is first of all, a narrow zone of enamel that is removed by the etching. This zone is called the etched zone and is approximately 10μm in depth. The second zone is named the qualitative porous zone and is 20μm in depth. The third zone is also approximately 20μm in depth and is called the quantitative porous zone. This zone possesses a lower negative value of birefringence than adjacent sound enamel, indicating a higher degree of porosity (see Figure 3).

The method used to determine the porosity volume in enamel is based on that proposed by Darling (1958). He proposed the use of a formula developed by Wiener (1912) which related volumes of intrinsic and form birefringence in a mixed rodlet body (as in enamel)

to the refractive indices of apatite and an imbibing fluid. The basis for such calculations is discussed later.

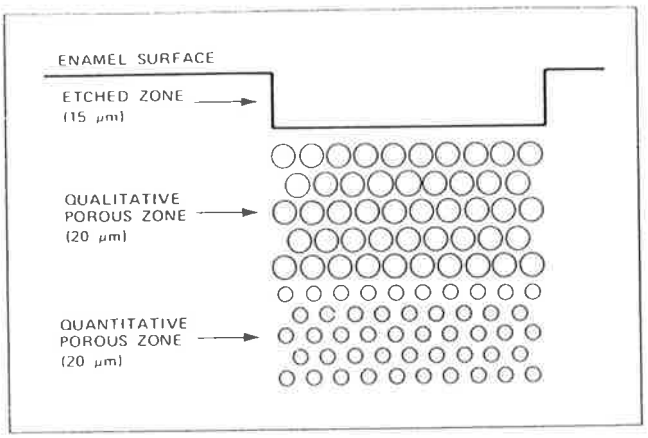


Figure 3. Acid-etching of sound enamel results in the formation of three distinct histologic zones (Silverstone et al., 1988).

(e) Light scattering

Light scattering by enamel has been found to be caused by the enamel crystallites in relation to their environments. Hence, the light conversion emerging from the specimens as a result of light scattering is expected to be related to the size and number of crystallites in a unit volume. This method has been used by ten Bosch et al., (1984) and Brinkman et al., (1988).

(f) Iodide permeability

This method of measuring changes in the permeability of tooth surfaces was introduced by Bakhos et al. (1977). These measurements are related to the pore volume of enamel. However, the relationship between permeability changes and the amount of mineral lost or gained is not yet clear.

(g) Wet chemical analysis

Arends and ten Bosch (1992) believe that the determination of calcium and/or phosphate in solutions in which a hard tissue is dissolved using an acid is, in principle, a good method for quantification of demineralisation and remineralisation of tissue. This technique is destructive and only a very large mineral loss or gain is measurable.

Among these, other methods have been used to obtain information on demineralisation and remineralisation. Scanning electron microscopy however, provides only qualitative data. Profilometric analysis has been used by Meurman et al. (1990) to examine erosion changes induced by sports drinks. The procedure of digital image analysis (DIA) has been tried by Mistry and Grenby (1993) for recording and assessing erosive changes on teeth. The authors concluded that, "The system provided a direct and highly sensitive means of quantifying the erosion of dental hard tissues."

Other methods recently explored include confocal laser scanning microscopy (Edgar et al., 1989), laser-induced fluorescence (Sundström et al., 1985), and ultrasound microscopy. The advantage of these methods is the ability to carry out the measurements without having to destroy the specimens. These are very promising areas of future research.

The methods of analysis selected for use in this study will be discussed in a later chapter.

1.7.3 (b) Etching Patterns Examined With Scanning Electron Microscopy Technique.

Three etching patterns have been described by Silverstone (1975) for sound enamel that has been exposed to phosphoric acid solutions and viewed with the scanning electron microscopy technique.

The type 1 pattern reveals a "generalised roughening of the enamel surface, but with a distinct pattern in which the prism cores have been lost preferentially, leaving the prism peripheries relatively intact". In the type 2 etching pattern, there appears to be preferential loss or damage of the prism peripheries. The type 3 pattern is one in which the etched region appears to demonstrate neither type 1 nor type 2 etching patterns, that is to say that, "this pattern may display a surface topography which cannot be related to surface morphology".

1.8 CONTROL OF EROSION

Only the dental means of control of erosion will be discussed, excluding therefore, the medical means of control of some of the conditions associated with erosion.

The obvious, and often successful way to protect human teeth against erosion by acids is to remove the acid challenge (Featherstone, 1989). However, this is most often easier said than done, as in many cases, a behavioural change needs to be achieved.

The ultimate aim in the control of dental erosion is the restoration of balance. If the erosive challenge is severe and frequent, the body's natural protection afforded by saliva is inadequate, but if the severity of the challenge, or its frequency can be reduced, then balance may be regained (Featherstone, 1989). Featherstone further adds that if fluoride is present at the correct concentration at the right time, it can enhance repair before the damage has gone too far, and the repaired mineral will be more resistant to subsequent acid attack just as it is in dental caries.

The underlying cause of the dental erosion must be determined. This can usually be arrived at through a thorough and carefully taken history of the patient. Many of the erosive factors can be controlled, such as diet and medicaments. However, in cases where erosion is the result of habitual regurgitation of gastric contents, control is not easily achieved. Other factors that may exacerbate erosion may be present and thus need to be taken into consideration, for example, Xerostomia.

The means of control of dental erosion are not new. Methods of control were suggested by Eccles et al. (1974) who stated that preventive measures generally recommended for dental erosion include topical application of fluoride, restriction of food and drink likely to produce erosion, modification of the method of consumption of food and drink, such as, cutting up fruit and drinking juice through a straw, and the use of neutral or alkaline mouthwashes immediately following the ingestion of fruit. Hay et al. (1962) also recommended the possibility of modifying commercially prepared beverages by the addition of fluoride, calcium, and phosphate to render them less harmful.

The literature on the effects of fluoride on the remineralisation of enamel is copious. It is generally believed that fluoride "accelerates the (initial) rates of lesion mineral accretion, regardless of the types of enamel defects studied, being either softened enamel (Koulourides et al., 1961; Koulourides & Reed, 1964; Feagin & Jeansonne, 1973) or sub-surface lesions (Ten Cate & Arends, 1977; Silverstone et al., 1981; Featherstone et al., 1981)". The caries preventive effects of fluoride have long been recognised and accepted. Literature reports that fluoride in low concentrations is effective in promoting mineral deposition and inhibition of mineral dissolution. In relation to caries, Larsen (1976, 1977) reported from a rat caries study that structurally incorporated fluoride was considerably less effective than free fluoride. Ten Cate (1990) concurred with this from his study and stated that, " lesion formation was significantly inhibited by the introduction of low levels of fluoride in the demineralisation environment. He also concluded that, "the real importance of the role of fluoride in the liquid phase on de- and remineralisation has only recently been recognised".

There are two methods most commonly described in the literature for controlling dental erosion.

1. Rinsing with an antacid solution, or sucking an antacid tablet immediately after an erosive episode has occurred.
2. Using a neutral fluoride mouthrinse immediately following an acidic attack.

Both methods of control can be combined. McIntyre (1992) states that theoretically, the neutral fluoride mouthrinse should come first to initiate the process of remineralisation by allowing the fluoride to be incorporated more deeply into the softened tooth surface. This should then be quickly followed by an antacid mouthrinse or tablet. The alkalinity of the antacid continues any remineralisation that can occur.

It is important to realise that fluoride may not totally prevent severe erosion from occurring. In a situation where the pH is below 4.5, even fluorapatite will be dissolved. However, fluoride should assist by reducing the severity of the attack.

Other means of gaining further protection intraorally have been suggested. At the Adelaide Dental Hospital, patients are advised not to brush their teeth for at least one hour following an erosive episode. In cases where there appears to be a frequently acidic environment, patients are recommended to use a concentrated neutral fluoride gel in the evening before they retire to bed. It has also been suggested for patients where periodontal disease is not a complicating factor, to leave the calculus covering the exposed lingual root surfaces to serve as a natural buffering barrier against the erosion (McIntyre, 1990).

Saliva is believed to be a major modifying factor in erosion (Bevenius, L'Estrange & Angmar-Månsson, 1988). Mannerberg (1961), Frostell (1973) and Bevenius et al. (1990) suggested that chairside evaluation of salivary parameters in erosion or erosion-susceptible patients would be valuable in determining the method of control. Chairside kits for assessing caries susceptibility have already been developed in Scandinavia. A chairside colourimetric test kit known as the Dentobuff (Frostell, 1980) can now be used to assess salivary flow rate and buffer capacity of stimulated saliva samples (Bevenius & L'Estrange, 1990). In a pilot study by Bevenius and L'Estrange (1990) on the use of chairside evaluation of salivary parameters in a group of patients with excessive toothwear, using the Dentobuff kit, their results indicated that, "impaired salivary function may be common in patients presenting with tooth surface loss....The corollary may also be true, that is, patients with salivary dysfunction may risk developing tooth surface loss".

(iii) A selection of methods to estimate volume loss.

(iv) Estimations of calcium loss with time, to see if these correlate with overall volume loss.

(b) The density of the residual lesion surface, to determine whether it has been left in a severely weakened state.

2. (A) Develop modifications of the "continuous erosion" model of endogenous erosion, in order to explore ways in which it may:

(a) More clearly simulate clinical erosion.

(b) Permit the testing of the ability of topical application of 1.23% APF Gel to achieve inhibition of the erosion process using these in vitro" test systems.

(B) Explore the potential of the 1.23% APF Gel, when applied in a variety of ways to inhibit erosive demineralisation of enamel, in terms of volume, depth and pattern of enamel surface loss.

(C) Analyse the residual surface "hardness" and "porosity" level in both control and test experiments, in order to assess whether the 1.23% APF Gel application results in altered density properties.

CHAPTER 3

ANALYSIS OF OUTCOMES OF THE EROSIVE DEMINERALISATION OF ENAMEL, USING THE "CONTINUOUS EROSION" METHOD OF THE ENDOGENOUS EROSION MODEL

3.1 OBJECTIVES

The "in vitro" model of endogenous erosion employed was that developed by Hunt et al. (1992). This involved the use of a test erosive solution which consisted of 0.06M hydrochloric acid and 2.2mM calcium hydrogen phosphate, with crowns of extracted human teeth.

The objectives were to test:

(a) The pattern, including volume and depth of enamel loss, using,

  (i) Photographic analysis of surfaces prior to, and following erosion.

  (ii) Scanning electron microscopic anaysis of the lesion to explore the nature of the pattern of mineral loss at the residual surface.

  (iii) A selection of methods to estimate volume loss.

  (iv) Estimation of calcium loss with time, to see if these results correlate with overall volume loss.

(b) The density of the residual lesion surface, to determine whether it has been left in a severely weakened state.

3.2 MATERIALS and METHODS

3.2.1 Specimen Preparation

Extracted human premolar and molar teeth were used for this experiment. Following extraction, they were stored in thymol water. Only intact teeth were selected to be cleaned with the ultrasonic scaler with the buccal and lingual surfaces of these teeth to be used. The teeth were hemisectioned with a diamond saw. To achieve this, dental sticky wax was used to mount the teeth onto a Perspex block, being careful not to allow any wax to cover the enamel surface which will be exposed to the test erosive solution. The Perspex block was clamped onto the bed of the diamond saw. The specimens were slowly guided through the motorised saw. Windows measuring 3mm x 3mm were marked onto the enamel surface using a fine lead pencil. A coating of waterproof nail varnish (California Colours, burgundy) was applied to all surfaces of the tooth with the exception of the 3mm x 3mm window.

A photographic record of the enamel surface of each specimen prior to erosive challenge was obtained with black and white photography and with coloured photography (Agfacolour Portrait 160) using the Wild Photomakroskop M400 microscope (see Figure 4).

3.2.2 Preparation of the Test Erosive Solution

As stated previously, the erosive agent to be used in this model consisted of a solution of 0.06M hydrochloric acid, to which was added 2.2mM calcium hydrogen phosphate to simulate phosphate ion levels present in human saliva. The erosive solution was prepared as required in one litre quantities as described below.

A one-litre volumetric flask was half-filled with double de-ionised water (DDW). With a pipette, 5.24 millilitres of concentrated hydrochloric acid was added to the flask. Double de-ionised water was then added to make up to one litre of solution and to result in a 0.06M hydrochloric acid solution. This was mixed well by shaking. Using a four decimal place balance, 0.378 grams of calcium hydrogen phosphate was measured out into a

(a) Interference microscopy.

(b) Pre- and post-impression taking and model construction.

(c) Hemisectioning to determine sample profile and depth of enamel loss.

(d) Wax replacement of lost enamel to determine volume loss.

Method (c) and (d) were selected. The second option still left the difficulty of estimating the volume of enamel lost. This could however, have been achieved by using an elastomer. Kelly and Smith (1988) made silicone rubber impressions of the samples at the beginning of the experiment, filled them with a contrasting colour of the impression material and sectioned them through the long axis. Measurements were then made using a travelling microscope. The other methods were deemed too complicated, difficult and time consuming while providing little useful extra information. Another method combining both options (b) and (d) was also tried on a small sample to test the efficacy of such a technique. A composite resin material was used to adapt a key to the surface of the tooth prior to inducing the erosive lesion. A light-bodied elastomer was then injected into the key and placed over the tooth to allow the elastomer to replace the lost enamel surface. By calculating the specific gravity of the elastomer, and weighing the elastomer which replaced the lesion, the volume loss of enamel could then be calculated. This proved to be too difficult for the small amount of tooth substance loss we were dealing with.

The resulting erosion lesions varied in the degree of depth loss, and representative specimens were selected for this analysis (see Figure 5). The method employed involved the use of a soft dental wax (Kerr's blue inlay casting wax) to recontour the tooth surface to its original profile using the margins of the window as reference points (see Figure 6). Five wax-ups were completed for four selected specimens at the completion of the experiment. The wax patterns were removed and weighed on a four-decimal place balance. Repeat wax-ups were made, removed, and weighed for the individual teeth. The statistics were calculated using the Statview programme. A standard deviation of only 8.2526×10^{-5}

was calculated from the weights of the wax patterns for single samples, and hence this simple method was employed.

In the final calculations, three wax-ups for each of the eight specimens (one selected as representative of each time exposure period) were completed, withdrawn and weighed (see Figure 7). The specific gravity (Mass/Volume) of the wax had been previously calculated by determining the volume of the stick of wax when immersed in alcohol, and weight in air using a two-decimal place balance. These measurements were repeated three times and the mean and the difference between them calculated to give the volume of alcohol displaced. The specific gravity or density values were then inserted into the formula (Volume = Mass/Density) to calculate the volume of loss of enamel in each of the test specimens.

Figure 4. Example of a prepared specimen prior to exposure to erosive solution (x20 magnification).

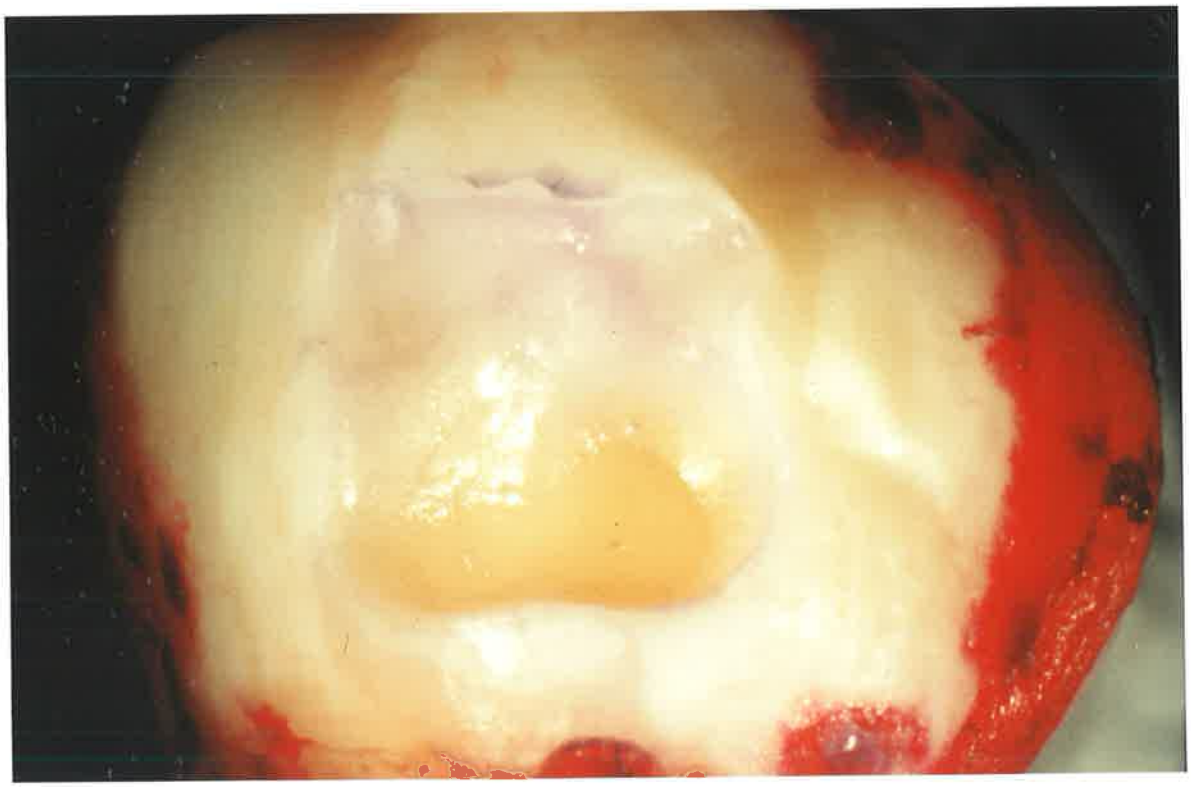


Figure 5. Example of a specimen showing depth of loss at the completion of the experimental procedure.

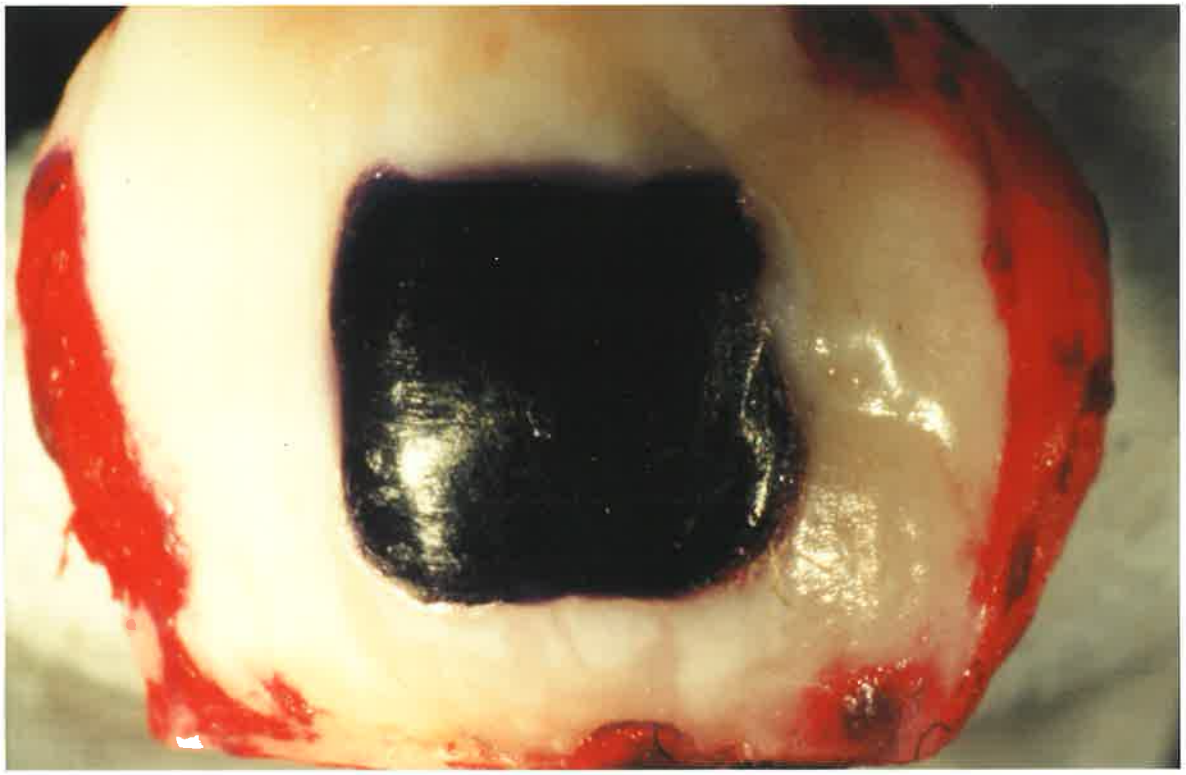


Figure 6. Wax replacement of surface loss for volume loss estimation.

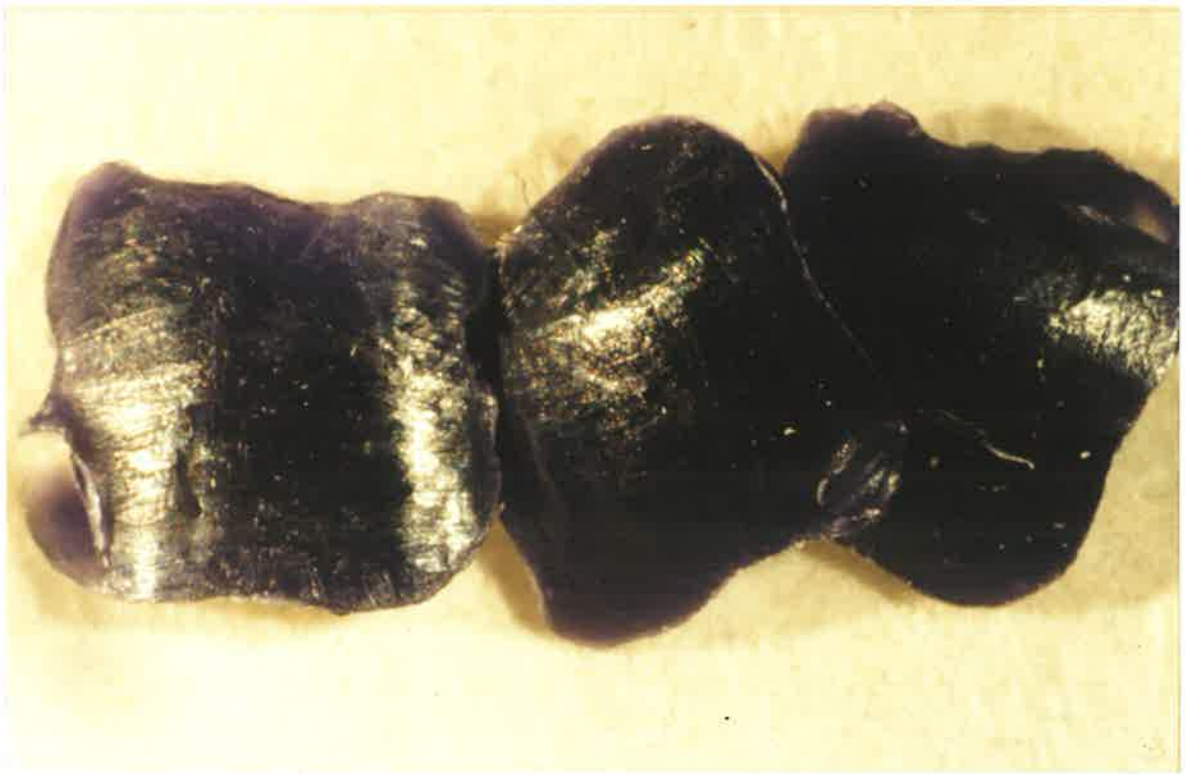


Figure 7. Three completed wax patterns for the one specimen.

3.2.4(d) Estimations of Calcium Loss with Time

The test solutions were determined by flame atomic absorption spectroscopy for calcium. A Varian Techtron AA-6D atomic absorption spectrophotometer (Geology Department, University of Adelaide) equipped with hydrogen lamp background correction was used for the determinations. However, background correction was not required for this work. Details of the instrumentation are given in the table below.

Instrument:	Varian Techron AA-6D
Background correction:	Not required
Hollow Cathode Lamp:	Calcium
Lamp Current:	5 mA
Spectral Band Pass:	0.2nm
Wavelength:	422.7nm
Flame Used:	Air-Acetylene (oxidising)
Burner Type:	Air-Acetylene (10cm)

Thirty-seven samples of the test erosive solution following the completion of the experiment were selected from the initial forty samples on the basis that only the exposed window area was eroded by the test erosive solution, to be analysed. As well as this, three specimen jars each containing forty millilitres of the fresh test erosive solution were also analysed as a control. The manufacturer's standard operating conditions were used for instrument set-up. The instrument was further optimised for burner position, aspiration rates, and flame conditions while aspirating the calcium standard solutions.

A range of calcium standard solutions were prepared for instrument calibration. The standards included solutions of 0.06M hydrochloric acid (HCl) to which was added calcium hydrogen phosphate ($CaHPO_4$) powder to form 2.2mM, 5mM, 10mM and 20mM standards. The basic solution of 0.06M HCl was made up and to which was added 0.378

grams, 0.86 grams, 1.72 grams and 3.44 grams of $CaHPO_4$ to prepare 2.2mM, 5mM, 10mM, and 20mM standards respectively.

After checking that the meter read zero absorbance when spraying double de-ionised water (DDW), the standard solutions were sprayed in order of increasing concentration and the resulting absorbance values read. The control and sample solutions were then sprayed followed by the standards again. This was repeated to provide three absorbance readings in total for each of the samples. The results were then averaged and entered into the computer programme to calculate the solution concentrations. The amount of calcium dissolved from the tooth into the test erosive solution was calculated from the difference between the concentrations of the experimental specimens and the control specimen.

In addition, the equivalent amount of hydroxyapatite lost from the tooth enamel was calculated, by dividing the amount of calcium lost in milligrams, by the molecular weight of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, of which, the calcium component comprises 4/10th of the molecular structure and weight (that is, dividing amount of calcium lost by 4/10). Since enamel consists of approximately 96% hydroxyapatite by weight, the equivalent enamel loss was calculated by multiplying the amount of hydroxyapatite loss previously determined, by the fraction of 100/96 (see Table 2 in chapter 4 of results). Hence, by knowing the specific density of enamel, the volume of enamel lost can be deduced from these values (see Table 4 in chapter 4 of results). The specific density of enamel is 2.8 (Orban, 1962). The volume loss calculated from the wax patterns and volume loss of enamel calculated from the calcium loss values, are later analysed to determine if there is any correlation between the two.

3.2.4(e) Profile of Changes in Hardness Across the Erosive Lesions

Three representative specimens were selected from each time category. The specimens were cleaned in an araldite/acetone mixture, followed by the infiltration procedure in a fresh solution of 100% araldite. A small-cubed ice-tray was used for embedding the specimens in an araldite mixture of one hundred millilitres of Araldite M and forty millilitres of Hardener HY 5160. The specimens were positioned in the ice-tray and the moulds were then

filled with the araldite mixture and allowed to polymerise in a 60°C oven for at least forty-eight hours.

When the araldite embedding was completed, each specimen was removed from its mould. The specimens were then hemisectioned vertically (occluso-gingivally) through the center of the erosive lesion using the Leitz saw microtome 1600 machine. One half was put aside to be further sectioned for polarised light microscopy and the other half was embedded and subjected to hardness testing.

The other half was re-embedded in the same araldite mixture in a flange form mould for hardness testing. The crown was embedded so that the cut section of the lesion was exposed. Each mould contained three specimens and each specimen was accordingly named. Following removal from the moulds, the cut surface of the specimens were serially polished on a Struers Abramin polishing machine using a series of 320-grade, 800-grade, and 1200-grade silicon carbide paper, followed by 9μm, 3μm and 1μm diamond abrasive spray (Struers, DP-suspension) on Struers polishing cloth (see Figure 8).

The hardness profile of each erosive lesion located within the window was evaluated. Six indentations were made with the long axis of the diamond parallel to the outer surface of the erosive lesion at 25μm intervals into the underlying enamel. This continued to 150μm within the enamel. A Knoop diamond on a Leitz Durimet small hardness tester was used under a 50 gram load. This was performed by the one operator. From the length of each diamond indentation, the Knoop hardness values were calculated.

$$\text{Knoop Hardness Number (KHN)} = \frac{14230 \times \text{force}}{(\text{length of indentation})}$$

The Knoop hardness numbers (calculated from the indentation lengths) were then converted to volume percent mineral using the empirically derived formula described by Featherstone et al. (1983).

$$\text{Volume \% Mineral} = 4.3\sqrt{KHN} + 11.3$$

This provided an indication of the degree of mineral loss across the depth of the erosive lesion.

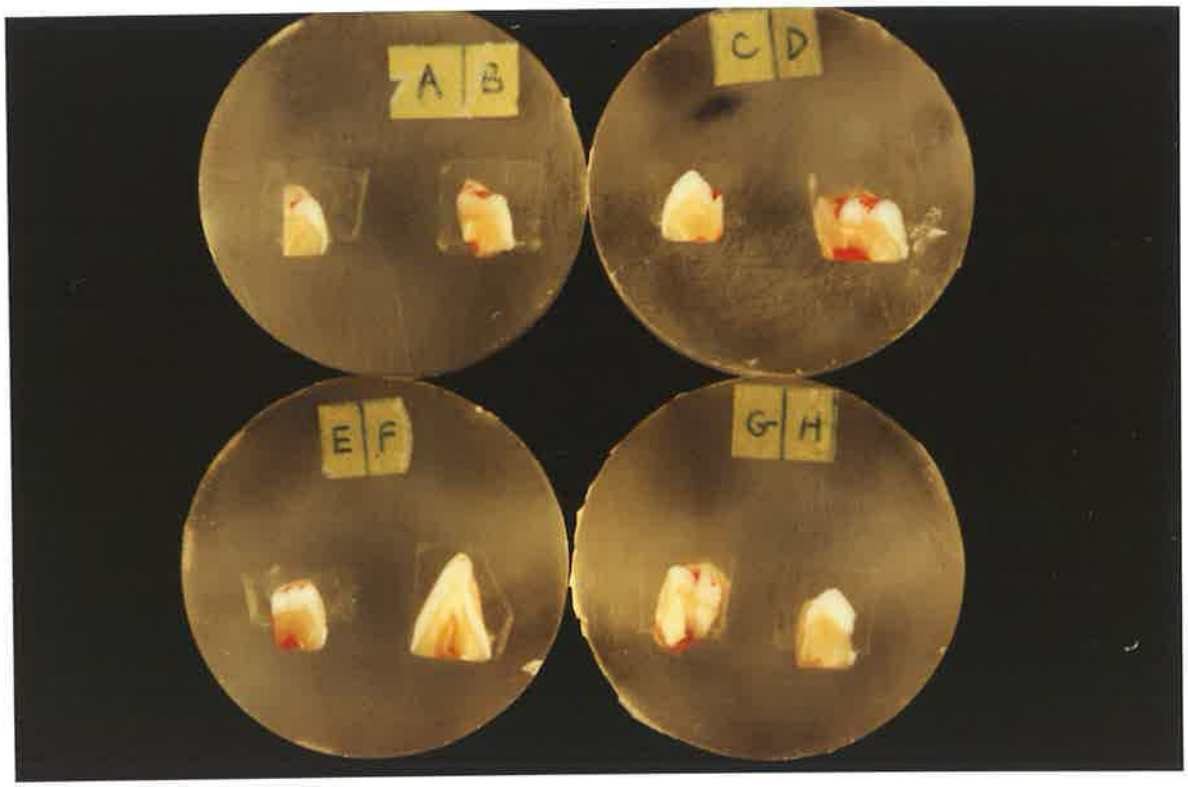


Figure 8. Prepared specimens from the "continuous erosion model" for microhardness testing.

CHAPTER 4

ANALYSIS OF OUTCOMES OF THE EROSIVE DEMINERALISATION OF ENAMEL, USING THE "CONTINUOUS EROSION" METHOD OF THE ENDOGENOUS EROSION MODEL

RESULTS

4.1 Visual and Photographic Assessment of Erosive Changes

Following the varying time exposures to the test erosive solution, the specimens demonstrated eroded surfaces which had a white, chalky appearance. The surfaces did not have a smooth, glassy topography but there were white islands of precipitate scattered throughout. In addition, the erosion surface was not even in depth. There was increased loss of tooth structure with increased time exposure to the test erosive solution.

The erosive process using this model demonstrated various stages of change with increasing time exposure to the erosive agent. A description of the resulting erosive surfaces representative of each category will be summarised in the table on the following page.

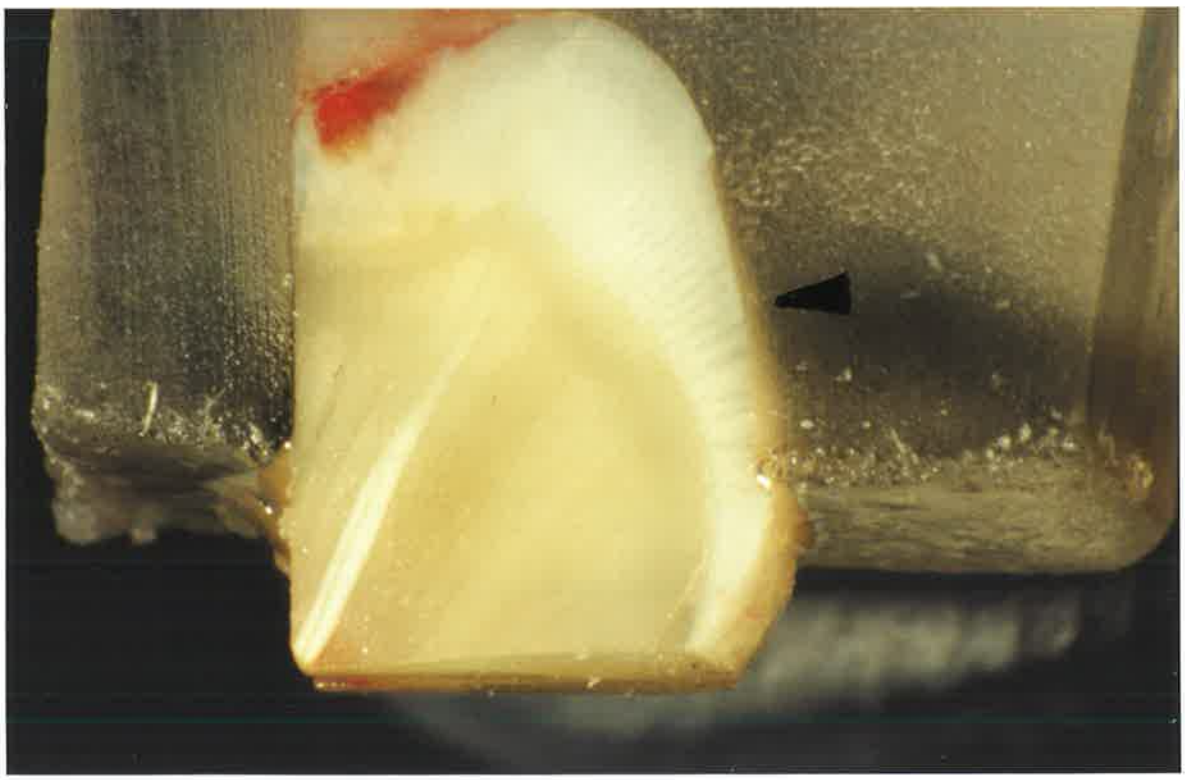
Specimen	Description of changes
A (2 hours)	Irregular surface following erosive process. Opaque surface, raised islands of precipitates. Less than 0.5mm depth loss when viewed under stereomicroscope (see Figure 9).
B (4 hours)	As above, but with increased bulk of mineral loss of approximately 0.5mm (see Figure 10).
C (8 hours)	Opaque surface which has a more even appearance. Mineral loss occurring under surrounding varnish resulting in caving in of varnish at margins of window (see Figure 11).
D (12 hours)	More pronounced bulk loss of mineral and caving in of surrounding varnish. Loss of enamel to level of DEJ at various parts exposing yellow colour of the dentine . Surface appears to have lost the opacity and seems more translucent (see Figure 12).
E (18 hours)	As above, but to a greater degree, with obvious erosion of dentine. Glassy appearance of erosive surface with still uneven loss of mineral (see Figure 13).
F (24 hours)	As above, but with greater bulk mineral loss (see Figure 14).

G (36 hours)	As above, but opaque appearance of surface present again (see Figure 15).
H (48 hours)	Greater surface of dentine exposure with severe depth loss and caving in of marginal varnish (see Figure 16).

Figure 9. Representative specimen following exposure to test solution for 2 hours

(a) Showing irregular surface of erosion lesion.

(b) Cross-sectional view showing depth of erosive lesion.



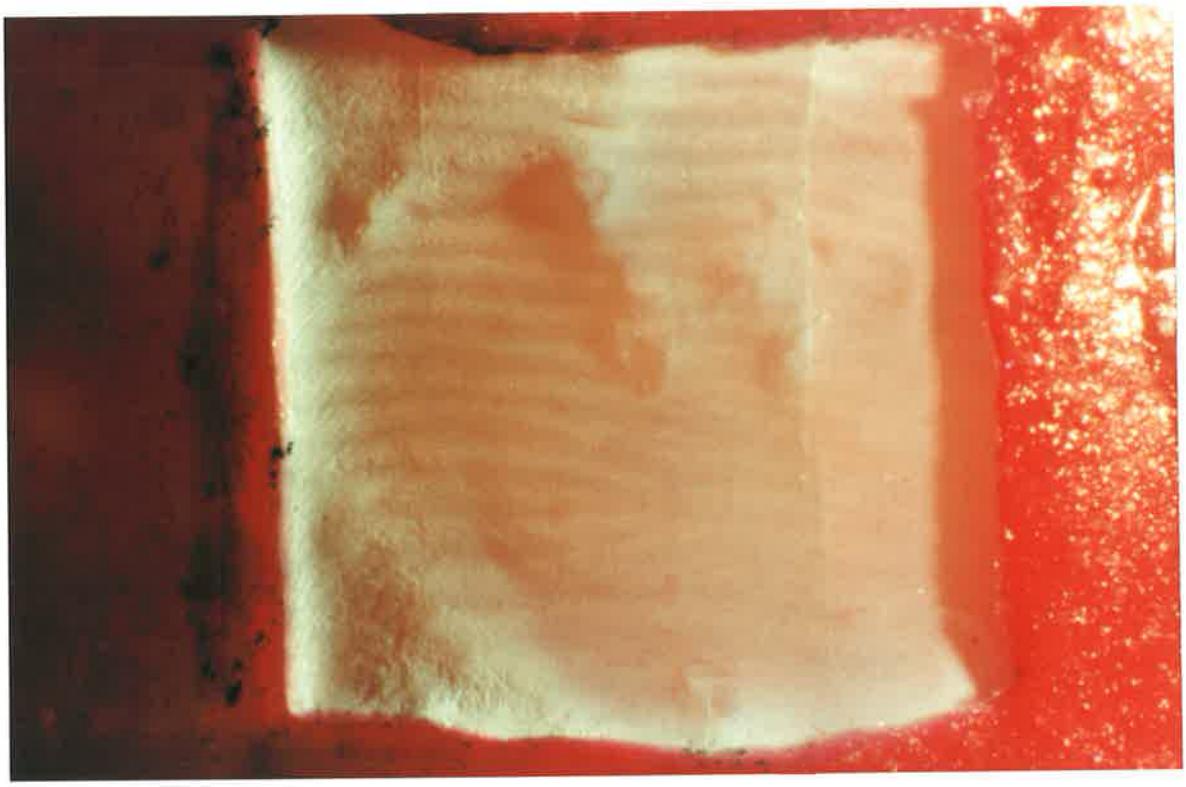


Figure 10. Representative specimen following exposure to test solution for 4 hours

(a) View of surface of erosive lesion.

(b) Cross-sectional view showing depth of erosive lesion.

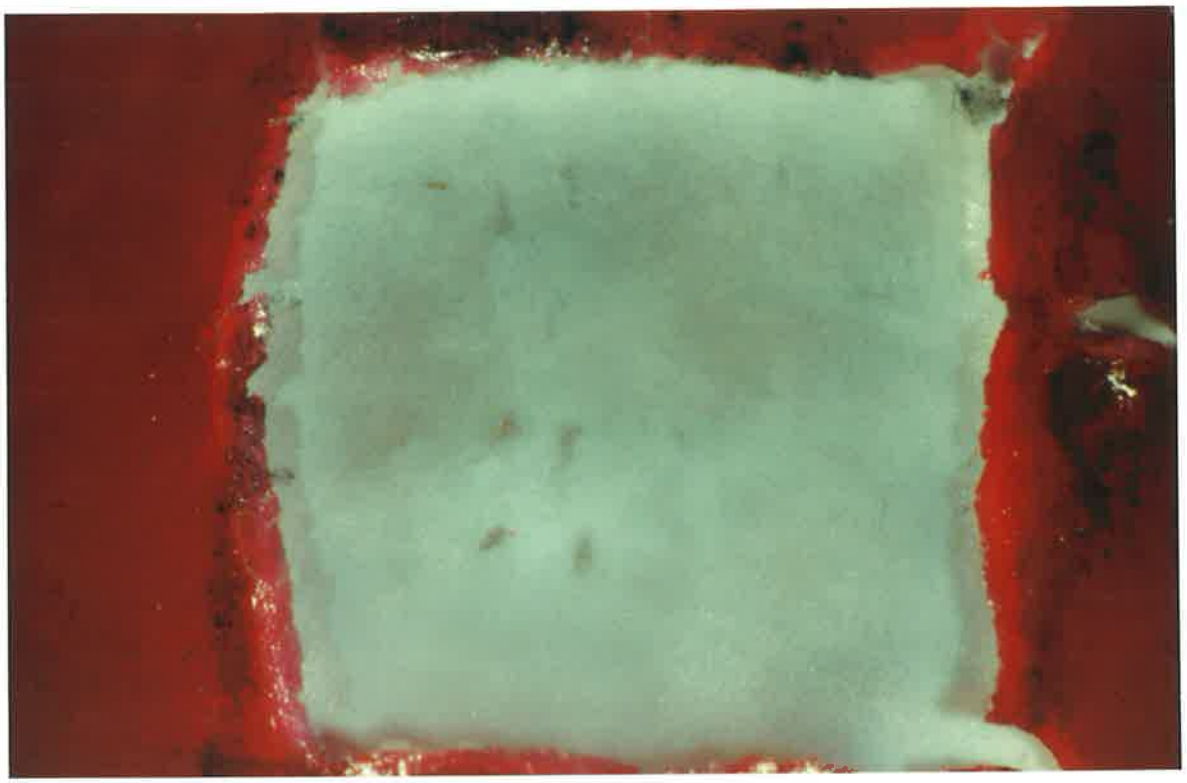
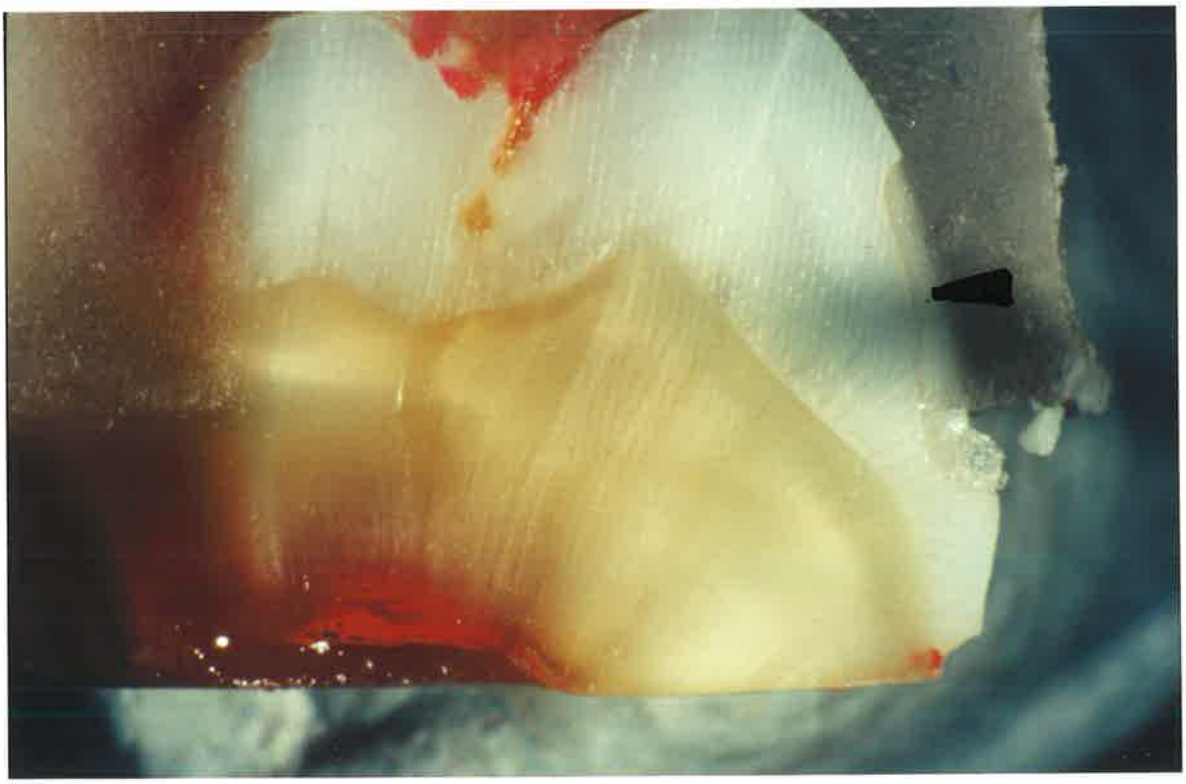


Figure 11. Representative specimen following exposure to test solution for 8 hours

(a) View of surface of erosive lesion.

(b) Cross-sectional view showing depth of erosive lesion.



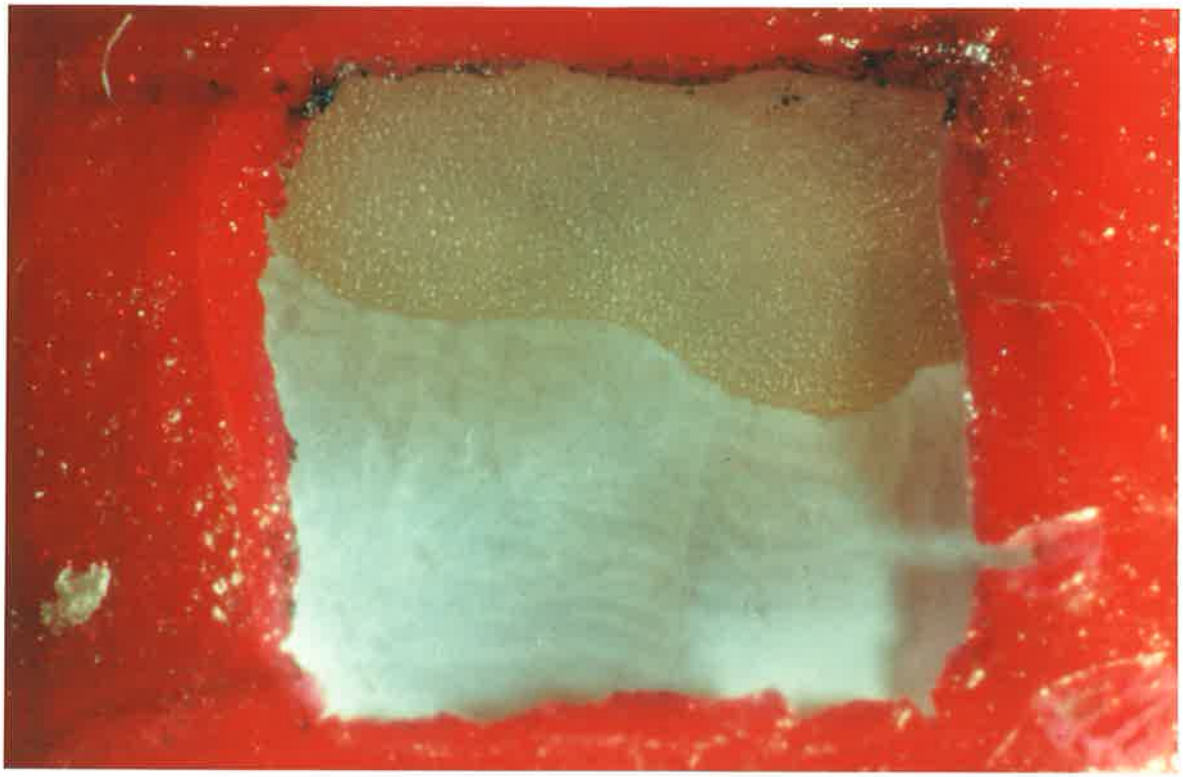
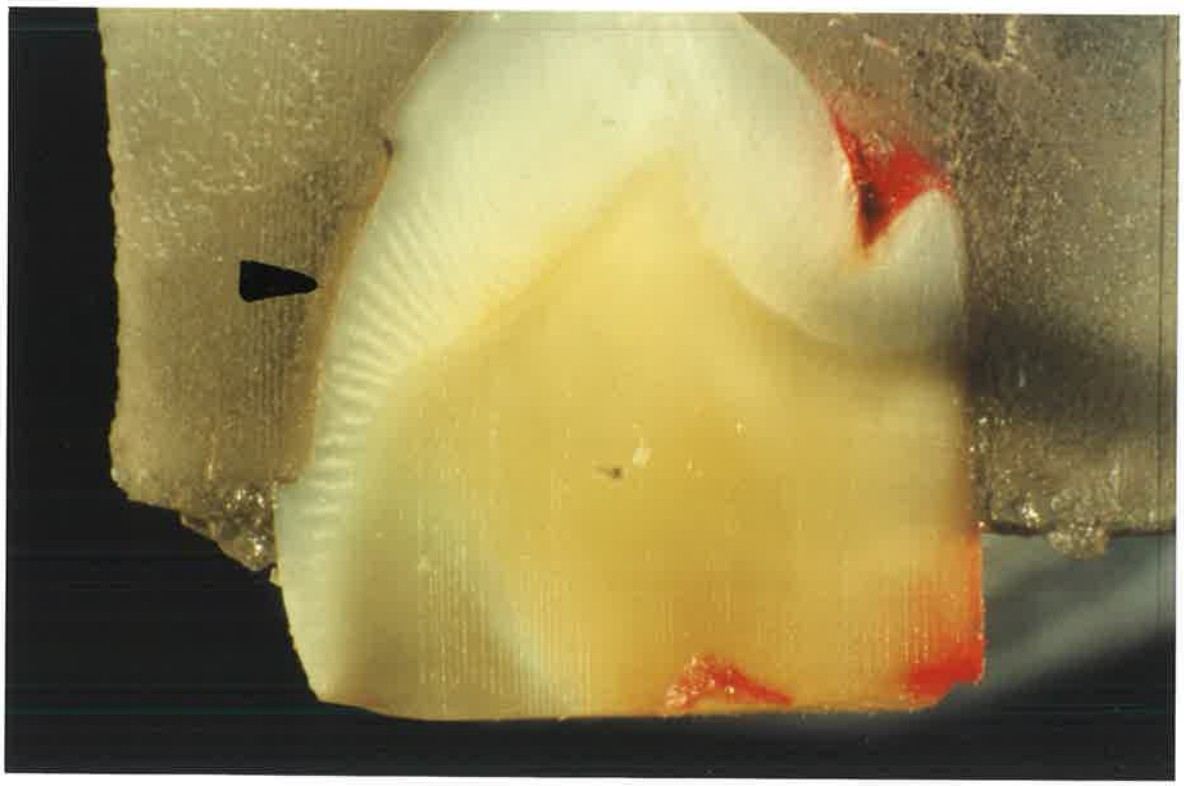


Figure 12. Representative specimen following exposure to test solution for 12 hours (a) View of surface of erosive lesion.

(b) Cross-sectional view showing depth of erosive lesion.



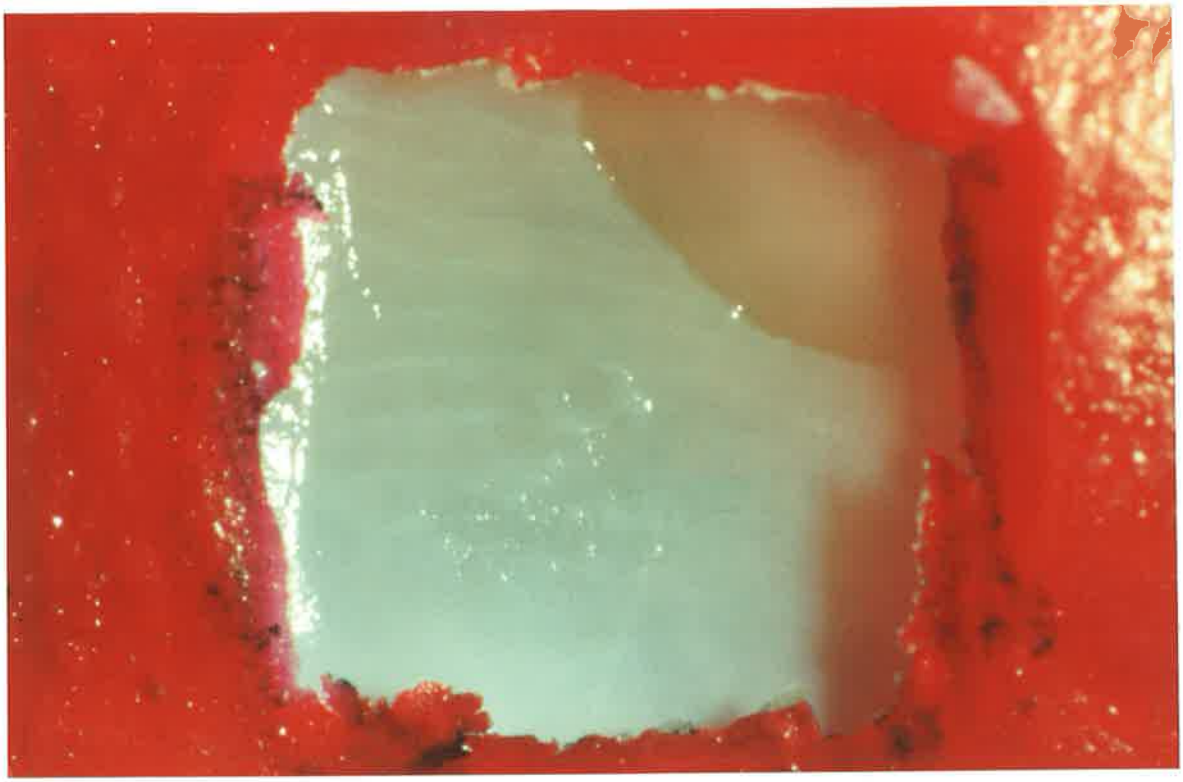
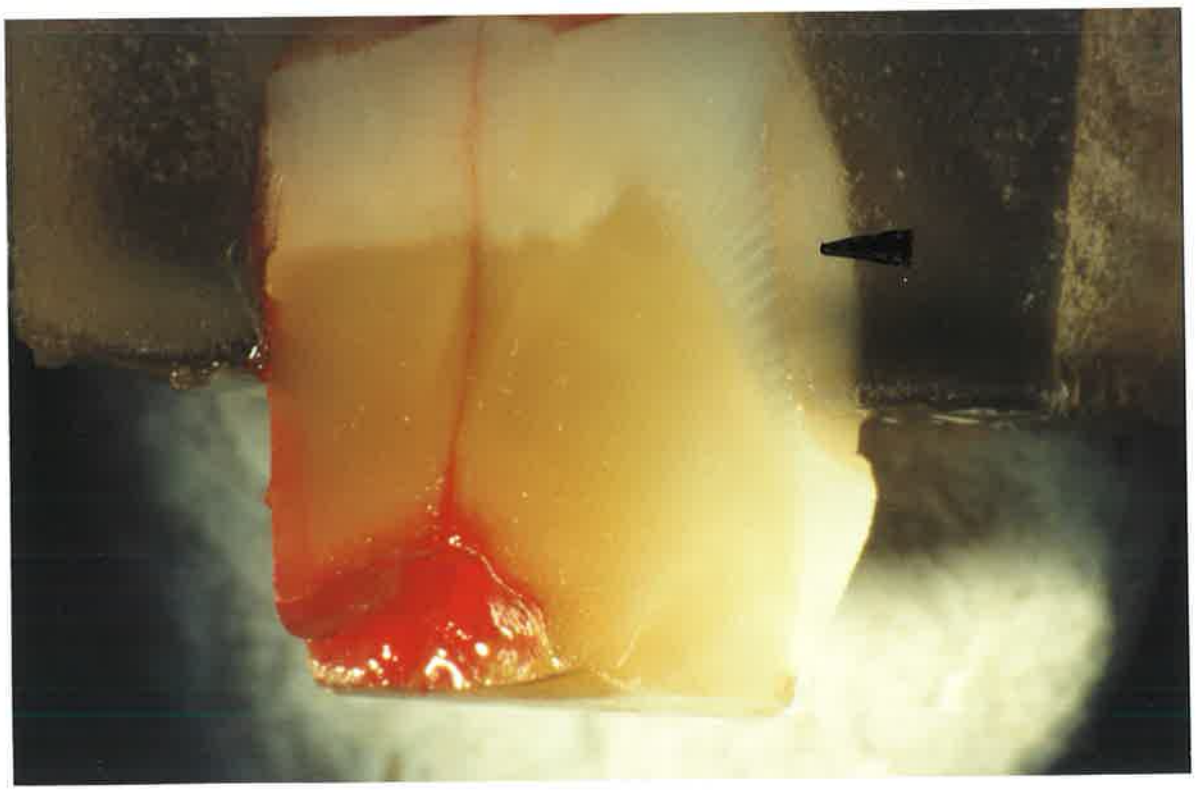


Figure 13. Representative specimen following exposure to test solution for 18 hours (a) View of surface of erosive lesion.

(b) Cross-sectional view showing depth of erosive lesion.



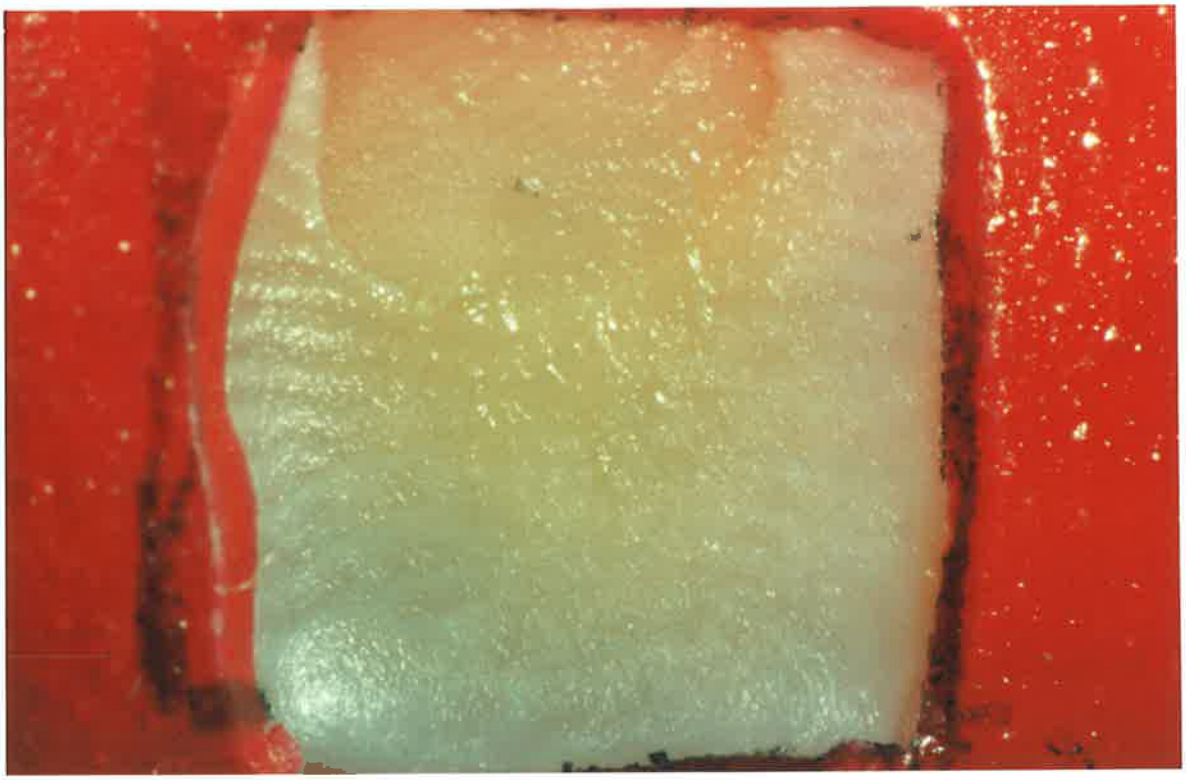


Figure 14. Representative specimen following exposure to test solution for 24 hours (a) View of surface of erosive lesion.

(b) Cross-sectional view showing depth of erosive lesion.

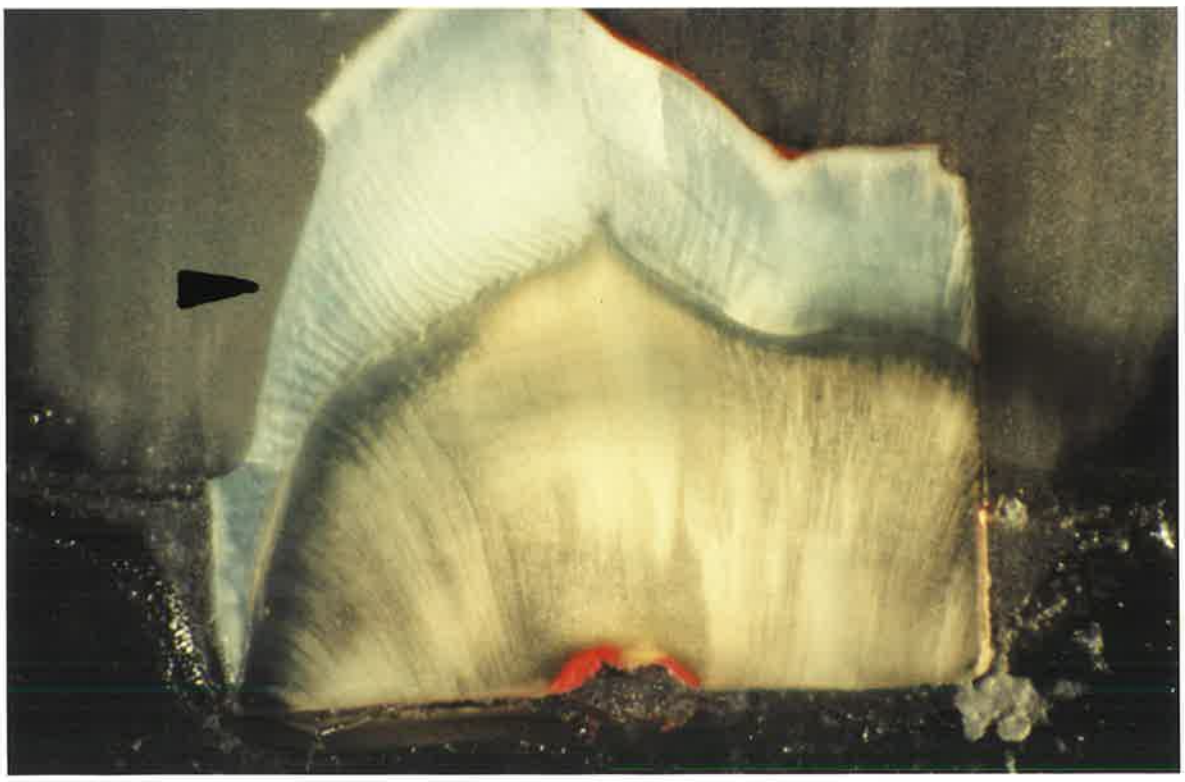


Figure 15. Representative specimen following exposure to test solution for 36 hours (a) View of surface of erosive lesion.
(b) Cross-sectional view showing depth of erosive lesion.

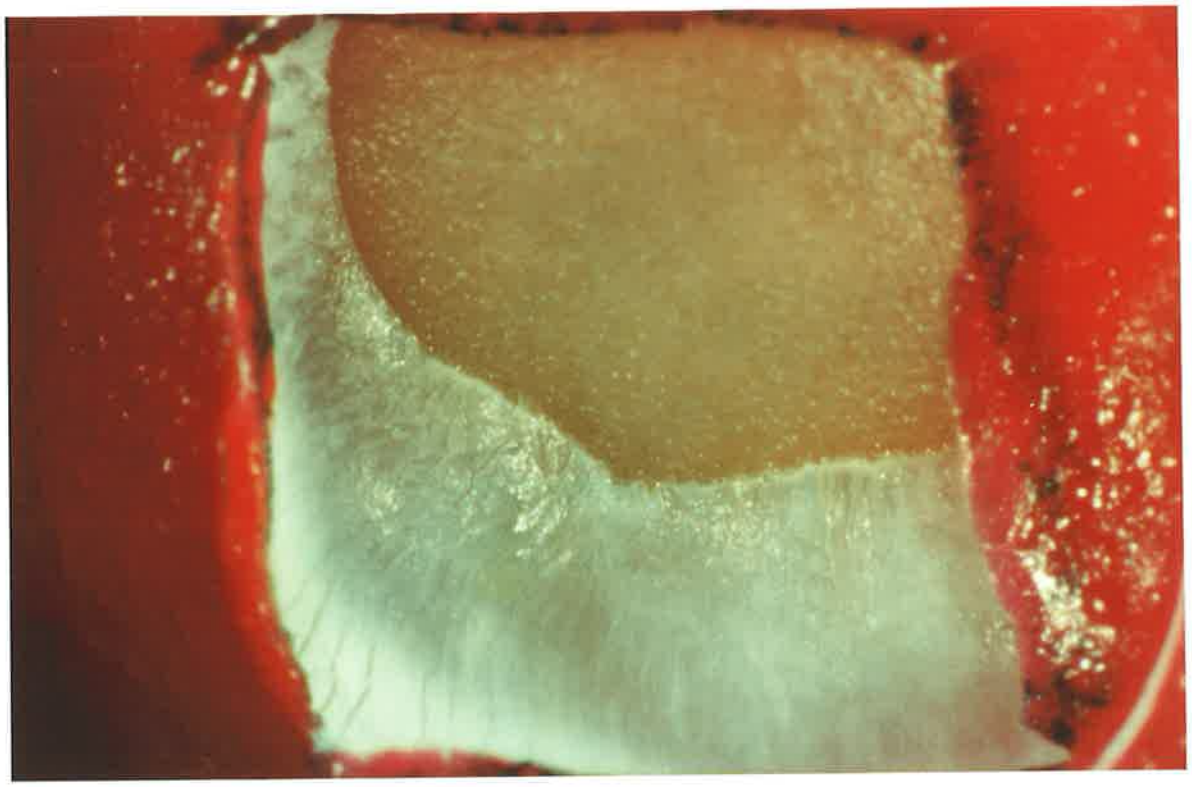
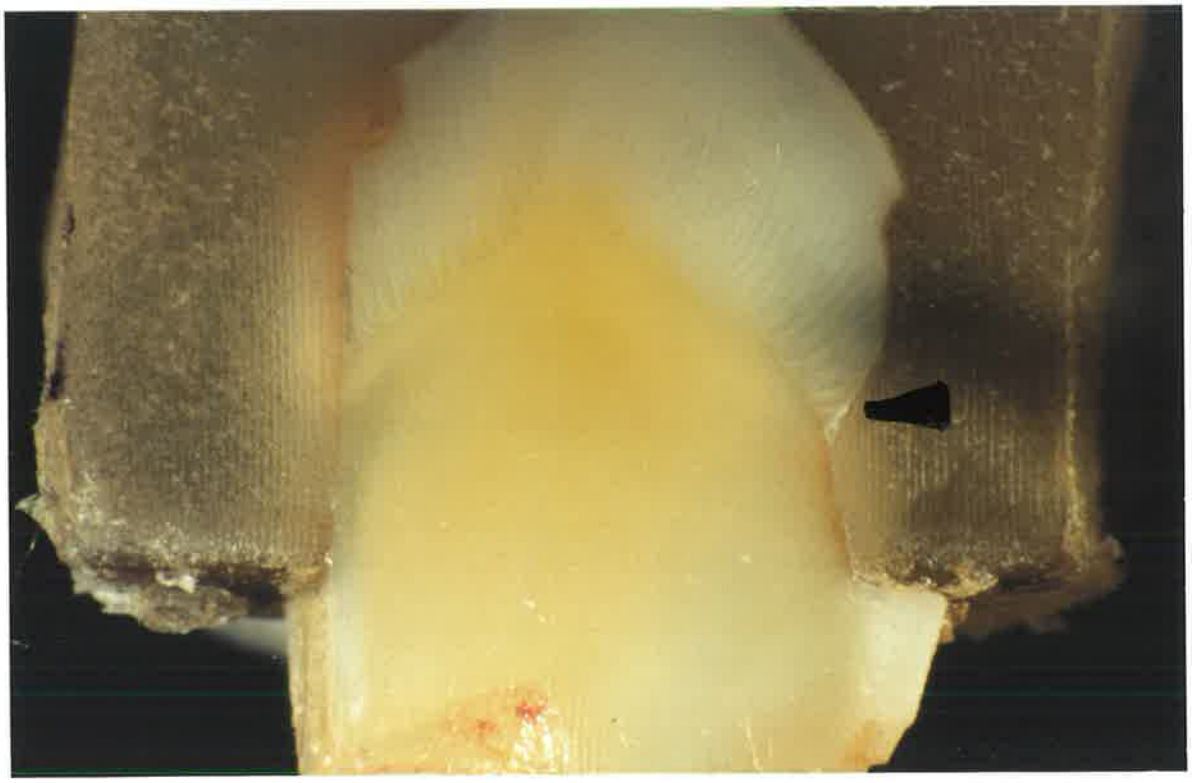


Figure 16. Representative specimen following exposure to test solution for 48 hours (a) View of surface of erosive lesion.

(b) Cross-sectional view showing depth of erosive lesion.

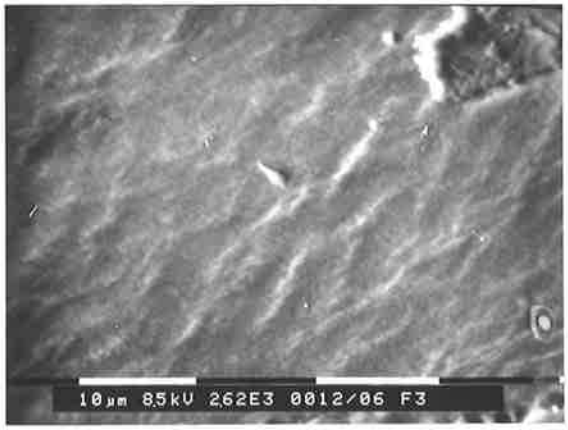


4.2 Scanning Electron Microscopy Analysis

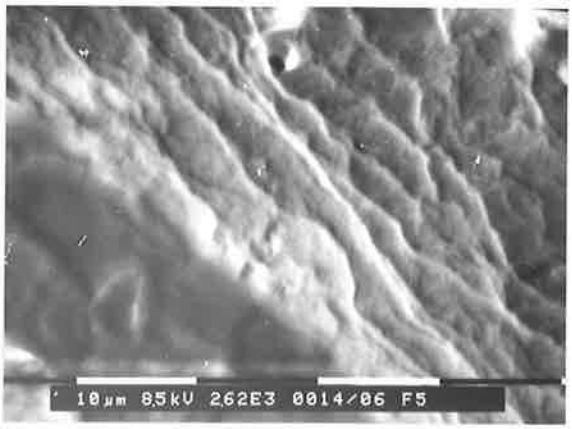
Under the scanning electron microscope, the prism structure of dental enamel could be viewed. There were some difficulties in obtaining a clear picture of the eroded surface in the specimens which had been exposed to the test erosive solution for a greater length of time. This was due to increased charging which seemed to be unalterable in our aim to obtain views of the eroded surfaces at the same magnification throughout.

With prolonged exposure of the specimens to the erosive solution, there was increase in loss of definition in the prism structure (prism core and periphery) of the enamel. This was seen in the specimens exposed to the acid for periods of 18, 24, 36 and 48 hours (see Figure 17). The eroded surfaces of these demonstrated a smeared surface with ridge-like appearance. In the specimens from the time exposure categories of 2, 4, 8 and 12 hours, the tubular structure of enamel was still evident, with the two latter category specimens showing not only intra-prismatic loss of tooth structure, but also inter-prismatic destruction (see Figures 18 to 21). Intra-prismatic destruction of the tubules preceded the inter-prismatic destruction, that is, longer acid immersion period resulted in dissolution of enamel prism cores followed by prism peripheries.

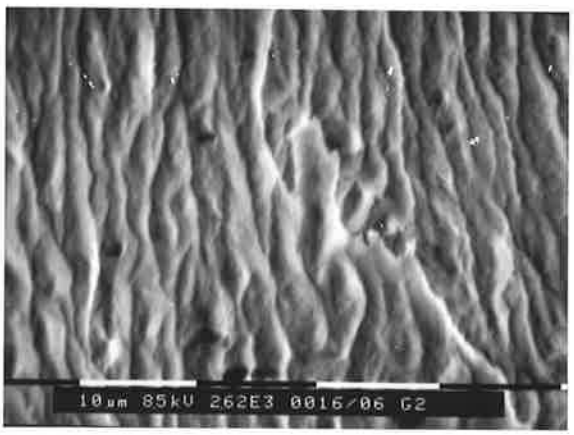
The micrographs appeared to demonstrate the three characteristic etching patterns as described by Silverstone et al. (1975). The specimens exposed to acid for 2 and 4 hours revealed a type 1 etching pattern. A type 2 pattern was seen on those with 8 and 12 hours of acid exposure. The specimens from the final acid exposure categories of 18, 24, 36 and 48 hours displayed a type 3 pattern in which the enamel possessed neither the type 1 nor 3 etching patterns.



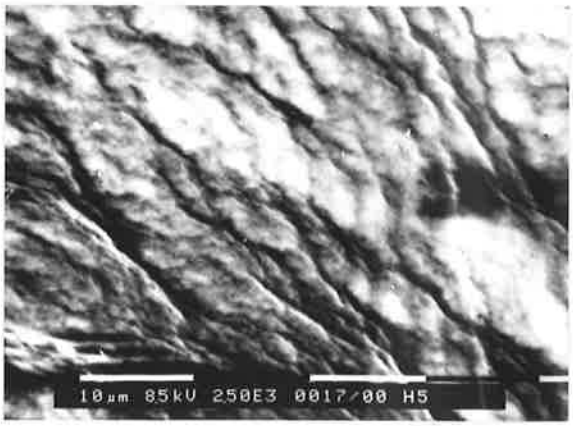
(a) After 18 hours



(b) After 24 hours



(c) After 36 hours



(d) After 48 hours

Figure 17. View of eroded surface following greater exposure times to test solution at 2.62×10^3 magnification.

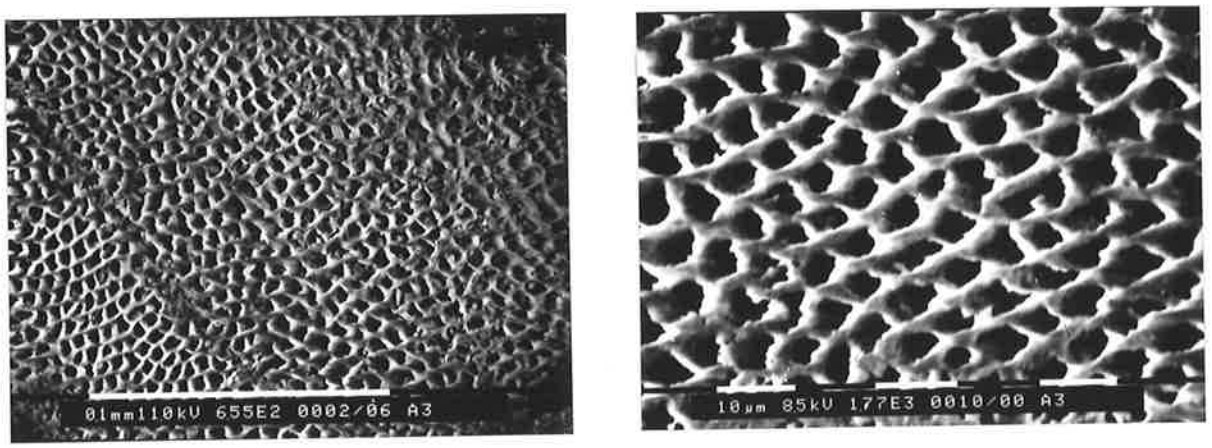


Figure 18. SEM view of eroded surface of lesion following exposure to test solution for 2 hours (a) At 6.55 x 10^2 magnification.
(b) At 2.62 x 10^3 magnification.

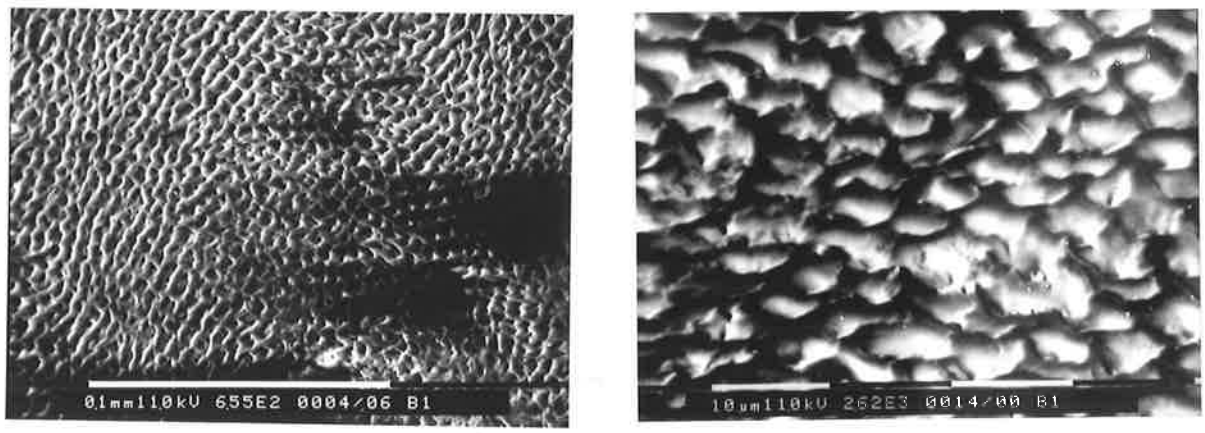


Figure 19. SEM view of eroded surface of lesion following exposure to test solution for 4 hours (a) At 6.55 x 10^2 magnification.
(b) At 2.62 x 10^3 magnification.

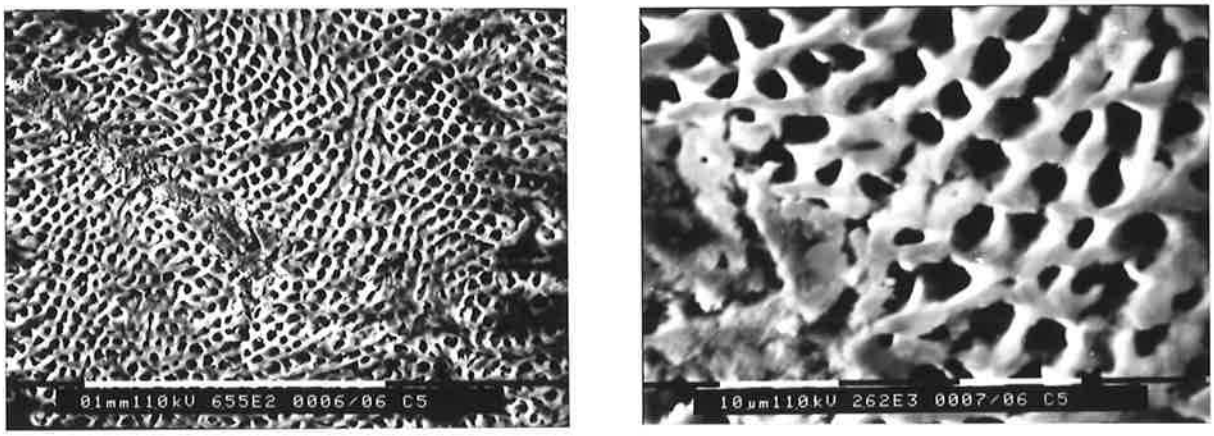


Figure 20. SEM view of eroded surface of lesion following exposure to test solution for 8 hours (a) At 6.55 x 10^2 magnification.
(b) At 2.62 x 10^3 magnification.

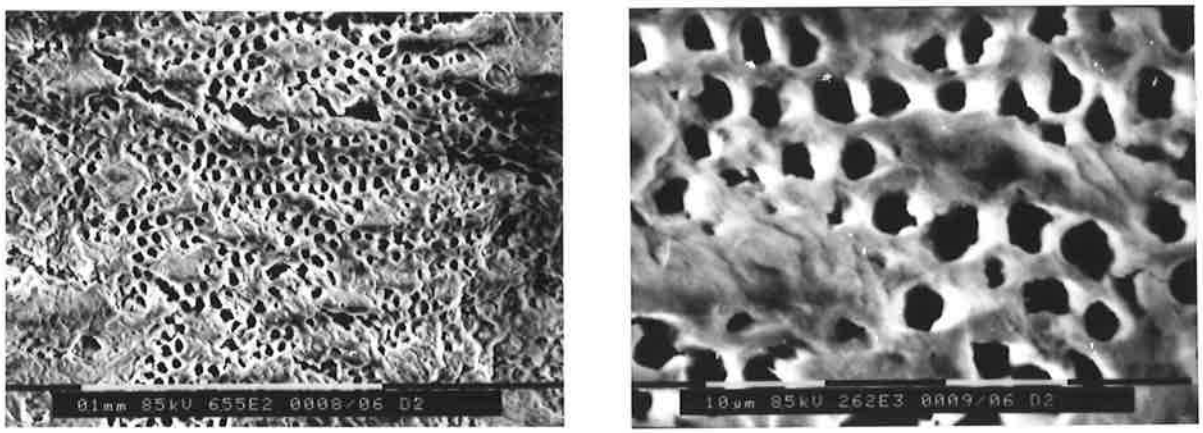


Figure 21. SEM view of eroded surface of lesion following exposure to test solution for 12 hours (a) At 6.55 x 10^2 magnification.
(b) At 2.62 x 10^3 magnification.

4.3 Estimations of Calcium Loss from the Tooth Surface With Time.

The results showed in general, an increasing loss of calcium from the tooth structure with increased time exposure to the test erosive solution. The calcium loss results for the 48 hour acid exposure specimens represented a deviation from this trend, and in some cases, mineral loss occurred under the surrounding varnish with loss of the protective varnish, resulting in increased areas of enamel being exposed to the erosive agent. The standard deviation values showed a mixed range of values, adding further difficulty in the analysis of this data (see Table 1, 2 and Figure 22). The equivalent hydroxyapatite and enamel loss calculations are tabulated on Table 2.

4.4 Estimations of Depth and Volume of Enamel Loss With Time.

The results were varied for this aspect of the experiment (see Table 3 and Figure 23). In general, there was greater depth loss and hence volume of enamel loss with increased period of exposure of the teeth to the erosive solution. Again, several specimens showed mineral loss beneath the varnish and a loss of the varnish, thereby resulting in greater surface of enamel being exposed to the erosive agent. The standard deviations for the measurements did not appear to be very large with this technique. However, the fact that only one representative sample for each time period of erosion was selected did not provide the opportunity to obtain a more representative mean value. One specimen only was available for each category as the remaining specimens were used for other tests being conducted concurrently.

In comparing the volume of wax lost and the volume of enamel loss which was calculated from the calcium loss data, there was some correlation between the two values for half of the specimens (see Table 4). There was a positive correlation for the specimens exposed to the test solution for 2, 4, 8 and 36 hours only. With the remainder specimens, the volume of wax lost did not support the values for the volume of enamel lost from the tooth.

TABLE 1.

TABLE OF ALL RESULTS OBTAINED FROM ATOMIC ABSORPTION SPECTROSCOPY FOR CALCIUM LOSS ESTIMATIONS IN THE "CONTINUOUS EROSION" MODEL

Sample No.	Exposure Time (hrs)	Absorbance Readings	Soln Conc. (mM)	Ca Loss (mM)
control-1	0	0.4747	2.25	0.00
control-2		0.4773	2.27	0.00
control-3		0.4797	2.28	0.00
Average		0.48	2.27	0.00
a-2	2	0.7507	4.26	4.26
a-3		0.914	5.78	5.78
a-4		0.7747	4.46	4.46
a-5		0.795	4.64	4.64
Average		0.81	4.79	4.79
b-1	4	1.022	6.95	6.95
b-3		0.834	5.00	5.00
b-4		0.8313	4.97	4.97
b-5		0.8943	5.58	5.58
Average		0.8954	5.63	5.63
c-1	8	0.9727	6.39	4.12
c-3		0.978	6.45	4.18
c-4		1.25	9.91	7.64
c-5		1.304	10.72	8.45
Average		1.1262	8.37	6.10
d-1	12	1.238	9.73	7.46
d-2		1.1637	8.70	6.43
d-3		1.17	8.79	6.52
d-4		1.1577	8.62	6.35
Average		1.1824	8.96	6.69
e-1	18	1.362	11.63	9.36
e-2		1.3073	10.77	8.50
e-4		1.552	15.00	12.73
e-5		1.319	10.95	8.68
Average		1.3851	12.09	9.82
f-1	24	1.858	21.54	19.27
f-2		1.4327	12.82	10.55
f-4		2.2157	30.08	27.81
f-5		1.7743	19.63	17.36
Average		1.8202	21.02	18.75
g-1	36	2.078	26.79	24.52
g-2		1.9633	24.03	21.76
g-3		2.1247	27.92	25.65
g-4		1.6893	17.78	15.51
Average		1.9638	24.13	21.86
h-1	48	1.852	21.40	19.13
h-2		1.495	13.93	11.66
h-3		1.6957	17.92	15.65
h-5		1.7903	19.99	17.72
Average		1.7083	18.31	16.04

TABLE 2.

TABLE OF AVERAGED RESULTS FOR CALCIUM LOSS ESTIMATIONS IN THE "CONTINUOUS EROSION" MODEL

Time (hrs)	*Ave Absorbance*	*Soln Conc (mM)*	*Ca Loss in 40ml (mM) ± sd*	*Ca Loss in 40ml (mg)*	*Equivalent HA loss (mg)*	*Equivalent enamel loss (mg)*
Control	0.48	2.27	0	0	0	0
2	0.8086	4.79	2.52 ± 0.59	2.52	6.3	6.3
4	0.8954	5.63	3.36 ± 0.80	3.36	8.4	8.4
8	1.1262	8.37	6.10 ± 1.97	6.1	15.25	15.25
12	1.1824	8.96	6.69 ± 0.45	6.69	16.73	16.73
18	1.3851	12.09	9.82 ± 1.71	9.82	24.55	24.55
24	1.8202	21.02	18.75 ± 6.15	18.75	46.88	46.88
36	1.9638	24.13	21.86 ± 3.93	21.86	54.65	54.65
48	1.7083	18.31	16.04 ± 3.25	16.04	40.1	40.1

Figure 22. GRAPH SHOWING THE RELATIONSHIP BETWEEN CALCIUM LOSS ESTIMATIONS AND EXPOSURE TIME TO TEST SOLUTION IN THE "CONTINUOUS EROSION" MODEL

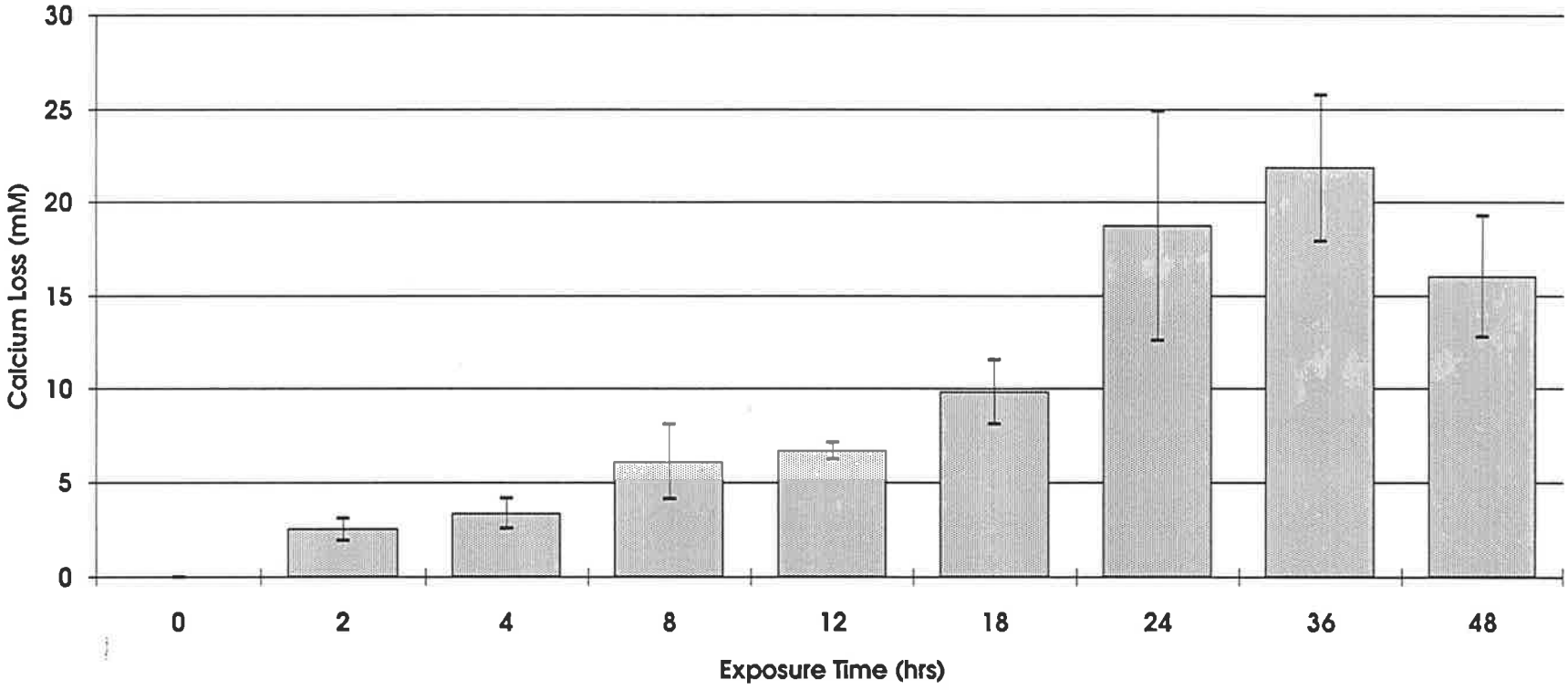


TABLE 3.

VOLUME LOSS ESTIMATIONS USING WAX REPLACEMENT METHOD FOR "CONTINUOUS EROSION" MODEL

Specimen	*Volume (mm^3) ± sd*
A (2 hrs)	1.5 ± 0.1
B (4 hrs)	3.4 ± 0.2
C (8 hrs)	3.9 ± 0.8
D (12 hrs)	3.7 ± 0.1
E (18 hrs)	7.2 ± 0.7
F (24 hrs)	3.7 ± 0.3
G (36 hrs)	8.1 ± 0.5
H (48 hrs)	7.9 ± 0.4

Figure 23. GRAPH SHOWING THE RELATIONSHIP BETWEEN VOLUME LOSS ESTIMATIONS USING WAX REPLACEMENT METHOD AND EXPOSURE TIME TO TEST SOLUTION IN THE "CONTINUOUS EROSION" MODEL

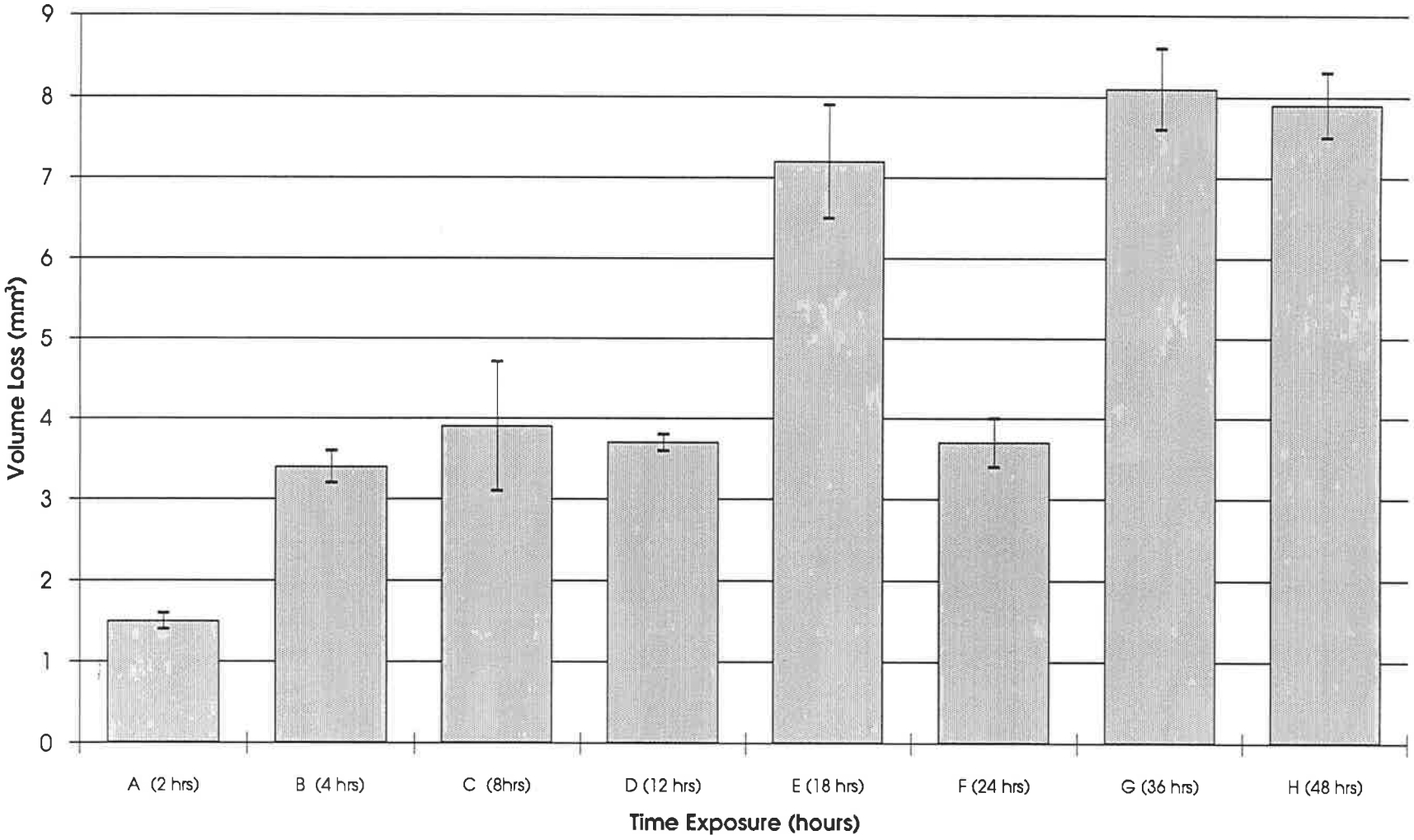


TABLE 4.

COMPARISON BETWEEN CALCIUM AND VOLUME LOSS ESTIMATIONS

FOR THE "CONTINUOUS EROSION" MODEL

Exposure Time (hrs)	*Ca loss in 40ml (mg)*	*Equivalent HA loss (mg)*	*Equivalent enamel loss (mg)*	*Volume of enamel loss (mm^3)*	*Volume of wax lost (mm^3)*	*Correlation (± 50%)*
Control	0	0	0	0	0	
2	2.52	6.3	6.3	2.25	1.5	Yes
4	3.36	8.4	8.4	3	3.4	Yes
8	6.1	15.25	15.25	5.45	3.9	Yes
12	6.69	16.73	16.73	5.98	3.7	No
18	9.82	24.55	24.55	8.77	7.2	Yes
24	18.75	46.88	46.88	16.74	3.7	No
36	21.86	54.65	54.65	19.52	8.1	No
48	16.04	40.1	40.1	14.32	7.9	No

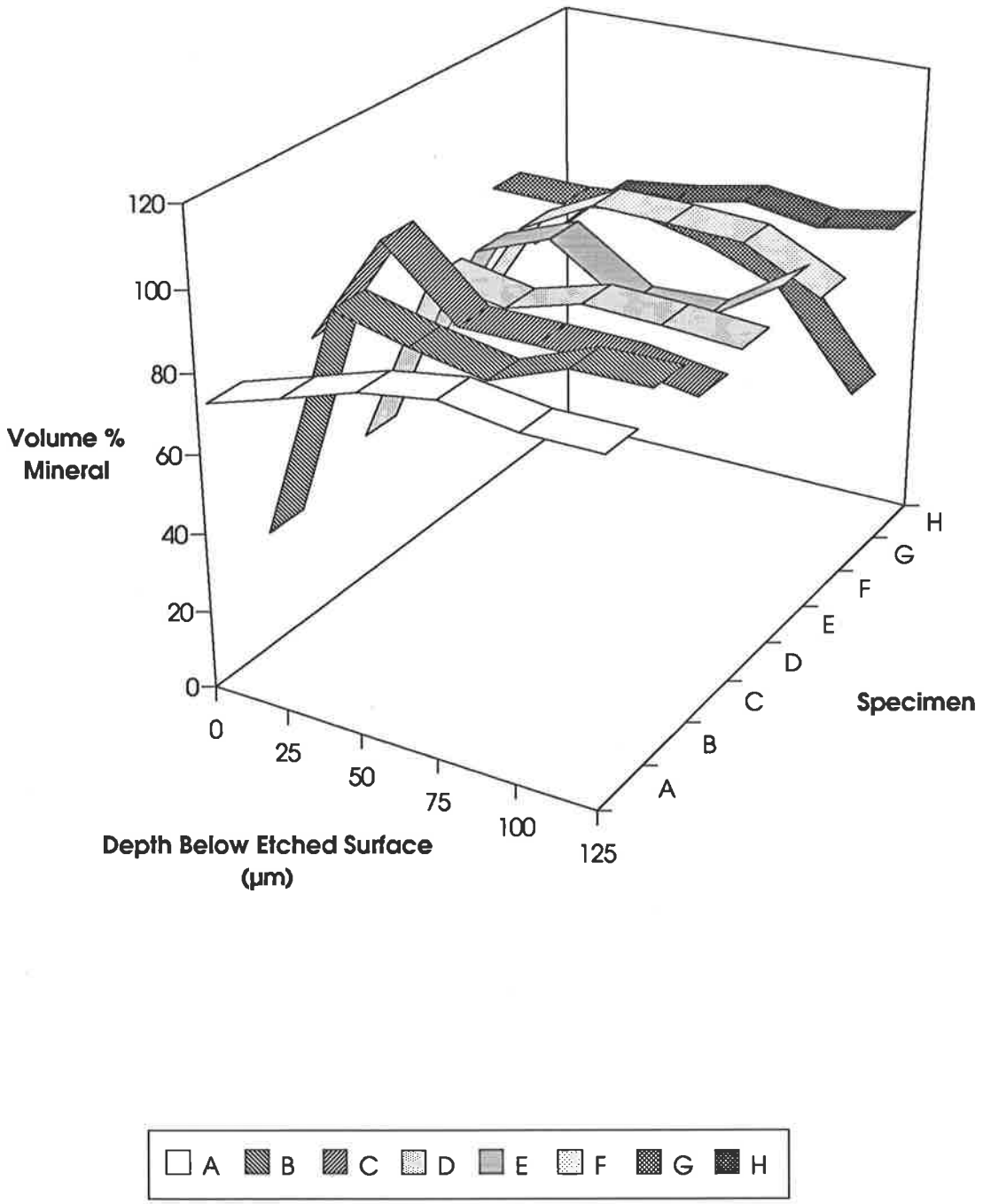
4.5 Profile of Changes in Hardness Across Erosive Lesions.

Calculations of the percentage of volume mineral loss through the erosive lesion revealed an increased loss of mineral at the surface of the lesions. The percentage of mineral loss then decreased at a depth of approximately 25-50μm beneath the eroded surface and the values remained relatively stable and similar throughout the remainder of the depth measured (see Table 5 and Figure 24). Only one representative specimen from each category of time exposure to the acidic solution was used. There were very variable results between the specimens. Specimen A (2 hours) demonstrated little difference in the percentage volume mineral content between the surface of the erosive lesion and further into the tooth structure. However, specimens D (12 hours), E (18 hours), F (24 hours) and H (48 hours) showed volume mineral percentages of the same order, with an initial decrease at the surface of the erosive lesion. Specimen B (4 hours) demonstrated a similar trend, but with a greater decrease in the superficial surface volume mineral content. The other two specimens C (8 hours) and G (36 hours), indicated a deviation from the trend observed. Whether the variability in these results were due to deficiencies in the method, or individual variations between the specimens, is not known.

TABLE 5.
VOLUME % MINERAL ESTIMATIONS FROM MICROHARDNESS VALUES FOR THE "CONTINUOUS EROSION" MODEL

Specimen	*Depth from surface (μm)*	*Volume % mineral*
A (2hrs)	0	71.3
	25	77.25
	50	83.41
	75	86.47
	100	83.84
	125	83.84
B (4hrs)	0	29.41
	25	90.84
	50	86.24
	75	82.77
	100	90.32
	125	90.49
C (8hrs)	0	71.65
	25	100.31
	50	83.48
	75	83.41
	100	83.12
	125	80.06
D (12hrs)	0	37.51
	25	83.84
	50	80.39
	75	85.7
	100	84.87
	125	83.48
E (18 hrs)	0	49.76
	25	83.19
	50	90.32
	75	77.73
	100	78.59
	125	91.46
F (24 hrs)	0	51.6
	25	82.07
	50	91.9
	75	91.46
	100	90.49
	125	80.72
G (36 hrs)	0	82.14
	25	81.73
	50	83.48
	75	78.78
	100	71.5
	125	47.46
H (48 hrs)	0	59.66
	25	77.01
	50	79.22
	75	83.12
	100	80.26
	125	83.84

Figure 24. GRAPH SHOWING THE RELATIONSHIP BETWEEN VOLUME % MINERAL CONTENT AND DEPTH FROM SURFACE IN THE "CONTINUOUS EROSION MODEL"



CHAPTER 5

DEVELOPMENT OF A "CYCLIC EROSION" METHOD OF THE ENDOGENOUS EROSION MODEL, TO INVESTIGATE THE ABILITY OF 1.23% APF GEL TO INHIBIT THE PROCESS

5.1 Objectives

The objectives were to:

(A) Develop modifications of the "continuous erosion" model of endogenous erosion, in order to explore ways in which it may :

(i) More clearly simulate clinical erosion.

(ii) Permit testing of the ability of topical application of 1.23% APF Gel to achieve inhibition of the erosion process using these "in vitro" test systems.

(B) Explore the potential of the APF Gel, when applied in a variety of ways to inhibit erosive demineralisation of enamel, in terms of volume, depth and pattern of enamel surface loss.

(C) Analyse the residual surface "hardness" and "porosity" level in both control and test experiments, in order to see whether the 1.23% APF Gel application results in altered density properties.

5.2 Assessment of the Usefulness of the Continuous Exposure Model of Erosion in Permitting Analysis of the Ability of 1.23% APF Gel to Inhibit This Process

The basis of the continuous exposure model, as described in the previous chapter, was to expose enamel surfaces to the test erosive agent for varying though specific periods of time, and to assess the amount of mineral loss and any resultant change in the integrity of the remaining enamel surface.

One of the major difficulties encountered with the continuous exposure model was effective quantitative assessment of the volumes of mineral loss. For this reason, it was decided to move to a new system of assessment. This involved the determination as to when specific, discernible erosive changes could be seen microscopically on the exposed enamel surfaces, and recording the aggregate time period required to result in these changes. These are described later in this chapter.

A second difficulty with the continuous exposure model of erosion was noted in the pilot studies of the effectiveness of a single coating of 1.23% APF Gel for four minutes (as used clinically) in inhibiting the erosive process using the 0.06M HCl and 2.2mM $CaHPO_4$ erosive agent. No reduction in erosive action could be detected. Hence, it was recognised that the model would have to be modified to permit more frequent topical fluoride gel exposure. To permit this investigation to take place, it was decided to move to a cyclical model in which the teeth were exposed to acid for multiple shorter increments of time. This model is described below.

5.3 Development of the Cyclic Erosive Acid Model

Tooth crowns were prepared as described previously, with the additional precaution to keep both hemisectioned sections of the crown together in order to provide control and test specimens of enamel, having had similar exposures to oral fluids and nutrients.

In general, enamel surfaces were exposed to the test acid solution for multiple periods of 2, 4, 8 or 12 minutes. Following each period of exposure, the half crowns were washed under tapwater and dried before being exposed again (see Figure 25). To facilitate the process, groups of five half crowns were fixed to rectangular blocks of polystyrene using dental sticky wax in such a way as to permit ready bulk dipping into and removal from the acid solution. Such blocks were prepared with control and test crowns and mounted to form test pairs (see Figure 26). Figure 27 shows the surface of a prepared specimen prior to exposure to test solution.

Analysis showed that the rate of enamel loss was different between the "continuous" and "cyclical" exposure to the acid for the same period of time of exposure. As it is later shown, the average time required to reach the desired depth loss of 0.5mm in the "cyclic" variation of the erosion model was 200 minutes. The closest equivalents to this time period in the "continuous" method are the specimens exposed to the erosive agent for two hours (120 minutes) and for four hours (240 minutes). The specimens exposed for two hours result in a lesion depth just short of 0.5mm. It is some of the specimens exposed to the test solution for four hours that demonstrate a depth loss of 0.5mm and more in some cases.

5.4 Assessment of Erosive Outcome on Enamel

As stated previously, assessment of erosive outcomes were made by recording the total time period (number of cycles by the length of cycles) necessary for a specific, discernible stage of erosive demineralisation to be observed. The four specific stages were:

(i) Initial etch (see Figure 28).

(ii) Total surface etch (see Figure 29).

(iii) Observed loss of surface volume to 0.25mm depth (see Figure 30).

(iv) Loss of enamel to 0.5mm depth (see Figure 31).

At the end of each erosive cycle, enamel surfaces were checked using a graduated probe and viewed under the Zeiss OPMI 1-FC stereomicroscope for signs of one of

these four progressive stages to be evident. When so, the aggregate time taken to reach this stage was recorded. This method permitted a comparison of rates of enamel loss, rather than the more difficult estimation of volume of enamel loss at fixed times.

Figure 25. Set-up of materials used for the experiments.

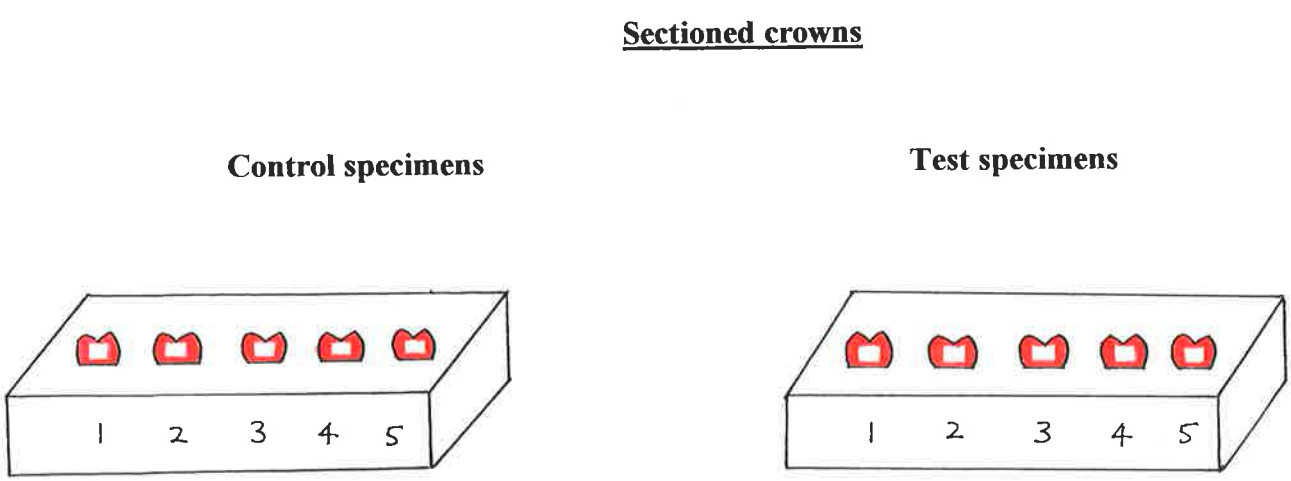


Figure 26. Diagrammatic representation of mounted specimens.

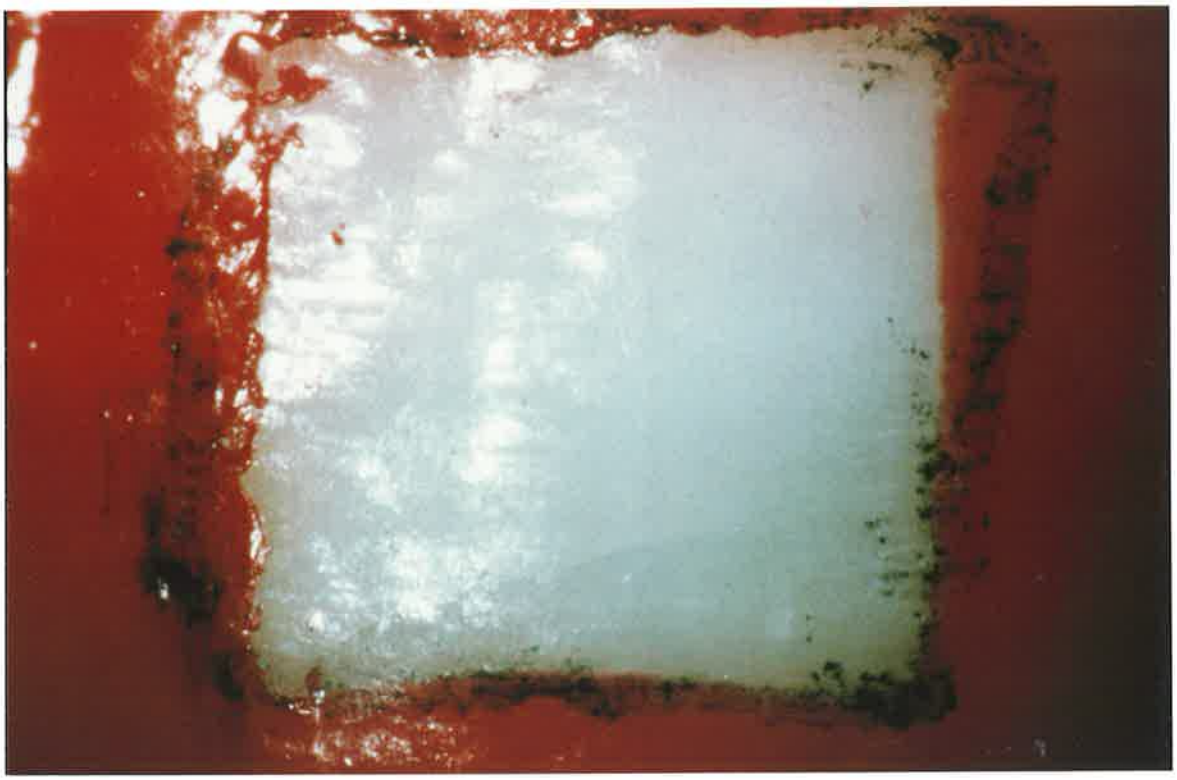


Figure 27. Example of specimen prior to exposure to test solution.

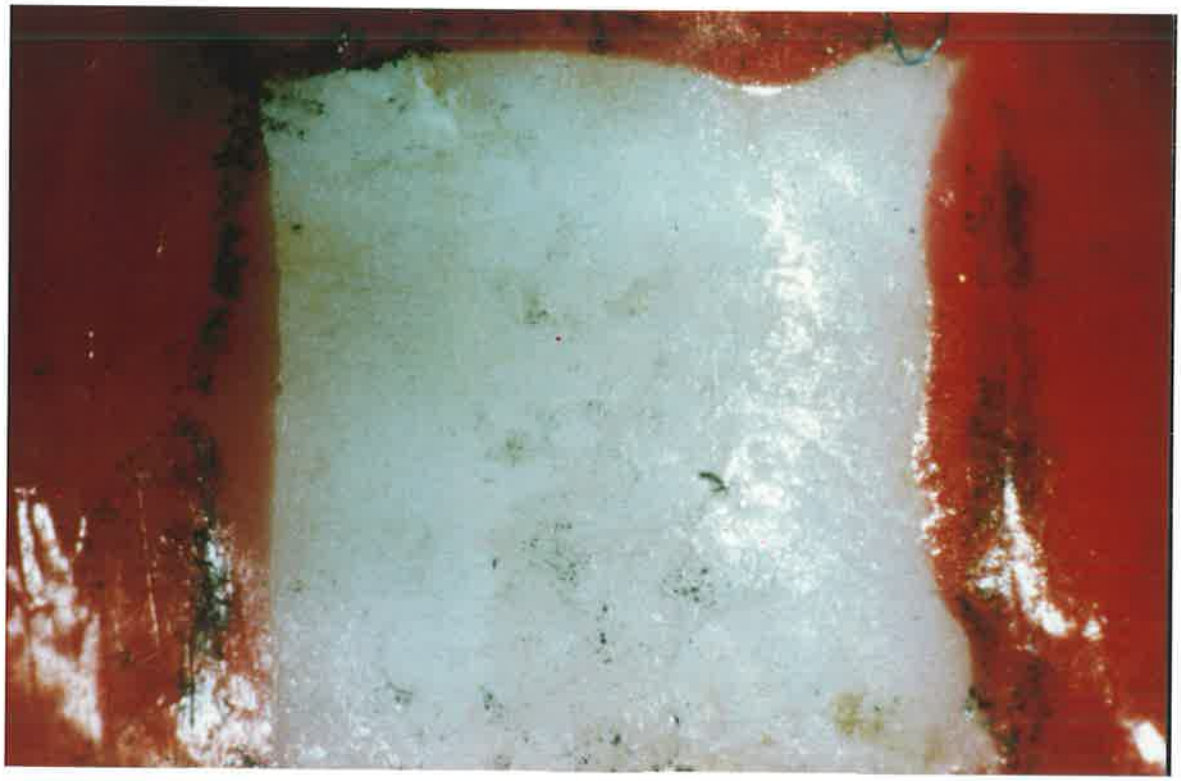


Figure 28. Example of a specimen showing initial etch.

Figure 29. Example of a specimen showing total surface etch.

Figure 30. Example of a specimen showing 0.25mm depth loss (sample tipped to show semi-profile view of margin of lesion).

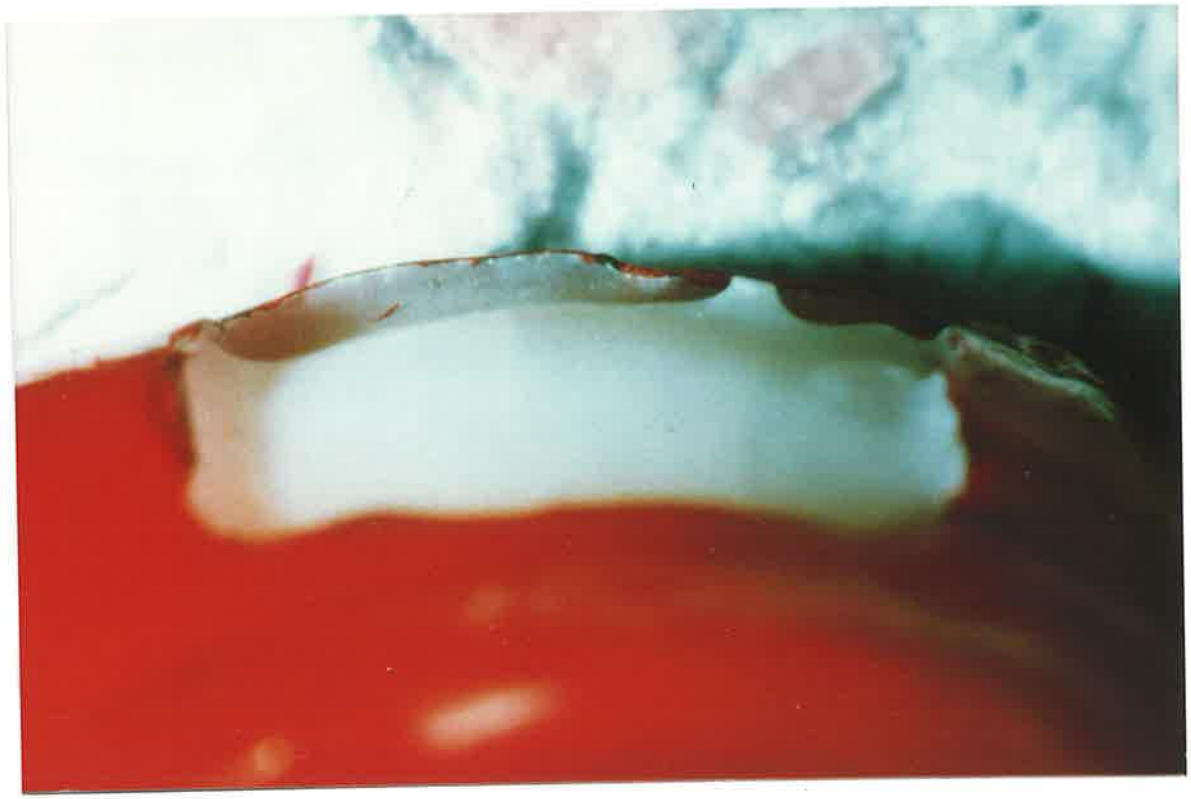


Figure 31. Example of a specimen showing 0.5mm depth loss (sample tipped to show semi-profile view of margin of lesion).

5.4(a) Cyclical Application of Fluoride with Time.

Twenty intact molar teeth were selected and prepared as outlined above.

Both the test and control experiments were carried out together. The test specimens were exposed to fluoride (acidulated phosphate fluoride gel 1.23%) for four minutes prior to acid exposure at every cycle, while the control specimens were only exposed to the acidic challenge. Fluoride was applied with a cotton bud swab to all the experimental teeth. After four minutes, the fluoride gel was removed by thorough rinsing under tap water for twenty seconds and gentle drying with an air gun. While the experimental teeth received fluoride, the control specimens remained on the benchtop. After the fluoride application was completed, both blocks of teeth were placed upside down into the bath containing the erosive test solution for the appropriate time category in a static state without agitation and incubated at 37°C. Following the acidic challenge, the blocks were removed from the acid bath and rinsed thoroughly under tapwater for twenty seconds and dried with the air gun. These steps were repeated until the required "endpoint" was reached.

The degree of erosive demineralisation was assessed with a graduated probe while viewed under the Zeiss OPMI 1-FC stereomicroscope. Prior to reaching the endpoint, the experimental and control specimens were viewed regularly for various stages of change. Each stage of change was recorded in terms of cycles, which began with a fluoride application followed by the acidic challenge. All the four stages of erosive change were observed on each of the tooth specimens. This was done so as to minimise the number of specimens required. At the completion of the experiment, the two groups of specimens were compared,

(i) To determine that the incremental rate of erosion using the intermittent model, was accumulatively similar to that seen in the continuous model, and

(ii) To assess the effect of fluoride on the process of erosive demineralisation.

5.4(b) Application of Fluoride Following Every Second or Fourth Cycle of Erosive Challenge

The aim of this experiment was to provide a model system which better simulated the clinical situation. In practice, it would be difficult and may constitute excessive use of topical fluoride, to topically apply concentrated fluoride gel prior to every erosive acid exposure. The test specimens were similarly exposed to a 2-minute cyclical acidic challenge. However, they were exposed to a topical application of 1.23% APF Gel at predetermined intervals before the acid challenge. Separate experiments each using five intact molar teeth were performed using two selected exposure frequencies of fluoride exposure.

Experiment A: 1.23% APF Gel applied after every 2nd 2-minute exposure to acid (resulting in a total of four minutes acid exposure following fluoride application).

Experiment B: 1.23% APF Gel applied after every 4th 2-minute exposure to acid (resulting in a total of eight minutes acid exposure following fluoride application).

Apart from the altered intervals of fluoride application, identical conditions applied and the same procedures as described in the previous study were followed. The same measurement criteria were used, and the four stages of change were observed and noted in terms of cycles. The control and test groups of experiment A and B were compared to evaluate the effectiveness of fluoride in controlling the process of erosive demineralisation. This was achieved through:

(1) Estimations of depth,

(2) Estimations of volume of enamel loss with time, and

(3) Calculations of changes in hardness and hence volume % mineral loss across the erosive lesions.

5.5 Analysis of Erosive Changes in Test and Control Specimens

5.5.1 Estimations of Depth and Volume of Enamel Loss with Time

The same method as in the previous study was employed to determine the volume of enamel loss using Kerr's blue inlay casting wax at the more advanced stage of erosion (i.e. 0.5mm).

Two teeth (one control and one experimental) were selected from each set of specimens exposed for the varying time periods to the test erosive solution and fluoride. The two halves were from the same tooth. In less than half of the specimens, the layer of nail varnish had lifted off with the continual exposure to all the various substances. As a result, the erosive demineralisation had proceeded outside the marked window and these specimens were therefore unable to be used to determine the degree of volume of enamel loss.

Three wax patterns were made for each specimen by the same operator to obtain an averaged result. The patterns were weighed on a four-decimal place balance, and the average weight values were inserted into the formula (Volume = Mass/Density) to calculate the volume loss of enamel. The values will be compared to evaluate the effectiveness of fluoride in controlling the progression of enamel erosion.

5.5.2 Profile of Changes in Hardness Across the Erosive Lesions

One representative specimen from each of the control and experimental groups of specimens was selected from each time category. The same embedding procedures were followed as for the previous experiment. The specimens were embedded in a small-cubed ice-tray using an araldite mixture of 100 ml Araldite M and 40 ml Hardener HY 5160 following the cleaning process in the araldite/acetone mixture, and infiltration process in 100% araldite solution. Polymerisation took place in an oven heated up to 60°C for forty-eight hours.

Following removal of the specimens from the moulds, the specimens were hemisectioned vertically through the center of the erosive lesion using the Leitz saw microtome 1600 machine. One half of each crown was put aside for polarised light microscopy, and the other half was embedded for hardness testing.

The crown halves for hardness testing were embedded in the flange form moulds so that the cut section of the lesions were exposed, using the same araldite mixture. Following complete polymerisation of the araldite mixture, the specimens were removed from the moulds and serially polished on a Struers Abramin polishing machine. The same polishing procedure was followed as for the baseline experiment using silicon carbide paper and diamond abrasive spray (see Figures 32 and 33).

A Knoop diamond on a Leitz Durimet small hardness tester was used under a 50 gram load to make six indentations from the outer surface of the erosive lesion and at 25µm intervals into the underlying enamel. This continued to 125µm within the enamel at 25µm intervals. The Knoop hardness values were then calculated from the length of each diamond indentation.

$$\text{Knoop Hardness Number (KHN)} = \frac{14230 \times \text{force}}{(\text{length of indentation})}$$

Using these values, the percentage of volume mineral loss through the erosive lesion was calculated (Featherstone, 1983).

$$\text{Volume \% Mineral} = 4.3\sqrt{\text{KHN}} + 11.3$$

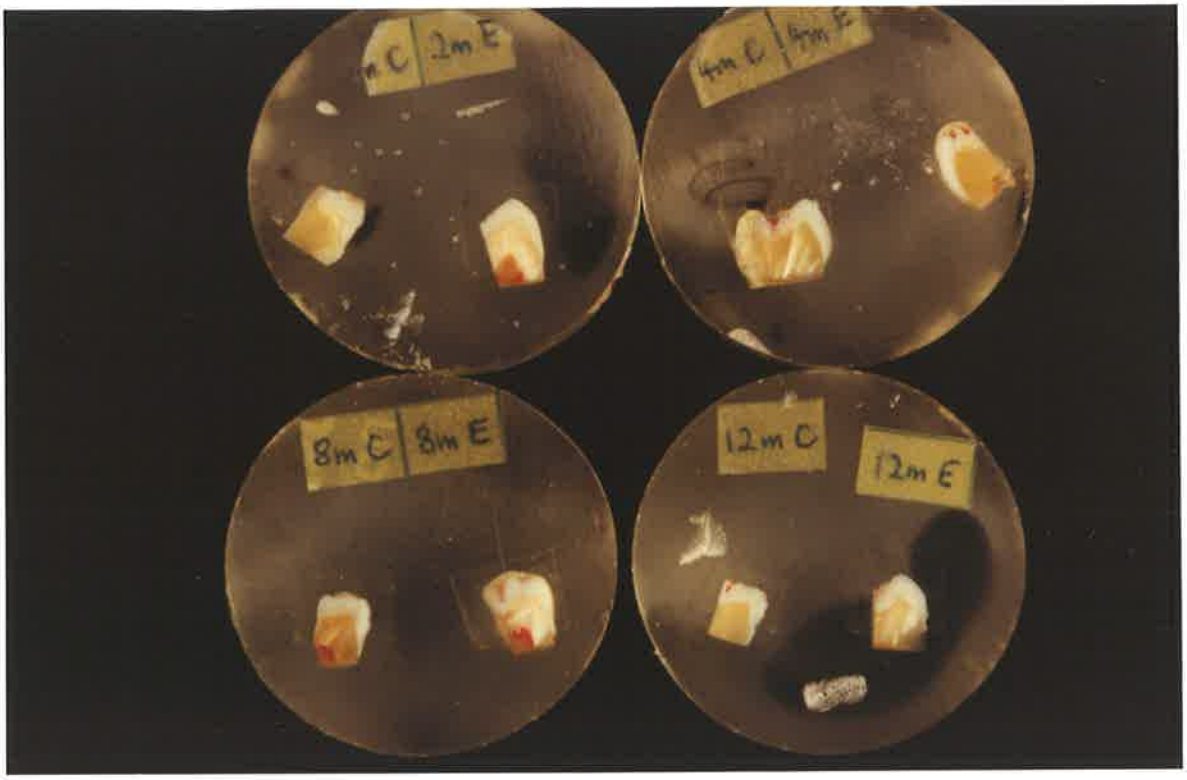


Figure 32. Prepared specimens from "cyclical erosion" model for microhardness testing.

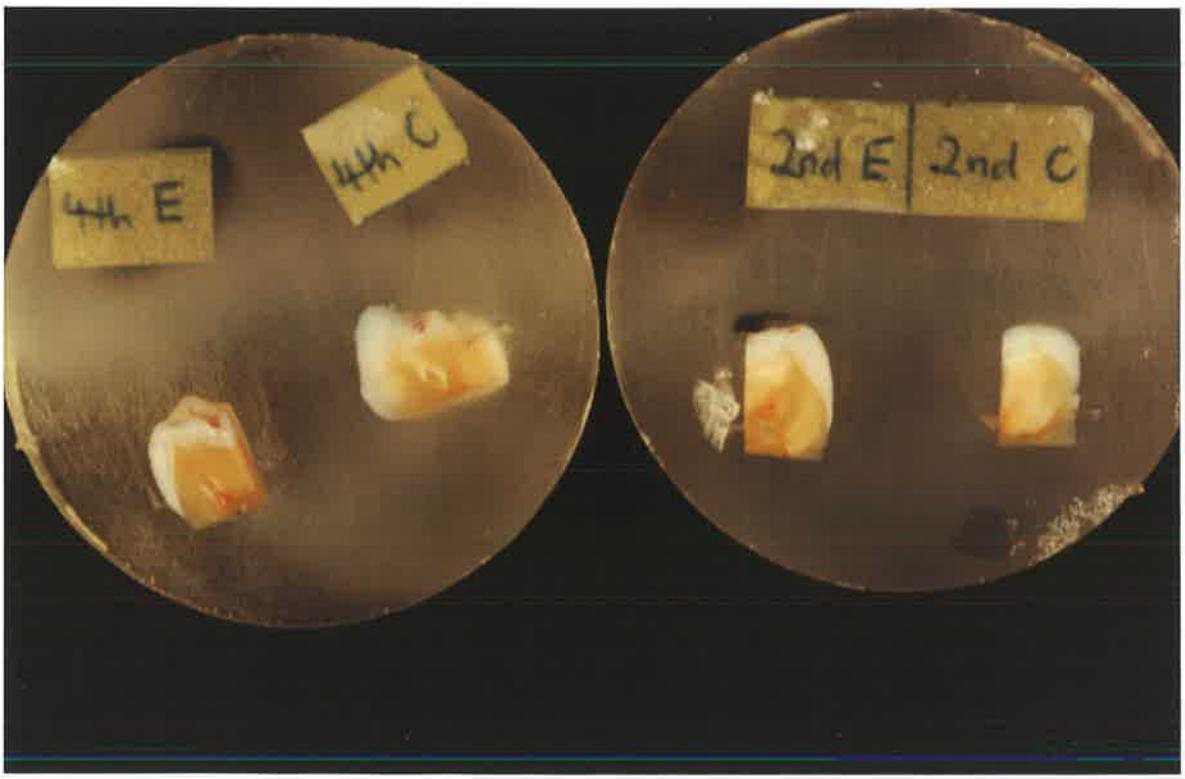


Figure 33. Prepared specimens from "intermittent erosion" model for microhardness testing.

5.5.3 Polarised Light Estimations of Porosity of the Enamel Under the Erosive Lesion

Preparation of Specimens

Sections through the erosion lesions on the crowns were prepared using a Leitz saw microtome 1600. The specimens were initially embedded in an epoxy resin block, after the eroded surface had been protected by a coat of nail varnish. The specimens were cut to a thickness of approximately 100μm in thickness using a "lapping" process and were mounted and secured on glass slides. Serial polishing was accomplished with a Struers Abramin polishing machine using a series of 320-, 800- and 1200-grade silicon carbide paper, followed by 9μm, 3μm, and 1μm diamond abrasive spray (Struers, DP-suspension) on Struers polishing cloth.

Quantitative Polarised Light Microscopy

This involves initially, measuring the total birefringence of a specimen at particular points throughout the structure of that specimen. The birefringence at any one point in the specimen can be determined using a compensator, a device which enables the degree of retardation of the emergent light from the path of the originating rays to be determined.

The degree of retardation is signified by $n_e - n_o$ (where n_e and n_o represent the difference in phase between the emergent and originating rays, which is measured using a compensator).

$$\text{Birefringence} = \frac{n_e - n_o \text{ (nm)}}{\text{Thickness of specimen } (\mu\text{m})}$$

In this experiment, retardation was measured using a Zeiss polarising microscope and an Ehringhaus Compensator.

Determining Pore Volume of Enamel Specimens

The total birefringence of enamel is largely due to the birefringence of the apatite crystallite component. The small amount of organic matter in enamel (1-2% by volume) diminishes the intrinsic birefringence of enamel by a very small amount. Another factor which may contribute to the total birefringence of enamel is water. Much of this is present as hydration layer around crystallites. If enamel is demineralised, as in caries or partial erosion, the volume of porous space in enamel may increase substantially, and contribute to the total birefringence, depending on the refractive index of the liquid material filling the spaces. The birefringence caused by the porous spaces in enamel is called form birefringence, as distinct from the intrinsic birefringence of the basic apatite prism (rodlet) system.

As the intrinsic birefringence of apatite is negative with respect to the morphological prism axis, and form birefringence is generally positive, the greater the pore space, the greater the effect on total birefringence. This is particularly noticeable if imbibing liquids of differing refractive index are used.

If enamel is porous, and is imbibed in a liquid having a refractive index similar to enamel (1.62), only the intrinsic birefringence will be observed. When the refractive index of the imbibing medium is different from that of enamel, the observed birefringence will be the sum of the negative intrinsic birefringence of the apatite and the positive form birefringence of the imbibed spaces or pores.

The value of the form birefringence is given by the Wiener formula:

$$n_e^2 - n_o^2 = \frac{v_1 v_2 (n_1^2 - n_2^2)}{(1 + v_1) n_2^2 + v_2 n_1^2}$$ where,

n_1 = refractive index of the rodlets (apatite in enamel)

v_1 = volume % of the rodlets

n_2 = refractive index of the pores

v_2 = volume of the pores

This formula may be used to determine from the observed total and intrinsic birefringence at a particular point, the relative volumes of rodlets and pores. To make this process simpler, variations of the original Wiener formula have been developed. One by Stokes (1963) is as follows:

$$\text{Form birefringence} = \frac{v_1 v_2 (n_1^2 - n_2^2)^2}{2n_1 (v_1 n_2^2 + v_2 n_1^2 + n_2)^2}$$

where, v_1 and v_2 are respectively rodlet and pore fractions %, and n_1 and n_2 are refractive indices of rods and pores.

This variation should be used only where small differences between n_1 and n_2 are present, as the mathematics used to derive the formula rely on n_1 and n_2 being nearly equal.

As stated earlier, when enamel is imbibed in a medium of refractive index 1.62, only intrinsic birefringence is observed. If imbibed in a medium of differing refractive index, the resultant birefringence represents the difference between intrinsic and form birefringence.

In this experiment, sections were imbibed in media of refractive index 1.62 (Thoulet's saturated solution of Mercuric Iodide in Potassium Iodide) and of 1.55 (the saturated solution diluted in KI till this refractive index is measured with a refractometer). Thus, from the differences in birefringence noted in the two liquids, which represents form birefringence, it is possible to determine the relative pore volumes present in enamel at particular locations in a specimen.

To achieve this, a graph is prepared from the above formula, in which,

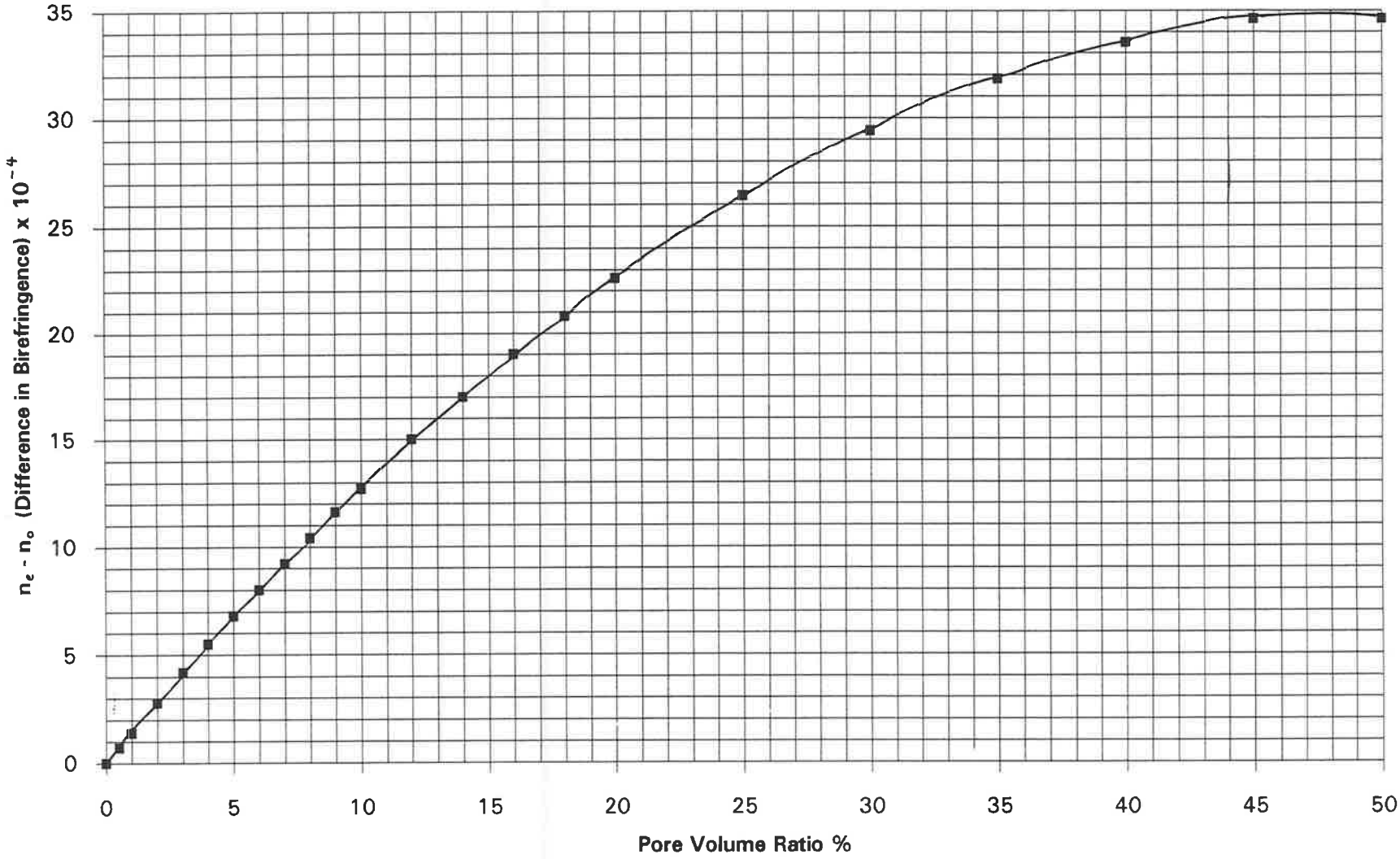
n_1 = 1.62 (refractive index of apatite and of imbibing medium)

n_2 = 1.55 (refractive index of the second imbibing medium)

and where a sequence of relative rodlet/pore volumes is entered, providing a resultant form birefringence value. The observed form birefringence of the specimen can then be checked against the graph to determine the relative pore volume % at that location in the specimen, which provides that result (see Figure 34).

In this experiment, birefringence was measured at various points inside the erosion lesion. These points were at 10μm from the eroded surface, at 10μm intervals up to 100μm, and at larger intervals to a depth of 300μm from the surface. Data were recorded at the eroded and normal enamel surfaces adjacent to the eroded areas, in both baseline and in the intermittent erosion series. Only a very small number of specimens were subjected to this analysis due to the difficulty and complexity of the procedure involved. Four specimens were studied in all, one control and one test specimen from the two minute cyclical exposure to the acid and fluoride, and one control and test specimen from the two minute intermittent exposure to the acid and fluoride.

Figure 34. Change in Birefringence in Enamel (Mixed Rodlet) System With Change in Imbibing Medium (RI 1.62 → 1.55)



CHAPTER 6

DEVELOPMENT OF A "CYCLIC EROSION" METHOD OF THE ENDOGENOUS EROSION MODEL, TO INVESTIGATE THE ABILITY OF 1.23% APF GEL TO INHIBIT THE PROCESS

RESULTS

6.1 Cycles and Equivalent Time Taken to Reach the Stages of Erosive Change Observed

A cyclic model of erosion and the effect of fluoride on the process of enamel erosion were compared in this study. One variation of the model involved the cyclical application of fluoride prior to exposure to acid for multiple time periods of 2, 4, 8 and 12 minutes. The second variation of the model involved the intermittent application of fluoride only at various determined intervals. These were at every second cycle consisting of two minute acidic challenge, and at every fourth cycle consisting of again, two minute acidic challenge.

The appearance of the eroded surfaces differed for the control specimens and for the experimental specimens to which fluoride was applied. The test specimens demonstrated opaque and precipitated surfaces, and the control specimens presented relatively smooth and glassy surfaces. However, the surfaces of the test samples exposed for a greater time period to the acid solution, appeared to be more even with fewer islands of precipitation.

On average, 200 minutes of exposure time to the test solution was required to reach the "endpoint" of 0.5mm depth loss on the control specimens irrespective of the variation employed within the cyclical and intermittent methods. The difference appeared to be the rate at which each depth of loss was reached in the separate test methods.

Cyclical Erosion Method

Initial etch was observed on a number of both the control and test specimens after the first cycle of exposure to the erosive agent, that is, after two minutes of acid exposure. Variation was noted in the number of cycles required to achieve total surface etch, 0.25mm depth loss and 0.5mm depth loss. The time required for the control specimens to attain total surface etch ranged from 8 to 40 minutes of acidic challenge. For the test specimens, this took a range of 8 to 48 minutes. On account of the average times, there appears to be little variation in the number of cycles required to show total surface etch. A depth loss of 0.25mm occurred only in the control specimens, exhibiting a range of 56 to 116 minutes to the acidic challenge. The 8-minute cyclic variation of the model required the least amount of time to reach this depth loss (56-72 minutes). Only the test specimens reached the depth loss of 0.5mm, requiring a range of 168 to 228 minutes of exposure time to the erosive agent (see Table 6).

Of particular interest on these results, was the high level of etch visually observable in those specimens which had been protected with fluoride. This was retained for a long period of time, even though it did not result in the same degree of mineral loss as in the control specimens.

Intermittent Erosion Method

In experiment A, initial etch was seen following the first cycle of acid exposure, that is, after four minutes. The results showed that a greater number of cycles and therefore range of time was needed to achieve total etch of the enamel surface. Again, only the control specimens reached the depth loss of 0.25mm, requiring 100 to 108 minutes of acid exposure, and for 0.5mm, requiring 180 to 204 minutes. The same observation as above was made, that is, the high level of etch visually observable on the specimens with fluoride protection, and a much lower degree of mineral loss compared with the control specimens.

The first signs of erosive change in experiment B specimens was observed as before, after the first cycle of acid exposure. One specimen alone exposed to fluoride application demonstrated 0.25mm depth loss, taking 144 minutes of exposure time to the acidic challenge. The time taken for the control specimens to reach this stage ranged from 96 to 144 minutes. A depth loss of 0.5mm was seen within a range of 232 to 248 minutes of acid exposure (see Table 7).

In all the experimental specimens (with topical fluoride application), only the first two stages of change were observed and erosion did not progress to quite reach the depth of 0.25mm. The graduated probe used to measure the depth of loss was not finely graduated enough to accurately detect finer measurements. Although the depth of 0.25mm of surface loss was not achieved on the experimental specimens, the graphs were plotted from this depth loss of 0.25mm to allow a comparison between the test and control specimens for the given time and to demonstrate the effect of fluoride on the progression of enamel erosion.

The number of cycles taken to reach initial and total etch did not appear to differ greatly whether or not the specimens were exposed to topical fluoride.

TABLE 6.

RECORD OF CYCLES TAKEN TO REACH THE FOUR STAGES OF OBSERVATION FOR THE " CYCLIC EROSION" MODEL

		Initial Etch	*Total Etch of Surface*	*0.25mm depth loss*	*0.5mm depth loss*
2m C	Cycles	1-7	8-14	36-45	90
	Minutes	2-14	16-28	72-90	180
2m E	Cycles	1-5	8-16	not	not
	Minutes	2-10	16-32	reached	reached
4m C	Cycles	1	2-10	25-29	48-57
	Minutes	4	8-40	100-116	192-228
4m E	Cycles	1	2-11	not	not
	Minutes	4	8-44	reached	reached
8m C	Cycles	1	2-5	7-9	21-26
	Minutes	8	16-40	56-72	168-208
8m E	Cycles	1	2-6	not	not
	Minutes	8	16-48	reached	reached
12m C	Cycles	1	1-2	8	18
	Minutes	12	12-24	96	216
12m E	Cycles	1	2-3	not	not
	Minutes	12	24-36	reached	reached

TABLE 7.

RECORD OF CYCLES TAKEN TO REACH THE FOUR STAGES OF OBSERVATION FOR THE "INTERMITTENT EROSION" MODEL

Experiment A

		Initial Etch	*Total Etch of Surface*	*0.25mm depth loss*	*0.5mm depth loss*
2nd C	Cycles	1	3-9	25-27	45-51
	Minutes	4	12-36	100-108	180-204
2nd E	Cycles	1	6-30	not	not
	Minutes	4	24-120	reached	reached

Experiment B

		Initial Etch	*Total Etch of Surface*	*0.25mm depth loss*	*0.5mm depth loss*
4th C	Cycles	1	2-6	12-18	29-31
	Minutes	8	16-48	96-144	232-248
4th E	Cycles	1	1-5	18+	not
	Minutes	8	8-40	144+	reached

6.2 Estimations of Depth and Volume of Enamel Loss with Time

In both variations of the erosion model (cyclical and intermittent models), the application of fluoride resulted in the reduction of depth of erosion, thereby decreasing the severity of erosion (see Figures 35 to 38). On viewing the corresponding specimens in terms of similar time exposure under the stereomicroscope, the application of fluoride intermittently at selected frequencies to the enamel surface appeared to be equal to the cyclical exposure in its effectiveness in reducing erosion. This is to say that, the degree of control of erosion is the same regardless of whether the tooth was exposed to fluoride at every cycle or at predetermined intervals, resulting in similar depth loss of the test specimens.

However, the results from the wax replacement technique for the calculation of volume loss did not appear to support the above observations (see Table 8). The effect of fluoride on the test specimens were shown less dramatically, and especially so in the group of specimens subjected to the cyclical erosion method (see Figure 39).

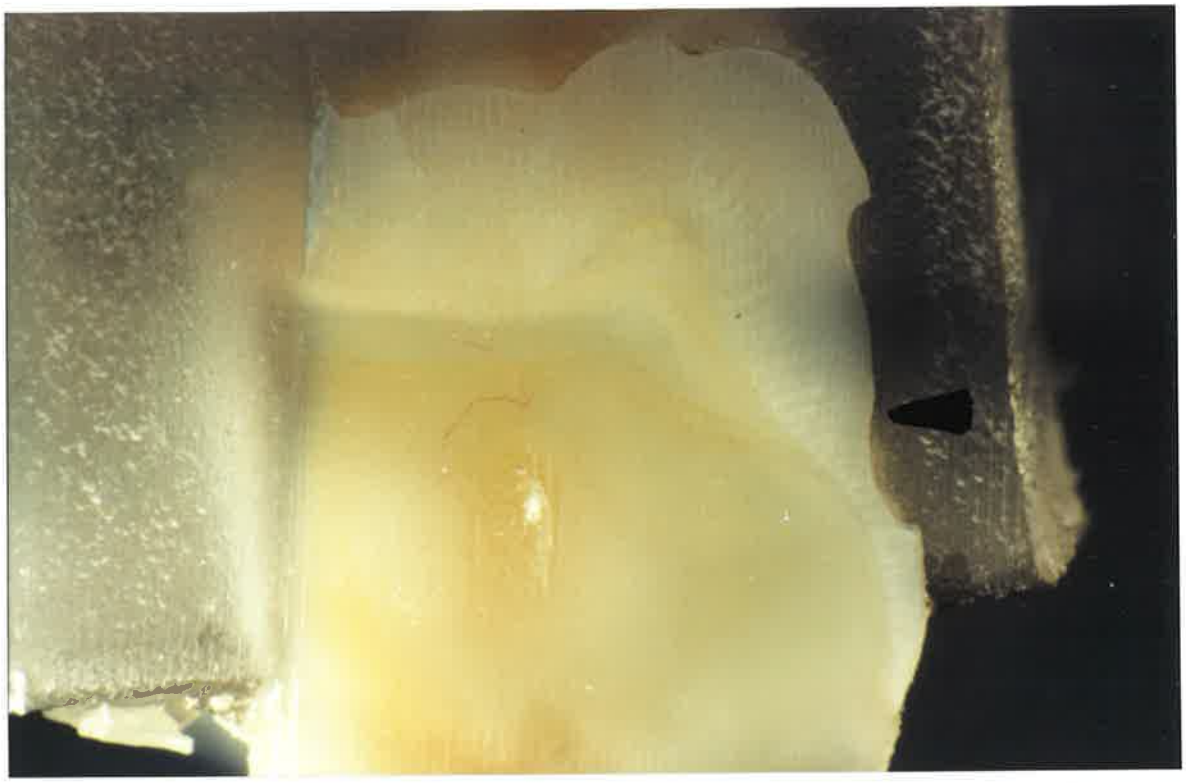


Figure 35(a). Cross-section of selected control specimen from 2-minute "cyclical erosion" model showing depth of erosion reached after 90 cycles of exposure time to test solution.

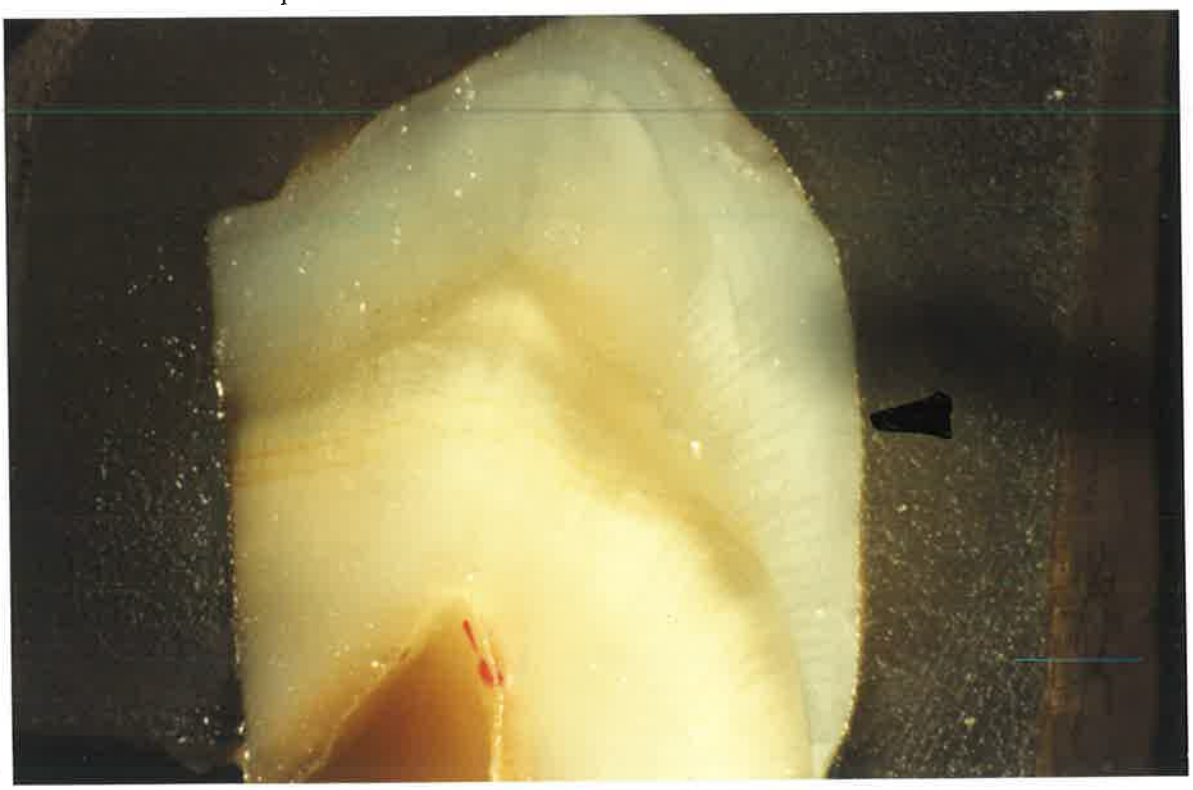


Figure 35(b). Cross-section of corresponding test specimen showing effect of fluoride in inhibiting progress of erosion for the same exposure period to the test solution.

Figure 36(a). Cross-section of selected control specimen from 4-minute "cyclical erosion" model showing depth of erosion reached after 192-228 cycles of exposure time to test solution.

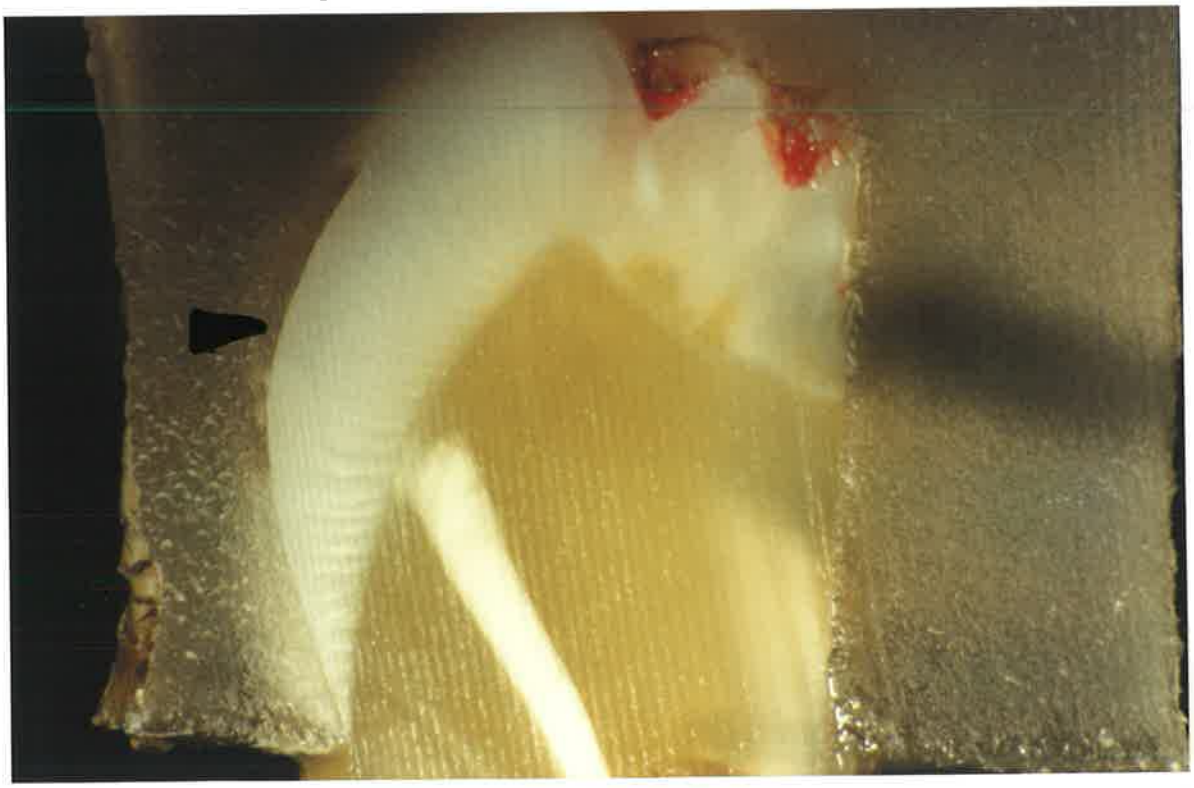


Figure 36(b). Cross-section of corresponding test specimen showing effect of fluoride in inhibiting progress of erosion for the same exposure period to the test solution.

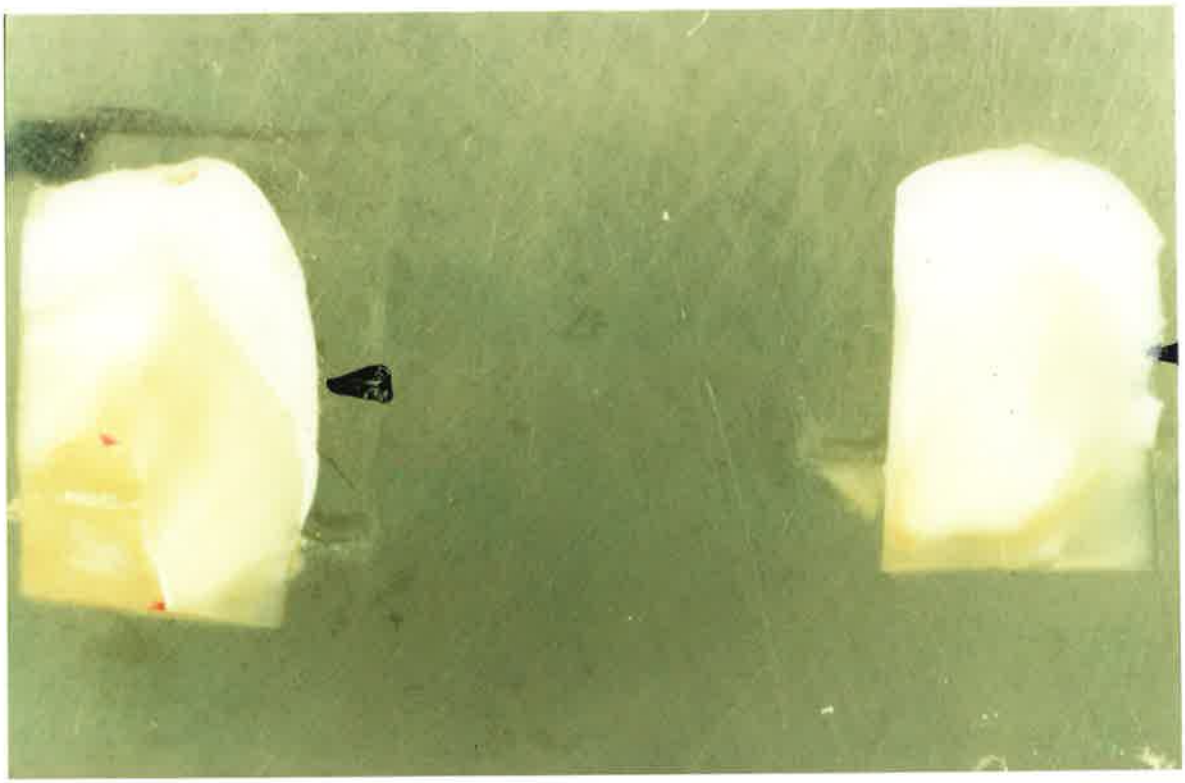


Figure 37. Cross-sectional views of representative control and test specimens from experiment A showing effect of fluoride in inhibiting progress of erosion (left : test specimen, right: control specimen).

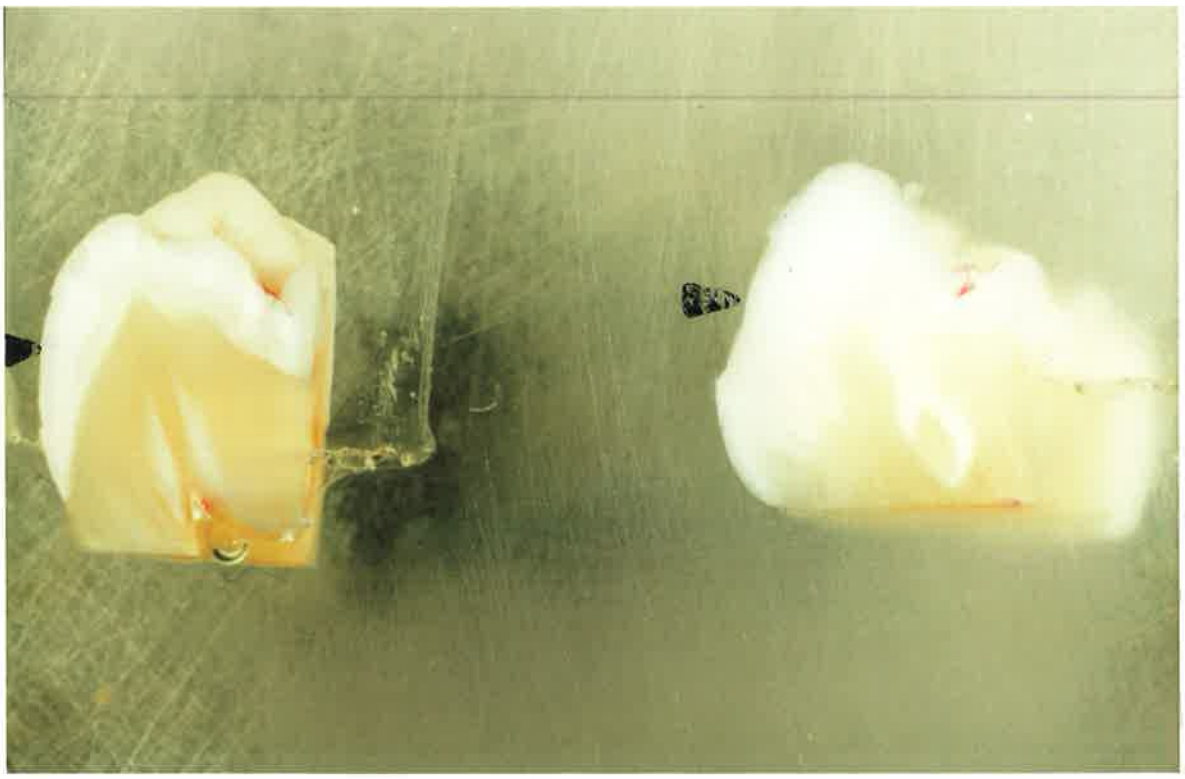


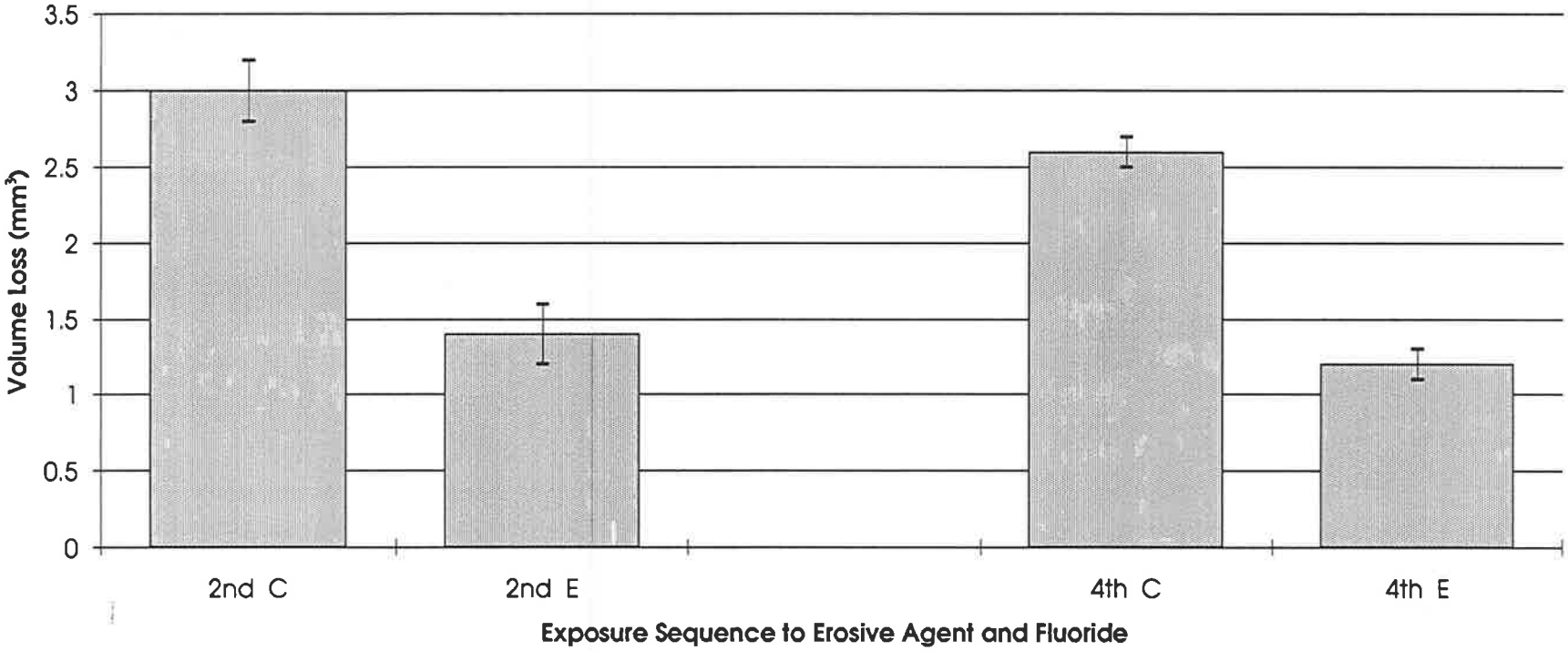
Figure 38. Cross-sectional views of representative control and test specimens from experiment B showing effect of fluoride in inhibiting progress of erosion (left : test specimen, right : control specimen).

TABLE 8.

VOLUME LOSS ESTIMATIONS USING WAX REPLACEMENT METHOD FOR THE "INTERMITTENT EROSION" METHOD

Specimen	*Ave Volume Loss (mm^3) ±sd*
2nd C	3.0 ± 0.2
2nd E	1.4 ± 0.2
4th C	2.6 ± 0.1
4th E	1.2 ± 0.1

Figure 39. RELATIONSHIP BETWEEN VOLUME LOSS ESTIMATIONS USING THE WAX REPLACEMENT METHOD AND EXPOSURE TIME TO TEST SOLUTION FOR THE "INTERMITTENT EROSION" MODEL



6.3 Profile of Changes in Hardness Across the Erosive Lesions

At the surface of the erosive lesion, the percentage of volume mineral loss was greater. Measurements extending 150μm further into the tooth structure appeared to show an increase in the percentage of volume mineral content which remained fairly stable and even throughout (see Table 9, 10 and Figures 40 and 41). The decrease in surface hardness seemed only to be evident at the superficial surface of the erosive lesion and did not progress further into the underlying enamel. This appeared to be so in all the specimens, control and experimental, from both methods of erosion, cyclical and intermittent. No conclusion could be drawn as to whether fluoride application resulted in increased hardness or conversely, increased porosity beneath the eroded surface.

It should be noted that the graphs of mineral density were drawn to represent the loss of mineral evident in the various specimens. That is, when fluoride was not used, approximately 0.5mm of enamel was lost, representing zero density over this depth. The surface mineral density profiles of the remaining enamel were then recorded.

TABLE 9.
VOLUME % MINERAL ESTIMATIONS FROM MICROHARDNESS VALUES FOR THE "CYCLICAL EROSION" MODEL

Specimen	*Depth below original surface (μm)*	*Average Vol % Min ± sd*
2m C	0	0
	500	41.98 ± 10.97
	525	74.49 ± 22.91
	550	81.45 ± 3.65
	575	81.49 ± 4.08
	600	76.31 ± 13.04
	625	83.65 ± 0.33
2m E	0	0
	125	57.78 ± 23.52
	150	79.49 ± 6.92
	175	69.9 ± 12.58
	200	86.4 ± 8.46
	225	85.87 ± 4.01
	250	87.7 ± 6.09
4m C	0	0
	500	46.39 ± 11.61
	525	100.74 ± 13.6
	550	86.72 ± 3.66
	575	92.47 ± 7.22
	600	83.48 ± 0.62
	625	82.47 ± 3.17
4m E	0	0
	125	58.78 ± 12.75
	150	85.07 ± 7.67
	175	86.17 ± 4.35
	200	81.54 ± 4.24
	225	89.53 ± 10.8
	250	85.68 ± 6.41
8m C	0	0
	500	48.11 ± 0.93
	525	76.58 ± 13.32
	550	79.72 ± 3.45
	575	83.72 ± 0.21
	600	83.55 ± 0.50
	625	88.36 ± 3.92
8m E	0	0
	125	43.29 ± 4.23
	150	89.38 ± 10.71
	175	75.17 ± 20.39
	200	89.28 ± 10.96
	225	87.42 ± 7.15
	250	87.83 ± 7.11
12m C	0	0
	500	53.34 ± 2.10
	525	80.07 ± 12.12
	550	89.56 ± 3.68
	575	80.61 ± 3.69
	600	86.07 ± 5.06
	625	86.05 ± 5.08
12m E	0	0
	125	42.63 ± 12.64
	150	74.92 ± 24.10
	175	86.24 ± 4.92
	200	83.7 ± 6.86
	225	84.71 ± 1.23
	250	84.19 ± 1.00

TABLE 10.

VOLUME % MINERAL ESTIMATIONS FROM MICROHARDNESS VALUES FOR THE "INTERMITTENT EROSION" MODEL

Specimen	*Depth below original surface (μm)*	*Average Vol % Min ± sd*
2nd C	0	0
	500	43.86 ± 12.79
	525	73.13 ± 3.26
	550	81.57 ± 7.80
	575	81.88 ± 7.93
	600	83.09 ± 6.62
	625	82 ± 3.18
2nd E	0	0
	125	43.36 ± 12.88
	150	80.32 ± 7.69
	175	87.08 ± 3.94
	200	87.2 ± 5.82
	225	87.57 ± 3.84
	250	85.28 ± 3.50
4th C	0	0
	500	57.49 ± 3.32
	525	84.63 ± 6.90
	550	86.17 ± 4.99
	575	84.96 ± 6.19
	600	84.87 ± 7.34
	625	91.49 ± 0.44
4th E	0	0
	125	58.44 ± 2.00
	150	87.79 ± 5.30
	175	85.22 ± 9.81
	200	84.4 ± 8.74
	225	87.89 ± 5.18
	250	92.06 ± 10.28

Figure 40(a). RELATIONSHIP BETWEEN VOLUME % MINERAL CONTENT AND DEPTH FROM ORIGINAL TOOTH SURFACE FOR "CYCLICAL EROSION" MODEL : 2-MINUTE CYCLES OF EXPOSURE TO TEST SOLUTION

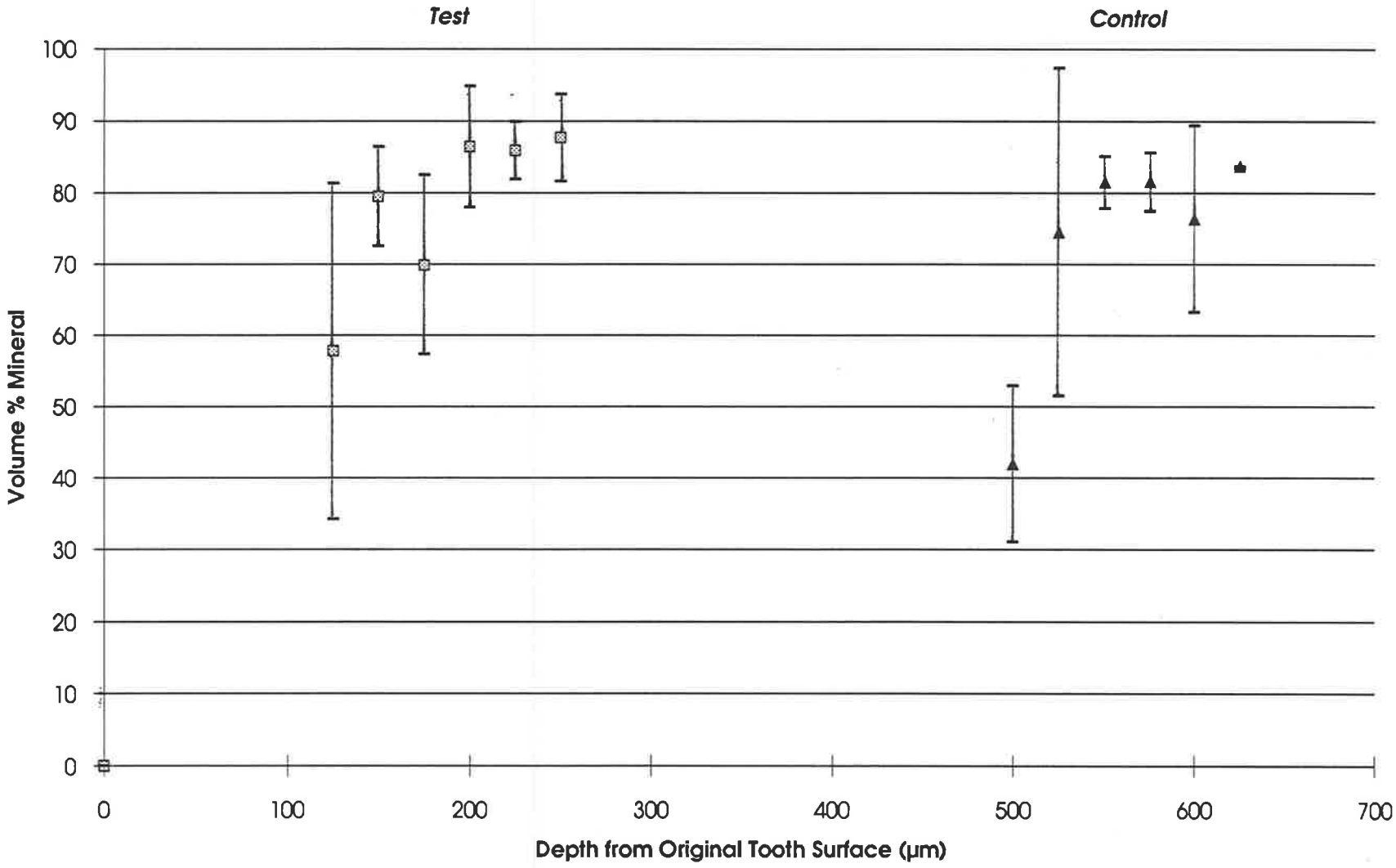


Figure 40(b). RELATIONSHIP BETWEEN VOLUME % MINERAL CONTENT AND DEPTH FROM ORIGINAL TOOTH SURFACE FOR "CYCLICAL EROSION" MODEL : 4 MINUTE CYCLES OF EXPOSURE TO TEST SOLUTION

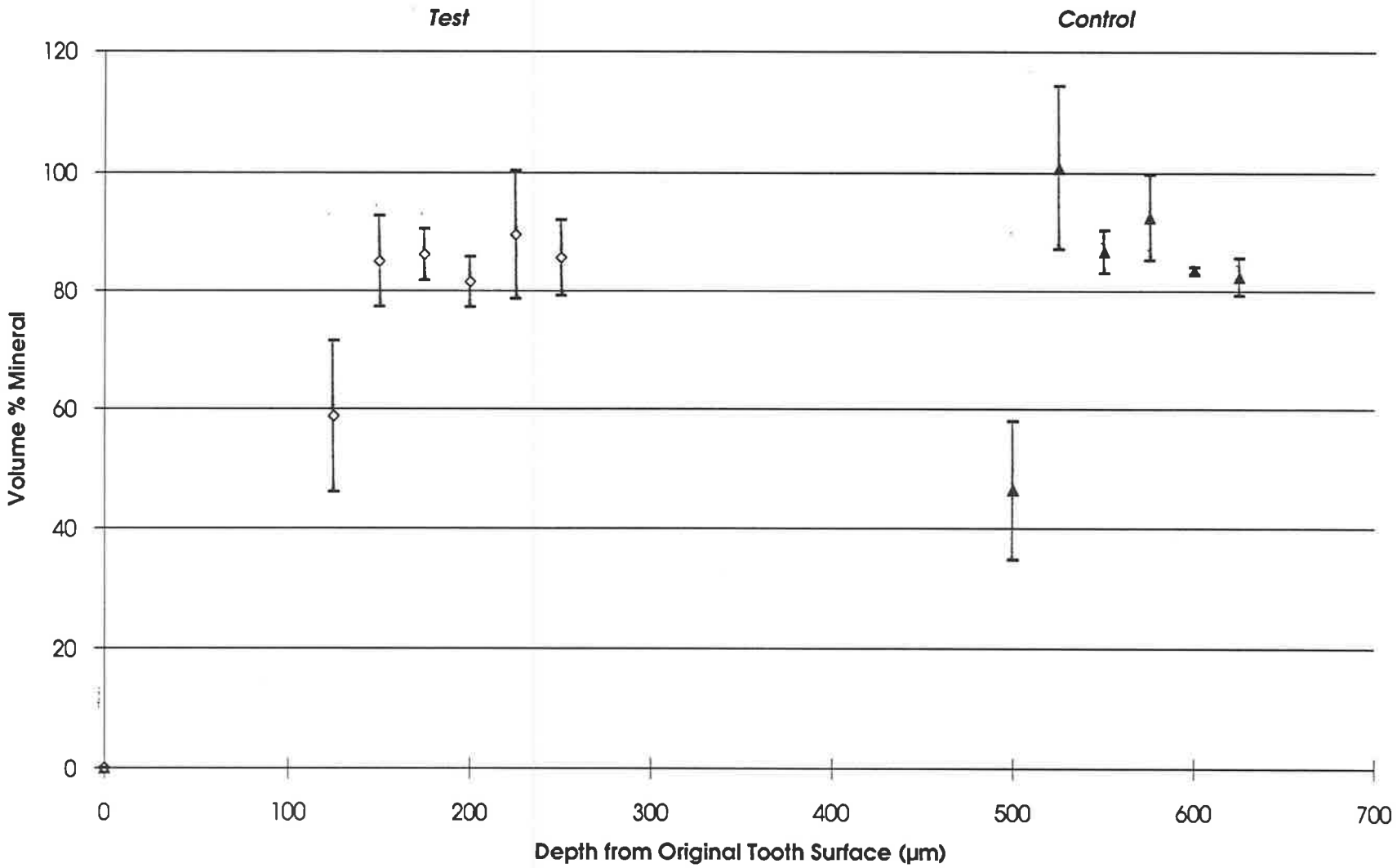


Figure 40(c). RELATIONSHIP BETWEEN VOLUME % MINERAL CONTENT AND DEPTH FROM ORIGINAL TOOTH SURFACE FOR "CYCLICAL EROSION" MODEL : 8-MINUTE CYCLES OF EXPOSURE TO TEST SOLUTION

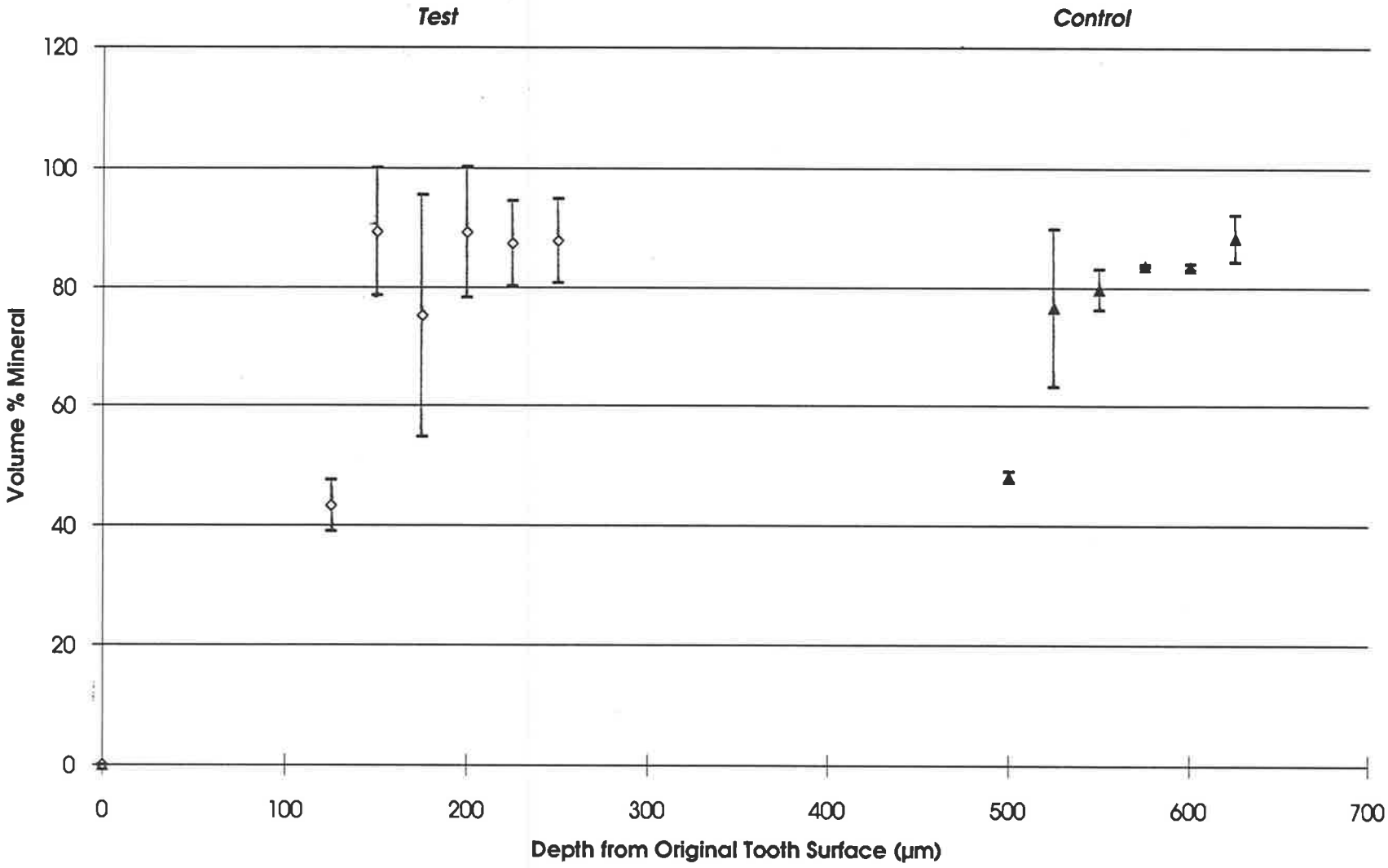


Figure 40(d). RELATIONSHIP BETWEEN VOLUME % MINERAL CONTENT AND DEPTH FROM ORIGINAL TOOTH SURFACE FOR "CYCLICAL EROSION" MODEL : 12-MINUTE CYCLES OF EXPOSURE TO TEST SOLUTION

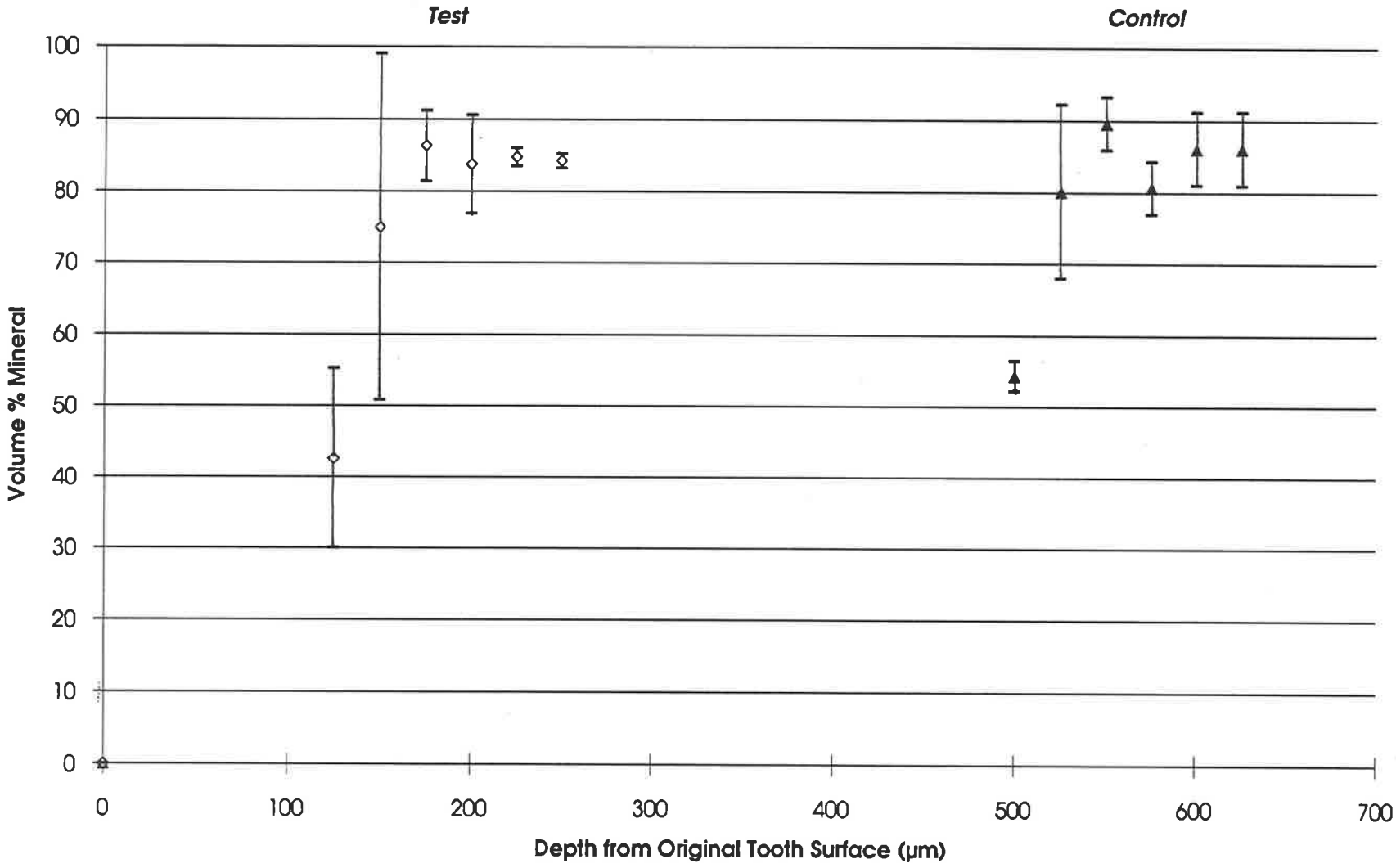


Figure 41(a). RELATIONSHIP BETWEEN VOLUME % MINERAL CONTENT AND DEPTH FROM ORIGINAL TOOTH SURFACE FOR " INTERMITTENT EROSION" MODEL : EXPERIMENT A

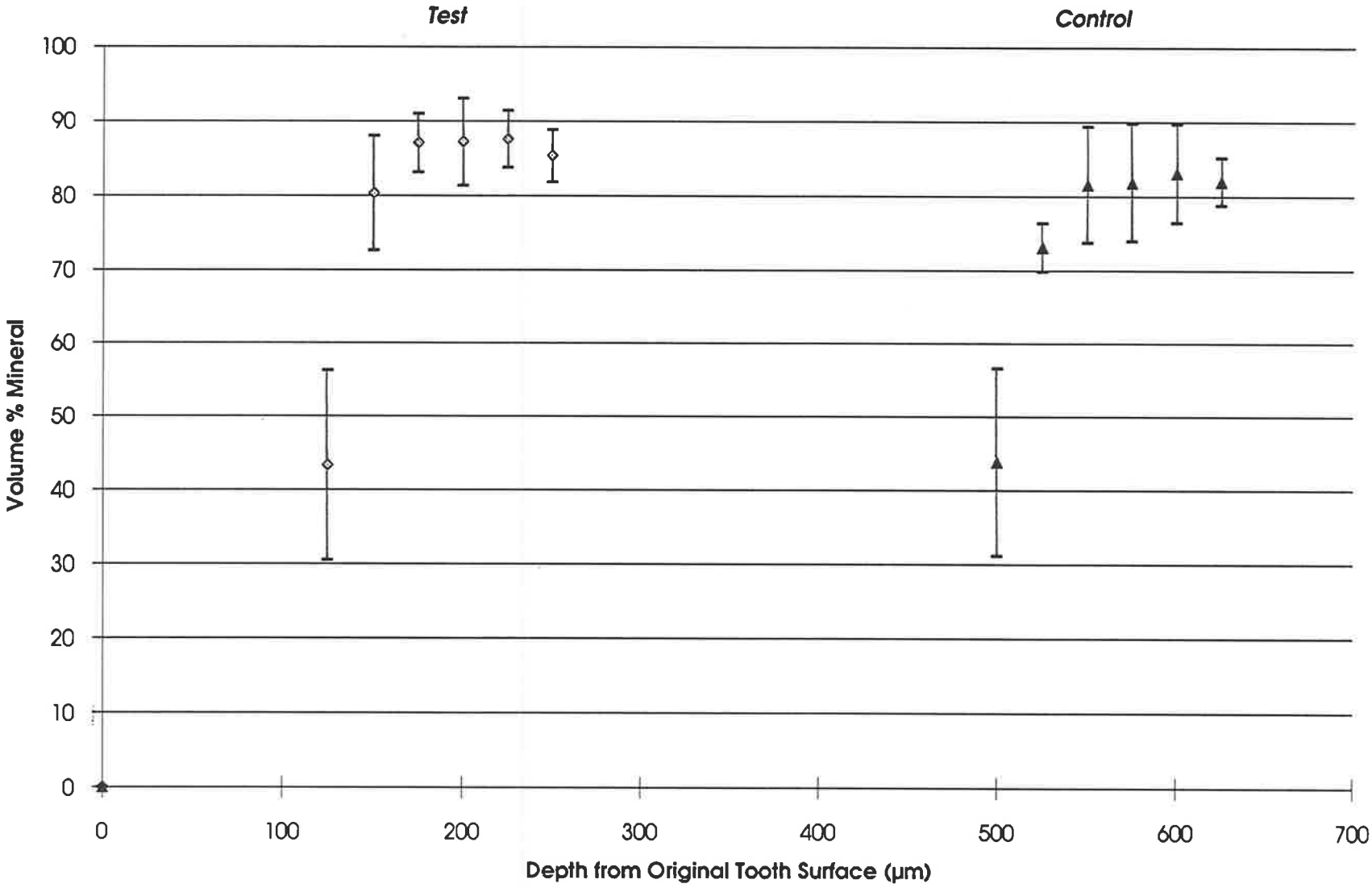
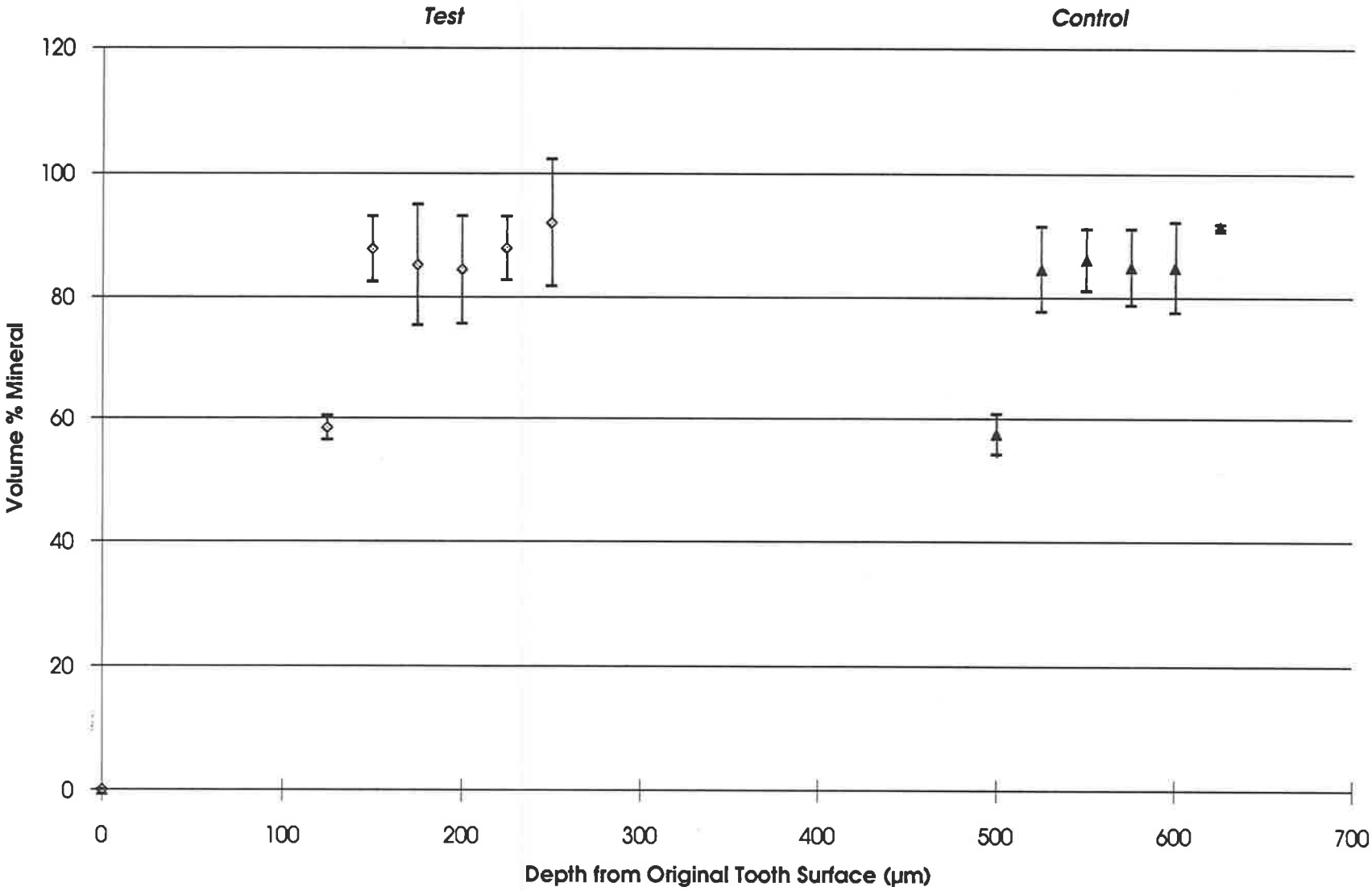


Figure 41(b). RELATIONSHIP BETWEEN VOLUME % MINERAL CONTENT AND DEPTH FROM ORIGINAL TOOTH SURFACE FOR "INTERMITTENT EROSION" MODEL : EXPERIMENT B



6.4 Polarised Light Estimations of Porosity of the Enamel Under the Erosive Lesion .

Control and Test Specimens from the two-minute Cyclical Model

The calculated results indicate that the erosion lesion contained only minor amounts of porosity, up to 3.65% of total volume at 10μm from the surface (see Table 11). Even the amount may be bordering on the limits of significance which may be obtained from these results, where even in deeper test points, similar differences were indicated. The measurements on normal enamel surfaces did not show such changes.

Control and Test Specimens from the two-minute Intermittent Model

The data here demonstrated a substantial degree of surface porosity, up to 10.2% of total volume at 10μm. The values remain relatively high and varied up to the depth of 60μm at 2.5% of total volume. The normal segment did not reveal any evidence of surface porosity of significance, though the readings were shown to be fluctuating through the depth of testing (see Table 12).

The results overall show a considerable variation in porosity levels, using this method, at various depths into the tooth enamel. Hence, the significance of the results at the surface may be questionable. Even so, the 10% pore volume recorded when topical fluoride had inhibited the depth of mineral loss, may be of significance, as will be discussed in the next chapter.

TABLE 11(a).

PORE VOLUME % ESTIMATIONS USING QUANTITATIVE POLARISED LIGHT MICROSCOPY FOR "CYCLICAL EROSION" MODEL : 2-MINUTE CYCLES OF EXPOSURE TIME TO TEST SOLUTION CONTROL SPECIMEN

Distance (μm)	Media RI	Compens	Reading	Difference	Difference / 2	Ehringhaus Ret (μm)	Birefringence R/Th x10^{-4}	Form Biref x 10^{-4}	Pore vol %Est
10	1.55	99	78.8	20.8	10.40	127	17	1.1	0.7
	1.62	99.2	79.1	20.1	10.05	119	15.9		
20	1.55	99.6	79	20.6	10.30	124	16.5	0.6	0.4
	1.62	99.4	79.3	20.1	10.05	119	15.9		
30	1.55	99.3	79.6	19.7	9.85	115	15.3	0.3	0.2
	1.62	99.3	79.4	19.9	9.95	117	15.6		
40	1.55	99.2	79.9	19.3	9.65	110	14.7	0.6	0.4
	1.62	99.4	79.6	19.8	9.90	115	15.3		
50	1.55	99.4	79.5	19.9	9.95	117	15.6	0.7	0.5
	1.62	99.3	79.7	19.6	9.80	112	14.9		
60	1.55	99.3	79.6	19.7	9.85	115	15.3	0.4	0.3
	1.62	99.2	79.6	19.6	9.80	112	14.9		
70	1.55	99.2	80	19.2	9.60	108	14.4	0.1	0.1
	1.62	99.1	79.7	19.4	9.70	109	14.5		
80	1.55	99	80.2	18.9	9.95	117	15.6	1.9	1.3
	1.62	99	80.2	18.8	9.40	103	13.7		
90	1.55	99.1	79.7	19.4	9.70	110	14.7	0	0
	1.62	99.2	79.9	19.3	9.65	110	14.7		
100	1.55	99.7	79.3	20.4	10.20	99	13.2	2.4	1.7
	1.62	99.4	79.4	20	10.00	117	15.6		
260	1.55	99.5	79.4	20.1	10.05	119	15.9	2.6	1.9
	1.62	100.4	78.7	21.7	10.85	139	18.5		
300	1.55	99.7	79.2	20.5	10.25	124	16.5	1.4	1
	1.62	100.1	78.8	21.3	10.65	134	17.9		

TABLE 11(b).

PORE VOLUME % ESTIMATIONS USING POLARISED LIGHT MICROSCOPY FOR "CYCLICAL EROSION" MODEL : 2-MINUTE CYCLES OF EXPOSURE TIME TO TEST SOLUTION WITH FLUORIDE APPLICATION AT EVERY CYCLE : TEST SPECIMEN

Distance (μm)	Media RI	Compens	Reading	Difference	Difference / 2	Ehringhaus Ret (μm)	Birefringence R/Th x 10^{-4}	Form Biref x 10^{-4}	Pore vol % Est
10	1.55	99.9	80	19.9	10	117	13.9	4.5	3.65
	1.62	97.6	81.2	16.4	8.2	79	9.4		
20	1.55	101.7	77.1	24.6	12.3	177	21.1	2.1	1.5
	1.62	101.4	78	23.4	11.7	160	19		
30	1.55	101.9	76.7	25.2	12.6	185	22	2.5	1.8
	1.62	103	76.4	26.6	13.3	206	24.5		
40	1.55	102.3	76.9	25.4	12.7	188	22.4	1.4	1
	1.62	102.5	76.4	26.1	13.1	200	23.8		
50	1.55	102.6	76.6	26	13	197	23.5	0	0
	1.62	103	77	26	13	197	23.5		
60	1.55	102.2	76.6	25.6	12.8	191	22.7	0.7	0.5
	1.62	102.3	77.2	25.1	12.6	185	22		
70	1.55	102.8	76.5	26.3	13.2	203	24.2	4.6	3.4
	1.62	101.5	77.7	23.8	11.9	165	19.6		
80	1.55	102.6	76	26.6	13.3	206	24.5	5.5	4
	1.62	101.2	77.8	23.4	11.7	160	19		
90	1.55	102.7	76.1	26.6	13.3	206	24.5	5.1	3.8
	1.62	101.1	77.5	23.6	11.8	163	19.4		
100	1.55	103.1	76	27.1	13.6	216	25.7	6.7	5
	1.62	101.1	77.7	23.4	11.7	160	19		
200	1.55	100	78.6	22.4	11.2	147	17.5	5.5	4
	1.62	98.7	80.1	18.6	9.3	101	12		
300	1.55	99.7	79.3	20.4	10.2	122	14.5	2.3	1.6
	1.62	100.5	78.5	22	11	141	16.8		

TABLE 12(a).

PORE VOLUME % ESTIMATIONS USING QUANTITATIVE POLARISED LIGHT MICROSCOPY FOR "INTERMITTENT CYCLIC EROSION" MODEL : 2-MINUTE CYCLES OF EXPOSURE TIME TO TEST SOLUTION CONTROL SPECIMEN

Distance (μm)	Media RI	Compens	Reading	Difference	Difference / 2	Ehringhaus Ret (μm)	Birefringence R/Th x 10^{-4}	Form Biref x 10^{-4}	Pore vol % Est
10	1.55	106.3	73.2	33.1	16.6	320	19	2.6	1.9
	1.62	106.8	71.5	35.3	17.7	363	21.6		
20	1.55	106.6	72.3	34.3	17.2	343	20.4	0.4	0.3
	1.62	106.8	72.8	34	17	336	20		
30	1.55	106.7	72.3	34.4	17.2	343	20.4	0	0
	1.62	106.7	72.4	34.3	17.2	343	20.4		
40	1.55	107.2	72.1	35.1	17.6	359	21.4	1.4	1
	1.62	106.4	72.5	33.9	17	336	20		
50	1.55	107.4	71.3	36.1	18.1	380	22.6	3.3	2.5
	1.62	106.2	72.8	33.4	16.7	324	19.3		
70	1.55	107.5	71.6	35.9	18	376	22.4	2.6	1.9
	1.62	106.2	72.5	33.7	16.9	332	19.8		
100	1.55	107.5	71.2	36.3	18.2	384	22.9	0.3	0.2
	1.62	107.4	71.3	36.1	18.1	380	22.6		
150	1.55	108.1	70.8	37.3	18.7	405	24.1	0.2	0.1
	1.62	108.1	70.8	37.5	18.8	409	24.3		
200	1.55	108.4	70.6	37.8	18.9	414	24.6	0.3	0.2
	1.62	108.1	70.6	37.5	18.8	409	24.3		
300	1.55	108.1	70.9	37.2	18.6	401	23.9	1.2	0.8
	1.62	108.4	70.3	38.1	19.1	422	25.1		

TABLE 12(b).

PORE VOLUME % ESTIMATIONS USING QUANTITATIVE POLARISED LIGHT MICROSCOPY FOR "INTERMITTENT CYCLIC EROSION" MODEL : 2-MINUTE CYCLES OF EXPOSURE TIME TO TEST SOLUTION WITH FLUORIDE APPLICATION AT EVERY SECOND CYCLE : TEST SPECIMEN

Distance (μm)	Media RI	Compens	Reading	Difference	Difference / 2	Ehringhaus Ret (μm)	Birefringence R/Th x 10^{-4}	Form Biref x 10^{-4}	Pore vol % Est
10	1.55	196.3	73	33.3	16.7	324	19.3	12.9	10.2
	1.62	99.1	80	19.1	9.6	108	6.43		
20	1.55	106.7	72	34.7	17.3	347	20.7	12.7	10
	1.62	99.7	78.3	21.4	10.7	134	8		
30	1.55	106.7	72.4	34.3	17.2	343	20.4	9.6	7.2
	1.62	101.9	77	24.9	12.5	182	10.8		
40	1.55	107.2	71.7	35.5	17.8	368	21.9	7.5	5.6
	1.62	103.6	74.8	28.8	14.4	242	14.4		
50	1.55	107.1	71.8	35.3	17.7	363	21.6	4.8	3.5
	1.62	105	73.8	31.2	15.6	283	16.8		
60	1.55	107.4	71.6	35.8	17.9	372	22.1	3.3	2.5
	1.62	106.2	73.2	33	16.5	316	18.8		
80	1.55	107.4	71.8	35.6	17.8	368	21.9	1.7	1.1
	1.62	106.4	72.2	34.2	17.1	340	20.2		
100	1.55	107.1	71.6	35.5	17.8	368	21.9	2.1	1.5
	1.62	106.4	72.7	33.7	16.9	332	19.8		
150	1.55	106.9	72.2	34.7	17.3	347	20.7	1.4	1
	1.62	107.4	71.6	35.8	17.9	372	22.1		
200	1.55	107.8	71	36.8	18.4	392	23.3	0.9	0.5
	1.62	107.4	71.5	35.9	18	376	22.4		
300	1.55	107.5	71.2	36.3	18.2	384	22.9	0	0
	1.62	107.6	71.3	36.3	18.2	384	22.9		

CHAPTER 7

DISCUSSION

7.1 The Study of Enamel Erosion

Dental erosion has only recently been recognised as a significant cause of tooth destruction. Extensive research has been conducted into dental caries, its mechanism, nature, kinetics, macroscopic, microscopic and histological analyses, and control. In contrast, there has been much less research conducted for dental erosion. Much of the knowledge and information on dental erosion has been a result of research methods similar to those used on caries. Numerous studies have been done to determine the various food substances and drinks which may cause dental erosion. Much has also been documented on the variety of endogenous causes of erosion. However, less work has been done to assess the outcomes of erosive demineralisation of enamel, and the effectiveness of prevention measures.

Not only are the mechanisms and effects of dental erosion difficult to investigate clinically, but difficulties also arise in the study of erosion "in vitro". Various "in vitro" and "in vivo-in vitro" model systems have been developed to aid in its study. Difficulties arise from the shortcomings of the various models to simulate closely the clinical situation of dental erosion and to account for as many if not all the intrinsic intra-oral protective factors and mechanisms. The quantity of tooth enamel lost as a result of erosive demineralisation is often small and diffuse, making quantitative measurements and analyses a difficult task.

In this present study, the aim was to investigate several aspects of the generation and control of endogenous enamel erosion using "in vitro" models. The "continuous erosion" model was developed to more accurately simulate clinical erosion with respect to the actual length of exposure time to the acidic challenge, and to permit testing of the topical application of 1.23% APF Gel to inhibit the erosive process using various frequencies of fluoride application. The second specific objective was to trial and evaluate the efficacy of a

variety of methods which might enable accurate measurement of volume of mineral lost from erosion, and which would permit analysis of the surface quality of the residual enamel at the base of the erosion lesion to be determined. These objectives were largely reached, being limited mainly by time and equipment available.

7.2 Development of the Endogenous Erosion Model

The "continuous erosion" in vitro model developed by Hunt and McIntyre (personal communication), provides a useful and rapid method of simulating the accumulative effects of erosive demineralisation seen intraorally. However, its major limitation is that it does not represent the periodic nature of erosive acid attack experienced by patients suffering the effects of endogenous erosion.

Dental erosion is normally a sporadic occurrence, with the acid affecting the tooth surfaces intermittently for a short period of time, either on a frequent basis or less frequently, and not continuously. Gastric acid would normally come into the mouth and be in contact with the tooth surfaces for only a few minutes before being neutralised by the salivary action. The inherent properties of saliva play a major role in guarding against the erosive challenge, and hence, the tooth surfaces are never exposed to an acidic environment for say, two hours at a stretch. In the mouth, any intake of a drink or foodstuff will be instantaneously mixed with saliva, resulting in a subsequent rise in the pH of the oral environment. So in fact, the pH only stays low for a very short period of time, probably of the order of one minute (Meurman et al., 1987). In addition, acidic drinks have been shown to stimulate salivary secretion, which in turn facilitates the buffering systems in the oral fluid (Tenovuo and Rekola, 1977).

Hence, it was decided to expose the tooth samples to the test erosive solution for time periods of only 2, 4, 8, or 12 minutes before thoroughly washing and drying the specimens. This was later again modified to provide a suitable and practical means of testing the effect of various fluoride application regimens in controlling the rate of progress of enamel

erosion, namely, their application after differing intervals of brief erosive challenge.

Unfortunately, it was not possible to determine quantitatively, the comparative time to reach a similar degree of mineral loss from enamel between the continuous and cyclic models, due to the need to change to a different method of estimating incremental stages of erosion in the cyclic model. However, a qualitative judgement of the time taken to reach a depth of 0.5mm of erosive demineralisation suggested that the "endpoint" may be reached slightly more quickly using the cyclic model. Approximately 200 minutes of accumulated 2-minute erosion periods in the cyclic model resulted in a depth of erosion similar to that seen from 4 hours (240 minutes) in the continuous model. The possible reasons are that, in the continuous erosion model, the sample remains still, and there is an obvious build-up of reacted products at the tooth surface. This possibly comprises of various acidic calcium phosphate salts, which would inhibit the diffusion of acidic ions into the enamel surface. In the cyclic model, these loose aggregations of salts are washed away, as they may be in the mouth by salivary or rinsing action. From this point of view, the cyclic model possibly more closely simulates the normal endogenous erosive process.

The selection by Hunt and McIntyre (1992) of the concentration of 0.06M HCl as representing the likely concentration of refluxed or vomited gastric acid hitting the lingual surfaces of the maxillary teeth, appears to be well justified. Hydrochloric acid is produced by the acinar cells lining the stomach at a concentration of approximately 0.12M (pH 1.1). The actual pH of gastric content reaching the oral cavity is not recorded, but is likely to be somewhat higher due to various dilution factors. Further research into this precise pH range of gastric acids reaching the oral cavity would help resolve this unknown. The concentration used also permits simulation of erosion experimentally, over a suitable time span, for example, "in vivo-in vitro" studies. Sufficient depth of erosion can be obtained over a three week period, the limit of time most subjects will wear appliances.

The other major factor affecting erosive demineralisation of teeth is the action of saliva. This was partly simulated by the addition of levels of calcium and phosphate ions into the erosive acid, similar to those seen intraorally. However, more precise accounting for

this factor would require use of an in vitro-in vivo model of endogenous erosion, as developed by Lekkas, Hunt and McIntyre (1992). This introduces a great number of complexities beyond those met in the use of the cyclic erosion model. Hence, it is proposed that the cyclic endogenous erosion model, as developed in this study, represents the most suitable method of simulating endogenous erosion, for the purposes of exploring parameters such as rate and mechanism of enamel erosion, and testing effectiveness of potential methods to protect teeth against erosive acids. The results gained can then be tested in "in vitro-in vivo" experiments. Overall, the "cyclic" model of endogenous erosion as developed in this study, was considered a very effective method of simulating clinical erosion "in vitro".

7.3 Estimation of Efficacy of Methods Tested to Measure Rate and Degree of Erosive Demineralisation, and Resulting Properties of the Eroded Enamel Surface.

7.3.1 Estimation of Volume of Enamel Lost Through Erosion

This has been a major problem in both clinical and laboratory studies of erosion. Yet, in order to understand the mechanisms and the rate of enamel mineral loss with time, it is necessary to have a reliable method for quantitation of volume of mineral loss. Such a method need also not to be too laborious, as large numbers of specimens are usually involved in such experiments. The methods which involve measuring the depth profile in one cross-section using profilometry, and those which involve sectioning impressions to digitise residual surface profiles, are extremely time consuming. Hence, it was considered initially that the **wax replacement method** may be the simplest, least time consuming method available in the local laboratories. The variability of results using this method related to a number of factors. These included:

(i) The small number of specimens used.

(ii) Lack of a record of the original surface profile.

(iii) Variability of volume loss between specimens within the same time category.

This resulted from :

(a) Variable background history of the extracted teeth in terms of exposure to fluoride and other trace elements.

(b) The invasion by the erosive acids into and under the protective varnish in a number of specimens.

(c) The location of the windows nearer the cervical region of enamel, resulted in exposure of dentine in the more advanced categories of erosion. As dentine collagen remains relatively intact after demineralisation, provided the collagen remains hydrated, this would cause aberrations in actual volume loss.

(d) A tendency for the more cervically placed enamel to dissolve more quickly in erosive demineralisation than the thicker, more cuspally located enamel (also a personal communication, Hunt, 1993).

Overall, this method has promise of providing a suitable measure for volume of enamel loss, providing :

(i) Means of at least five samples can be taken for each category of time of erosion.

(ii) Erosion windows are kept in a location at a uniform distance between cusp tip and CEJ on each crown (preferably nearer the cusp tip so as dentine is not exposed).

(iii) An initial impression of the tooth surface is taken, and a means found for using a soft wax or setting gel to fill the space left following erosive demineralisation, thus filling material then becomes separable from the body of the impression.

Despite these precautions, it must be remembered that in the early stages of erosion, volume of mineral loss will be so small as to be impossible to measure accurately. Hence, other methods need to be used in conjunction with wax replacement.

The method of estimation of volume lost through measurement of depth of mineral loss in the very early stages of erosion, either through a surface profilometer or by microscopic estimation of depth through a sectioned profile view (following sectioning through the lesion) are also such methods. These assume that volume is proportional to depth, and that depth of demineralisation is constant across the eroded lesion. Neither assumptions were found to be correct in these studies. Profile views shown throughout this report show considerable variability in depth throughout most lesions. This was also emphasised by Hunt and McIntyre (1992). However, those methods may help to provide a comparative estimation of depth in the early stages of erosive demineralisation.

Gross estimation of mean depth by use of a graduated probe, as used in some of these studies for lesions of the order of 0.25mm depth and greater, while crude, provided a semi-quantitative measure for comparative estimations between specimens at the macroscopic level.

The need to also measure rate of initial surface change, for example, etching, and the difficulties met in obtaining reliable data on volume loss, led to the decision to revert to an entirely different system of rate measurement when using the cyclic model of erosion. Unfortunately, this meant that many comparisons between the continuous and cyclic methods of erosion could not be made. However, wax replacement was also used for some of the more advanced lesions in the latter "cyclic erosion" study. The use of the concept of measuring comparative stages of demineralisation with time, and thus establishing a ranking order of erosion, while only semi-quantitative, was considered a very useful method. It takes into account the following points.

(i) The time taken to show the first traces of surface etching, perhaps one of the most important stages to recognise in the erosion process. At this stage, no measurable volume of enamel is lost, and thus could not be read by any measure of volume loss, except that a profilometer would measure surface roughness. Profilometry often requires that the surface initially be planed flat, removing the fluoride-rich surface layer, and hence also has its disadvantages in reflecting the clinical outcomes of erosion.

(ii) The second stage of complete surface etching, was also considered a very important one, which could not be measured easily by other methods.

(iii) Possible variations in the rate of progress of erosive demineralisation over various time periods, and between specimens.

(iv) Still permits measurement of depth or volume of mineral loss to be made in the later stages of erosion, to enhance the comparative ranking of specimens.

Hence, while only semi-quantitative, this method was found to be useful in comparative studies between specimens and variability in erosive conditions. It is hoped that a more technologically advanced measure of volume loss will eventually be available, at costs affordable to researchers in erosion. A digital image analyser or ultrasound techniques, may provide such methods.

7.3.2 Estimation of Calcium Loss With Time

It was considered that, in "in vitro" studies, estimations of calcium loss with time may provide a more reliable measure of volume loss than any direct measure. This was on the basis that calcium is one of the most prevalent elements in the enamel mineral hydroxyapatite. A measure of the mass of calcium lost should be proportional to the mass of hydroxyapatite lost, and thus to the amount of enamel mineral lost. As enamel mineral constitutes 97% of the weight of enamel, the total weight of enamel lost should be able to be measured, and thus volume of enamel loss estimated from knowledge of the specific density of enamel.

These measures, as seen in Table 1 and 2, and Figure 22, show a consistent pattern of loss, except for the final measure (48 hours). It is difficult to explain this final result. The correlation of these readings with those from wax replacement data, is not good, particularly at the more advanced stages of erosion. This may be explained in a number ways.

(i) It is well known that nail varnish is semi-permeable to water, and presumably acidic ions in water. Thus, calcium may have been dissolved from the total tooth surface. This was not evident when surface profiles of hardness and

porosity were calculated from microhardness data and quantitative polarised light microscopy data of "normal" surfaces underneath the varnish. However, a small amount across the total specimen volume may have influenced the results.

(ii) When lesions reached into dentine in some specimens, apatite was dissolved from dentine, leaving collagen filling the volume until severe drying caused its shrinkage. This may affect the correlation between calcium and volume loss from enamel.

(iii) It is evident that some mineral is dissolved from the remaining enamel surface of the erosive leison. This would also affect the correlation.

It is not possible to say from the data presented, whether calcium loss is a possible alternative method of estimation of volume of mineral loss, even if a waterproof varnish is used and dentine is not involved. A more accurate measurement of volume loss would be necessary before any attempt at quantitative estimation of correlation can be made.

7.3.3 Analysis of Surface Changes to the Residual Enamel at the Base of the Erosion Lesion

7.3.3(a) Visual, Photographic and SEM Analysis

(1) *Visual examination*

Visual examination, using the stereomicroscope, provided useful information, particularly in the comparison of results between erosion categories. The presence of amorphous salts on the base of the lesions, particularly following use of the continuous erosion method, was an important observation. Also, the variable pattern of depth of erosion was evident from the stereomicroscopic examination. A further observation of interest was that, when topical fluoride inhibited erosive demineralisation, it resulted in the enamel surface looking very etched, almost severely hypoplastic. Yet, little enamel was lost from such surfaces. It may be that the increased porosity seen in such specimens when quantitative polarised light microscopy measurements are used, is seen visually in this way. This property needs further investigation.

(2) *Photographic Records*

Surface photography using both coloured and black and white films, provided a disappointing record of erosive lesion depth characteristics. Etching patterns were clearly revealed, but the depth profiles proved difficult to record despite widespread experimentation with lateral lighting and angling or tilting of the specimens. Only when lesions were vertically sectioned, did photography show sample depths of lesions.

(3) *SEM Analysis*

It was considered that for erosion lesion sequence analysis (that is, of the same specimen following increasing times of erosive acid challenge), the method of McCormack et al. (1991) should be used. This involved analysis of uncoated specimens, using low accelerating voltages. The clarity of the images obtained were quite inferior to those obtained by conventional techniques. This may be because of the lack of experience with this technique, or that the eroded surfaces were chemically activated by the acidic challenge. Even so, the

results provided some evidence of degradation similar to that seen by Silverstone (1975). In retrospect, it may be preferable to take elastomeric impressions of the eroded specimens and make dies of these for metal coating for traditional SEM analysis.

7.3.3(b) Microhardness Testing

In the absence of microradiographic equipment, microhardness testing was used to determine whether there was evidence of residual demineralisation into the floor of the erosive lesion. The Knoop diamond method used had the disadvantage that measurements could be made only at 25µm from the lesion floor, and at 25µm intervals into the remaining enamel. Results indicated that any residual loss of hardness was only evident close to the base of the erosive lesion. Again, the results suffered from the availability of insufficient test specimens.

In retrospect, it would have been preferable to have used a "scratch test" method for estimation of hardness and a greater number of specimens. The results emphasise the characteristic of erosion of enamel, that it is largely a surface dissolution phenomenon when such strong acids are involved, in contra-distinction to what happens with weaker acids in generating carious lesions.

It is recognised that clinically, such eroded lesion surfaces would be exposed to the reparative processes of saliva following the acid attack. Thus, the results obtained do not represent those likely from clinically eroded surfaces. This measurement was made however, more to gain information on the mechanism of the erosive demineralisation process, than to represent what occurs clinically. The latter case would be simulated by exposure of the eroded surfaces to an artificial saliva.

7.3.3(c) Quantitative Polarised Light Microscopy

This method proved to be very time consuming, and the results obtained from sections of both normal and eroded specimens were masked by variable data from deeper

within the specimens. Major difficulties were as follows.

(i) Obtaining thin sections for polarised light microscopy (approximately 100μm thick) proved very difficult, and even when obtained, were easily fractured.

(ii) The obtaining of extinction points at varying depths with differing imbibing agents was largely subjective, and extremely time consuming.

(iii) Interpretation of the resulting data in terms of degrees of porosity under the lesion is difficult, and is based on assumptions which are currently being strongly challenged (Theuns et al., 1993).

(iv) Again, only a small number of specimens were able to be tested.

Despite these difficulties. if there was any validity of the surface data, it indicated that protection of enamel by topical fluoride may result in increased porosity into the surface enamel following protection by topical fluoride. This may not be surprising, as a high level of fluorapatite and fluoride-enriched apatite may be formed. This usually results in a certain degree of porosity because of the differing sizes of the fluorapatite and hydroxyapatite crystallites, and differing levels of crystallisation when fluoride is present (Frazier et al., 1967). The highly hypoplastic appearance of such lesions, as discussed previously, supports this interpretation.

The measures of porosity by polarised light microscopy and density by microhardness analysis are clearly differing physical properties, though some degree of co-relationship might be expected. The measurement of density by microradiography might provide further information on surface density profiles.

Summary of Efficacy and Usefulness of Measures of Residual Hardness and Porosity of Eroded Enamel

The difficulty and unreliability of quantitative polarised light microscopy make it a low priority choice in estimation of surface porosity. Perhaps the main measure of use would be radiodensity, and this would require access to microradiography. It is proposed that microradiography analysis of density profiles of mineral be explored in future tests of this

parameter.

Overall, the data presented indicate little residual damage to the enamel at the floor of the cavity, even when it has not been subjected to salivary repair. The porosity present at the surface appears to be confined to within around 50μm depth in most cases , and is thus probably easily reparable by salivary contact, as appears to occur with enamel which has been lightly etched with phosphoric acid.

7.4 Analysis of the Protective Effects of 1.23% Gel "In Vitro" Prior to Acid Exposure, on Degree of Erosive Demineralisation.

The development of the cyclic exposure model permitted testing of the ability of topical fluoride gel to inhibit erosion. As it is unrealistic to expext that APF Gel might be applied by a patient before every endogenous erosion episode, the further variations of intermittent fluoride application should model more closely what is clinically feasible. Also, it would lead to less concern about excessive ingestion of such concentrated gels. Surprisingly, the level of inhibition of erosion seemed significant even when applied after every 8 minutes (aggregate) of erosive challenge. When control specimens had been eroded to 0.5mm depth, those protected with fluoride gel had not even progressed to reach half that depth of enamel loss. This was equivalent to approximately one quarter the volume of enamel loss. The marked reduction could be seen very clearly by direct visual inspection and using stereomicroscopy. This finding gives some degree of hope to patients suffering from endogenous erosion, that it may be possible to slow down the rate at which susceptible enamel surfaces are dissolved away. It is essential that such tests also be carried out in an "in vivo-in vitro" model, such as developed by Lekkas et al. (1992), in order to test these findings in circumstances more closely resembling those found intraorally. Evaluation of effectiveness also needs a greater degree of quantitation of the data resulting.

The ability of topical fluoride gel to provide such a high degree of protection is surprising, when considering the strength of this erosive challenge (0.06M HCl at a pH of

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