THE AMOUNTS OF FLUORIDES (ALKALI-SOLUBLE AS WELL AS INSOLUBLE) GAINED ON AND IN ENAMEL OF THIRD MOLARS FROM A HIGH FLUORIDE AREA

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I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

Signature: ..... Date: 25/10/92.....

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CHAPTER I

i) OBJECTIVES

To determine the amounts of alkali-soluble and insoluble fluoride gained in/on *in vivo* enamel of erupted as well as unerupted human third molars from subjects with a high fluoride background, (F^->1,8ppm). The subjects had, throughout their lives, received fluoride mainly through drinking water with no additional fluoride supplementation other than possibly from the use of a fluoride toothpaste.

Furthermore, to compare the above results to those obtained in a similar survey in South Africa by Grobler and Kotze (1990) from subjects with a low fluoride background.

ii) HYPOTHESIS

It is hypothesised that the fluoride concentration of the enamel in a high fluoride area (F^->1,8 ppm) will be higher than that of a low fluoride area (F^- <0,10 ppm). Secondly, that the effect of topical applications of F^-, eg. by way of toothpaste, will be different to that from a low fluoride area. The amount of alkali soluble and insoluble fluoride on the surface enamel will be different to that from a low fluoride area.

iii) SUMMARY

A total of 25 third molar teeth (erupted [9], as well as unerupted [16]), from subjects

who had lived continuously since birth in an area where the water fluoride concentration was more than 1,8 ppm, were studied. (The range was 1,8 ppm - 2,64 ppm of F^-). The subjects had no systemic fluoride supplementation. Tooth brushing with a fluoride-containing dentifrice and, perhaps, occasional fluoride mouth rinsing was the only additional exposure to fluoride.

The acid-etch biopsy technique was used to determine the fluoride and calcium concentrations at various depths on the enamel surface. The fluoride concentration of the buffered etch solution was determined with an adapted fluoride ion-selective electrode technique, and the amount of calcium by flame atomic absorption spectrophotometry.

Six consecutive etchings were done on the mesio-buccal and mesio-lingual cusps of each tooth; the teeth were then washed in an alkali and the same procedure repeated on the disto-buccal and disto-lingual cusps. The depth of etch of each biopsy was calculated assuming that human enamel contains 37% Ca and has a density of 2,95g/ml.

It was previously reported, (Grobler & Joubert, 1988), that the enamel fluoride levels of the mesio-buccal and mesio-lingual sides did not differ from that of the disto-buccal and disto-lingual sides.

The average etch depth and fluoride concentration value as calculated from the values for the two cusps per tooth were used for statistical analysis. The mean etch depths (μm) and mean enamel fluoride concentrations of alkali-washed and unwashed enamel of both erupted and unerupted teeth were tabled, together with the standard deviations and range for each etch. Contrary to the results obtained from a low F^- area, no significant difference ($p>0.05$) could be found in the etch depth between erupted and unerupted enamel in this study.

Graphs were plotted by a line fitted to the mean enamel fluoride concentration and mean etch depths values of unwashed erupted, unwashed unerupted, alkali-washed erupted and alkali-washed unerupted third molar teeth. These graphs were compared to the graphs obtained in a comparable study done by Grobler and Kotze (1990), on erupted and unerupted third molar teeth from a low fluoride area (F^- < 0,10 ppm).

Results indicate that the enamel fluoride concentration in the bulk of the enamel of teeth from a high fluoride area (> 1,8 ppm), is higher than that of teeth from a low fluoride area (< 0,10 ppm).

In contrast to the teeth from a low fluoride area, where there was a significant increase ($p<0.05$) in the fluoride concentration of the outer layer (± 4 μm) of erupted enamel when compared to that of the unerupted enamel, no notable increase in the F^- content of the enamel was observed in the present study of teeth from a high fluoride area ($p>0,05$). There was, in addition, no significant ($p>0.05$) difference between the enamel fluoride content of alkali-washed and unwashed, erupted and unerupted teeth, which showed that very little CaF_2-like material was gained by the enamel after eruption.

In both studies the subjects had brushed with a fluoride dentifrice for a period of 1 - 16 years. It was expected that this topical exposure would increase the surface enamel concentration in the high fluoride area similar to the increase found in the low fluoride area. However, this was not the case, and as all the teeth from the high fluoride area exhibited some degree of fluorosis, it was concluded that ***posteruptive fluoride uptake by fluorotic human enamel without severe enamel loss is limited.***

This is in agreement with work done by Richards, Fejerskov, Baelum and Likimani (1989).

CHAPTER II

The influence and distribution of fluoride on/in enamel.

(i) Historical background.

In the early years of this century, in Colorado U.S.A., attention was drawn to the occurrence of "mottled enamel", an abnormality in which the enamel, usually only of the permanent teeth, acquired an unsightly brown pigment (McKay, 1916).

After careful observation, it became clear that "mottling" only occurred in people born in, and who spent their childhood in, a certain well-defined geographical location or district. Teeth that were calcified during residency in other parts had a normal appearance, even after a prolonged stay later in the districts where pigmentation occurred endemically. It later became apparent that some of these areas had the same source of water supplies, and thus it was concluded that mottled enamel was probably caused by some yet unknown element in the drinking water. An analysis by routine methods at the time, however, failed to detect any known constituent common to water supplies which could cause mottling, but nearly twenty five years later, in 1931, chemical analysis of the water indicated that **fluoride** was found in a number of these water supplies (Jenkins, 1978).

At the same time, Smith (1932), found that when fluoride was added to the diet of rats, the effects on their teeth were similar to those produced by adding water from areas where mottling occurred, strongly supporting the view that fluoride was the agent responsible for the condition.

(ii) **Chemical nature and natural sources of fluoride.**

Fluorine, together with chlorine, bromine and iodine are elements known as "halogens", which are very widely distributed in nature, mainly in mineral form. Examples, in the case of fluorine, are fluorspar (CaF_2), cryolite ($AlF_3.3NaF$), and fluoro-apatite [$Ca_{10}(PO_4)_6F_2$] (Mellors, 1961; Driessens,1982).

Fluorine is an element with great electron-affinity (Murray,1976), and readily forms ionic compounds with metallic elements. As a halogen it presents unusual properties in that it readily forms reversible compounds with hydrogen ions (H^+) to produce hydrofluoric acid (hydrogenfluoride; HF). Depending on the pH of a solution, F^-(free fluoride), $H^+F_2^-$ or undissociated HF will be found therein as products of dissociation.

Fluoride (F^-) is the ionic form of fluorine. Since fluoride is very widely distributed in nature, and consequently is present in virtually all plants and in the bodies of all animals, it is present in varying amounts in all foodstuffs and in all ground waters. According to Jenkins (1978), it would be virtually impossible to prepare a fluoride-free diet. It is estimated that water containing 1 ppm of F^- contributes 1,0 - 1,5 mg of fluoride to the daily intake of adults (Jansen van Rensburg,1990).

(iii) **Fluoride metabolism.**

F^- and HF are the forms of fluorine of physiological importance in the body. The acid-base status of the body (blood) has a crucial effect on these two forms of fluoride. HF is the moiety for diffusion through cell membranes. The diffusion of fluoride (as HF) between body compartments is determined firstly, by the pH-gradient and secondly by the concentration gradient of F^- between the compartments. Fluoride tends to accumulate in the more alkaline compartment till a diffusion equilibrium for HF is established (Whitford, 1989). Fluoride also shows a great affinity for calcium-phosphate and will thus accumulate in calcified tissues.

The main sources of F^- intake for humans is by means of drinking water, food and F^- containing substances that are absorbed by the gastro-intestinal tract (GIT). Fluoride absorption takes place mainly in the stomach, and to a lesser extent in the duodenum (Whitford 1989; Priest & Van de Vyver, 1990). The reason for this is that compared to the rest of the GIT, the pH gradient, and also the concentration-gradient for F^- between the gastro intestinal lumen and the plasma, is the greatest in the stomach. This enhances rapid and almost complete absorption of F^-, (in the form of HF), from the stomach. The diluting effect of plasma and extracelluar fluids has the result that the plasmafluoride content, (plasma F^-), has no effect on the F^- absorption from the GIT (Whitford,1989).

Diet composition has an effect on F^--absorption, and absorption of F^- from fluoride containing substances is most complete on a relatively empty stomach. Calcium, phosphate, magnesium and aluminium form combinations with F^- that are less soluble, and thus can reduce absorption in some circumstances by up to 50% (Whitford,1989; Priest & van de Vyver, 1990).

Although milk is rich in calcium, the F^- absorption from milk, although slower, is still complete (Whitford,1989; Priest & van de Vyver,1990). The absorption of F^- from water varies from 90 - 100% (Whitford, 1989).

F^- can also be absorbed by the oral mucosa, eg. during a fluoride treatment by a dentist. The high fluoride content, (19-1000 $\mu g/g$), as well as the low pH of the topical agents used, create an ideal circumstance for the diffusion gradients of HF through the epithelial cells of the oral mucosa into the systemic circulation (Whitford,1989).

By means of diffusion of HF through the cell membrane, F^- can be absorbed by the lungs, (F^--containing dust particles, smoke from coal fires and polluted air), and in exceptional circumstances also through the skin (insecticides, industrial accidents) (Murray,1976).

As stated, absorbed fluoride rapidly enters the bloodstream, and it has been shown that approximately 75% of the fluoride is transported in the plasma, while 25% is bound to erythrocytes (Carlson,Armstrong & Singer, 1960). Fluoride exists in both ionic and bound forms in the plasma, with the bound form being present in larger quantity but physiologically unavailable. The unbound or ionic form varies according to the concentration of fluoride in the drinking water. When the water contains 0.25 ppm F^-, plasma may contain 0.01 ppm ionic fluoride, but when the water contains 1.2 ppm fluoride, the plateau value of ionic fluoride in plasma increases to 0.025 ppm (Ekstrand,1978), and is the only form of F^-in the plasma that has physiological value.

Although fluoride is excreted in faeces and sweat and is lost by way of hair, skin, and saliva, the kidneys are the most important organs for the excretion of fluoride, and are second only to plasma-bone-fluoride exchange as the most important regulator of the plasma fluoride concentration (Whitford, 1989).

The rapid excretion of F^- by the kidneys (20-30 % of an oral dose within 4 hours) is an

important safety mechanism in cases of severe fluoride poisoning (Mellberg & Ripa,1983; Jansen van Rensburg, 1990).

Accumulated evidence from numerous studies shows that the prolonged use of fluoride at recommended levels does not produce harmful physiological effects in the human except for dental fluorosis. This has been found true even when individuals consume drinking water containing up to 8,0 ppm F^-, - about eight times the recommended amount (Mellberg & Ripa, 1983). However, as for every chemical, there are limits for fluoride ingestion beyond which harmful effects occur. A summary list of the toxic effects of various levels of fluoride is given in Table 1. 2 (page 9).

Symptoms of acute toxicity occur rapidly, and present as diffuse abdominal pain, diarrhoea, vomiting, excess salivation, and thirst. Rapid measures to reduce fluoride absorption should be started by inducing vomiting and then large volumes of calcium as in lime, water or milk, should be given. Administration of aluminium hydroxide gels are exceptionally good for binding fluoride in cases of excessive ingestion (Smith & Hodge, 1959).

Chronic health problems of the soft tissues related to prolonged ingestion of fluoride in moderate excess have not been encountered, as fluoride does not accumulate in soft tissue and is in equilibrium with plasma fluoride content. When excessive fluoride, e.g. in drinking water containing more than 8,0 ppm F^-, is ingested daily for many years, symptoms of skeletal fluorosis may occur, eg., increased bone density, the joints stiffen and become painful with restriction of movement (Mellberg & Ripa, 1983).

Whitford (1990) discusses new information concerning the metabolism and toxicity of fluoride and quotes data from the first longitudinal study of the major pharmacokinetic features of fluoride that indicate that the ability of the skeleton to remove fluoride from extracelluar fluids declines rapidly during the period of growth.

During early infancy, 90% of a fluoride dose is taken up by calcified tissues; after growth has ceased, only 50% is retained by the skeleton. These findings have relevance to acute fluoride toxicity and to dietary fluoride supplementation schedules.

Whitford (1990) concludes that the "probable toxic dose" (PTD) of fluoride, i.e. the minimum dose that could cause toxic signs and symptoms, including death, and which should initiate immediate therapeutic intervention and hospitalization, is **5 mg/kg** of body weight. As currently packaged, many dental products contains sufficient fluoride to exceed the PTD for children.

Table 1. 2

Toxic Effects of Fluoride in the Human

Concentration or dose of	Medium	Effects
2 ppm or more	Water	Mottled enamel
5 ppm	Water	No osteosclerosis
8 ppm	Water	10% osteosclerosis
20-80 mg/day	Water or air	Crippling fluorosis
50 ppm	Food or water	Thyroid changes
100ppm	Food or water	Growth retardation
More than 125 ppm	Food or water	Kidney changes
2,5-5,0 g (acute dose)	Food or water	Death

[Data from Smith & Hodge,1959]

(iv) **Fluoride in saliva and plaque.**

Plasma levels of F^- remain relatively constant, and this is mainly ascribed to the rapid uptake of F^- by the skeleton and excretion by the kidneys (Priest & van de Vyver, 1990), although it may rise for a few hours after ingestion of a large dose of fluoride.

The F^- content of saliva and sulcular fluid, although in lower concentrations, still shows a positive correlation with the plasma F^- (Whitford,1989). The rapid rate of fluoride absorption is reflected in its appearance in the saliva soon after ingestion of measured fluoride doses; the peak concentration being after one hour (Shannon & Edmonds,1978).

See Figure 1. 2.

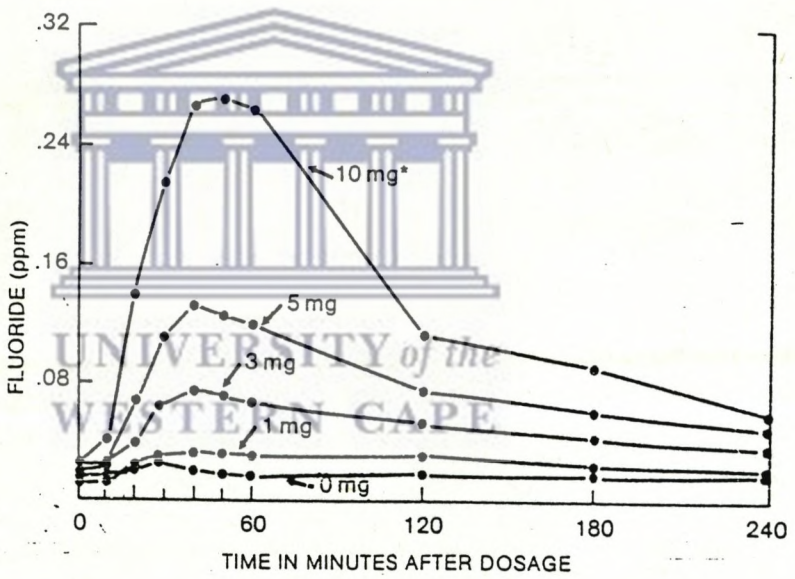

Figure 1. 2: Effect of exogenous fluoride on parotid fluid fluoride levels.

(Shannon & Edmonds, 1978)

* Oral fluoride dosage.

The normal concentration of fluoride in salivary gland secretions is about 1μmol/L and is always lower than the corresponding gingival fluid and plasma levels. An elevation of plasma F^- consequent to increased intake is accompanied by a corresponding increase in gingival fluid and saliva concentrations (Geddes & Bowen, 1990).

As fluoride is present in oral fluids, it accumulates in dental plaque. The sources of plaque fluoride include saliva, gingival fluid, certain foods and liquids in the diet, topically applied agents, and possibly demineralising dental hard tissue. The level of fluoride in plaque is usually 50-100 times more than that in the whole saliva, and is taken from the saliva as a function of pH gradients across the cell membranes, since most caries-inducing bacteria have a higher intracellular pH than the surrounding oral fluids. Fluoride in plaque exists in ionic and bound forms. The factors influencing the interconversions between ionic and bound forms are, to date, inadequately investigated and poorly understood (Geddes & Bowen, 1990).

It has been conjectured that fluoride from plaque can be released to interact with the underlying hard dental tissues to suppress demineralisation and enhance remineralisation. However, conclusive experimental evidence to support this is lacking. If plaque does act as a "store" of fluoride available for rapid release, then the factors which control the process and the concentrations of fluoride available for enamel protection or repair are of clinical importance (Geddes & Bowen, 1990).

Fluoride thus affects the potential cariogenicity of plaque in a number of ways:

High concentrations will eliminate sensitive bacterial populations. Sublethal concentrations will alter carbohydrate metabolism in ways which will include reduced acidogenicity and altered extracelluar, insoluble, polysaccharide production and possibly adhesion. In addition, sublethal concentrations of F^- may alter acid tolerance of *S. mutans* and other organisms, leading to a less acidogenic plaque flora (Geddes & Bowen, 1990).

The microbial ecosystems of dental plaque are complex, and the consequences of the plaque-mediated effects of fluoride on caries are multifactorial but, overall, can result in a slowing of the pH decrease and possibly influence the composition of the acid end-

products of sugar metabolism. If fluoride continues to be available, the reduced acidogenicity will, in turn, reduce the chance of population shifts within the plaque ecosystem which otherwise would favour domination by acidogenic and aciduric strains. These metabolic and ecological adaptations are lost if fluoride is withdrawn. (Geddes & Bowen, 1990)

The role fluoride plays in cariostasis by affecting plaque metabolism has been overshadowed in recent years by studies of its influence on remineralisation and demineralisation. Nevertheless, it is plausible that the anticaries effect of fluoride is partly biological (Geddes & Bowen 1990).

(v) **The distribution of fluoride in teeth.**

The influence and distribution of fluoride on/in teeth has been extensively studied ever since it became known that it has an anti-cariogenic effect and is the causative agent for "mottling" or fluorosis (McKay, 1916; Ainsworth, 1933; Dean, 1938; Weatherall, Deutsch, Robinson & Hallsworth, 1977; Richards, Fejerskov, Baelum & Likimani,1989b; Cutress & Suckling, 1990). The discovery of this unique property of fluoride in reducing caries so markedly had led to a great deal of research on its concentration in the oral structures and the effects which it exerts upon them. The fluoride content of enamel and dentine depends on the amount of fluoride ingested in drinking water and food during the mineralisation of the tooth (McClure & Likins, 1951; Brudevold, Gardiner & Smith 1956; Isaac, Brudevold, Smith & Gardiner,1958; Jansen van Rensburg,1990) as it was found that up to 90% of fluoride is deposited in the dental tissues prior to the eruption of the teeth. The plasma fluoride content is the fluoride source of the dental tissues. Thus the tissue with the higher and longer (over time) blood supply, will end up with the higher F^- content (Murray, 1976).

The concentration of fluoride in dentine is between two and three times that in enamel, and also increases in relation to the water F^- content. (See Table 2. 2)

Table 2. 2.

Fluoride concentration (ppm) in permanent teeth in relation to the fluoride content of the water supply.

Fluoride Conc. of water (ppm)	0,0 - 0,3	1,1 - 1,2	2,5 - 5,0
Enamel	100	130	340
Dentine	240	360	76

(Data from McClure & Likins, 1951)

Fluoride is not evenly distributed throughout the enamel, and it was found that the concentration of fluoride on the extreme outer surface of the enamel in permanent human teeth is sometimes ten times higher than that of the enamel as a whole, and decreases with each successive layer of enamel (Brudevold, Gardner & Smith, 1956; Isaac *et al.*,1958; Berndt & Stearns,1978).

Brudevold *et al.*,(1956), in an effort to understand the mechanism of action of fluoride to reduce dental caries, studied successive layers from the surface inward in the enamel of deciduous teeth, erupted as well as unerupted permanent teeth of different ages, and of teeth with "mottled" enamel.

Their findings were summarised as follows :

" In all these teeth a high concentration of fluoride was found in the outermost layer of enamel and a low level was found in the bulk of the enamel.

The findings suggest that small amounts of fluoride are laid down in the enamel during formation, and that only the outermost enamel picks up fluoride once calcification has been completed. Appreciable amounts of fluoride accumulate in the outer enamel of unerupted teeth. There also appears to be a posteruptive pickup. The outermost crystal layers of enamel may reach fluoride concentrations as large as 1 per cent. Deciduous teeth contain less fluoride in the outer enamel than permanent teeth.

Mottled enamel contains higher concentrations of fluoride than that of normal teeth at any distance from the enamel surface. The caries-reducing effect of fluoride is believed to be due to the high concentration of fluoride in the surface enamel.

Little protection is believed to be afforded by the low concentrations which were found in the bulk of the enamel."

Work done by Niibu, Nakagaki, Kuroso and Weatherell (1991), on the distribution of fluoride across human primary enamel, from the surface to the amelodentinal junction (ADJ), shows an interesting fluoride profile throughout the whole thickness of the primary enamel, including prenatal, neonatal and postnatal enamel, which is similar to that of permanent enamel - the highest concentration being found on the enamel surface and decreasing towards the interior. (See figure 2. 2.)

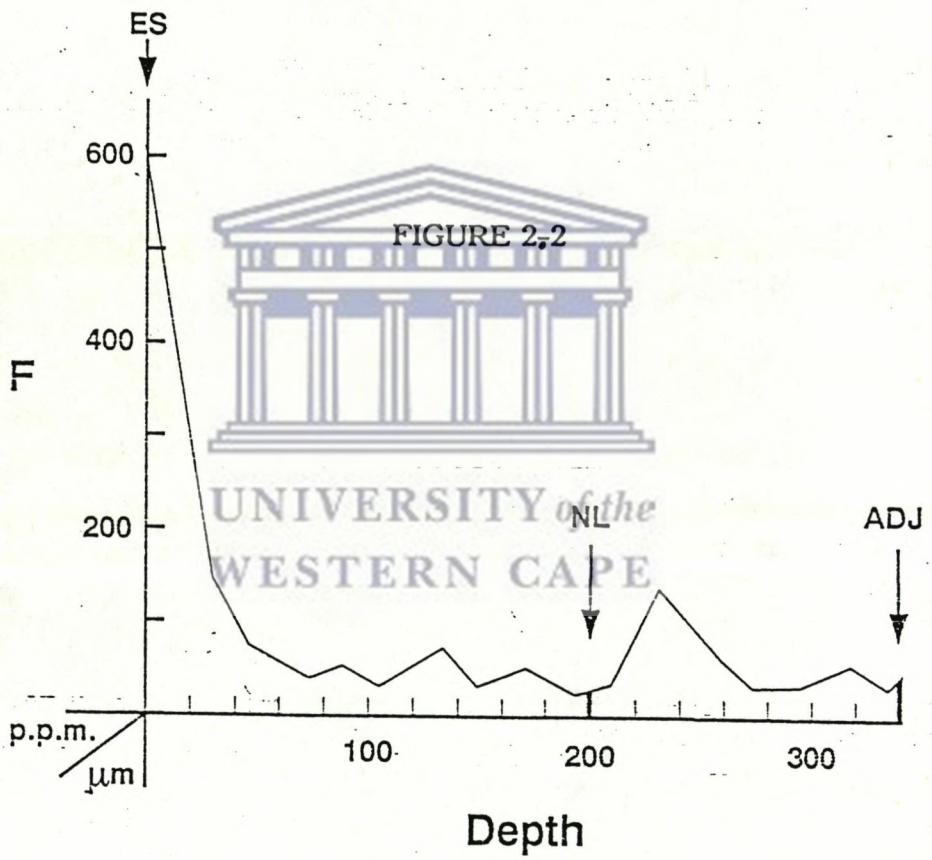

FIGURE 2.2

Figure 2. 2. : Profile of fluoride concentration across primary enamel. The Y-axis represents the fluoride concentration (ppm), and the X-axis the depth from surface to the interior (μm).

ES, enamel surface; NL, neonatal line; ADJ, Amelodentinal Junction.

(Niibu, Nakagaki, Kuroso and Wheatherall, 1991. p 604)

Jenkins and Speirs (1953) compared the fluoride content of the surface enamel to that of the subsurface enamel by etching with acetic acid, and found that the fluoride content of the surface enamel of fluorosed teeth was more than twice that of the surface enamel of teeth from a low fluoride area. These authors also concluded that all the fluoride of the enamel was acquired pre-eruptively and that the fluoride content does not vary with age. However, Brudevold *et al.*,(1956), and Isaac *et al.*, (1958), making use of a sampling procedure of grinding rather than etching, showed that post-eruptive uptake does take place and that it is thus desirable to take age into consideration. Weatherell *et al.*, (1977), stated that there is a continuous uptake of fluoride throughout the life of teeth, but that mature enamel absorbs fluoride with difficulty, although there is evidence that it does penetrate into the deeper layers. Brudevold *et al.* (1956), as well as Isaac *et al.* (1958), found that the highest concentration of fluoride is in the external or outer enamel and that there is a steady decrease from the surface inwards until a level is reached where the concentration remains fairly constant.

Weatherell,Robinson, Schaper and Kunsel (1983), found that the fluoride distribution in, and the enamel fluoride content of fluorosed teeth at any level from the surface was considerably different to that found in normal teeth. Recent literature (Richards *et al.*,1989; Cutress & Suckling, 1990) found the same trend in fluorotic enamel as opposed to sound enamel. This was also confirmed in the present study.

It was suggested by Brudevold *et al.* (1956), that fluoride deposition in the enamel takes place in three stages :

1. Small amounts are deposited throughout the enamel matrix during the period of calcification,
2. After calcification is completed, the bulk of the enamel loses contact with the tissue fluids and, as a result, the fluoride acquisition from tissue fluids is limited to the external surface. This part of the enamel continues to pick up fluoride until

eruption, and it is likely that the outer crystal layers of the enamel may reach a high degree of fluoride saturation in the pre-eruptive period, provided the fluoride level of the plasma is high enough and the period between tooth formation and eruption is sufficiently prolonged.

3. Fluoride uptake from drinking water, food, saliva, toothpastes and application of fluoride containing lacquers etc. continues throughout the life span of the erupted tooth, but is restricted to the more outer surface.

The authors also stated that there is no evidence that appreciable amounts of fluoride will penetrate more than 0,1mm (100μm) into intact enamel in the pre-eruptive or posteruptive period. Recent research (Grobler & Kotze,1990) in a low fluoride area, indicate that posteruptive F^- uptake would not exceed 20μm in sound enamel.

Results reported (Isaacs *et al.*, 1958), show that the inner enamel from unerupted and erupted teeth from persons from equivalent fluoride areas have a similar fluoride content. Since the amount of fluoride deposited in the enamel pre-eruptively is dependant on the fluoride level in the blood, it is conceivable that an increase in the fluoride level of the drinking water would result in an increase in the deposition of fluoride during tooth formation.

(vi) Dental fluorosis.

Dental fluorosis refers to developmental defects of the enamel induced by excessive fluoride intake. Clinically, in a mild form, it appears as white opacities over the enamel surface. Although severe fluorosis was first observed in the form of brown stains, it is now known that the defect is directly related to the concentration of fluoride in the drinking water or other excessive ingestion of fluoride, and takes different forms at different levels of fluoride intake. With increasing severity, the irregular opaque areas may merge until extensive areas appear chalky white, becoming pigmented later in severe cases (Jenkins, 1978).

The term "mottling" is only appropriate for the brown stains caused by a high intake of fluoride, and "fluorosis" is a preferable term to cover all grades of the defect.

Pitting can occur, either as minute depressions or as single or multiple cavitations indicating a loss of the outermost surface of the enamel. Pitting and larger surface destructions of enamel are post-eruptive features and not true hypoplasia of the teeth, and increase in severity with age (Jenkins, 1978).

Fluorosis occurs symmetrically within the dental arches, with the premolars being most affected, followed by the second molars, maxillary incisors, canines, first molars, and mandibular incisors (Mellberg & Ripa, 1983).

Dean, (1934) classifies fluorosis into six categories according to the severity of the condition, and rates each tooth according to a fluorosis index (figure 3. 2.). Fluorosis becomes apparent and is considered as "mild" from fluoride concentrations of 1 - 1,2 ppm in the drinking water, while concentrations of >4,0 ppm in the drinking water result in severe fluorosis.

TABLE 3. 2.

Dean's Dental Fluorosis Classification

Category	Dental Fluorosis score	Description of Enamel
Normal	0,0	Smooth, glossy, pale creamy white translucent surface
Questionable	0,5	A few white flecks or spots
Very mild	1,0	Small opaque, paper-white areas covering less than 25%
Mild	2,0	Opaque, white areas covering less than 50% of surface
Moderate	3,0	All tooth surfaces affected, brown stain, excessive attrition.
Severe	4,0	All tooth surfaces affected, brown stain, pitting.

(Data from Dean, 1934)

Several variations of Dean's classification have been developed to increase its sensitivity for diagnosing the severity of fluorosis, or to improve the recording procedure. Thylstrup and Fejerskov (1978) were the first to relate the histopathological features and the clinical appearance of fluorotic teeth. They examined the teeth of 120 children living in an area with 3,5 ppm F^- in the water supply and classified them into 9 grades of dental fluorosis.

Normal enamel was graded as 0, pre-eruptive changes in the degree of opacity 1-4, and posteruptive changes 5-9. They thus extended the range of scores of the Dean index from 6 to 9 categories and justified their Thylstrup-Fejerskov (TF) index ranking with histological evidence.

(See Table 4. 2.- page 20):

THYLSTRUP-FEJERSKOV INDEX (TF-INDEX)

Score :

0 The normal translucency of the glossy creamy-white enamel remains after wiping and drying of the surface.

1 Thin white opaque lines are seen running transversely across the tooth surface. Lines are found on all parts of the surface. The lines correspond to the position of the perikymata. In some cases, a slight "snowcapping" of incisal edges and cusp tips is found.

2 The opaque white lines are more pronounced and frequently merge to form small cloudy areas scattered over the whole surface. "Snowcapping" of incisal edges and cusp tips is common.

3 Merging of the white lines occurs, and cloudy areas of opacity occur spread over many parts of the surface. In between the cloudy areas white lines can also be seen.

4 The entire surface exhibits a marked opacity, or appears chalky white.Parts of the surface exposed to attrition or wear may appear to be less affected.

5 The entire surface is opaque, and there are round pits (focal loss of outermost enamel) that are *less than* 2 mm in diameter.

6 The small pits may frequently be seen merging in the opaque enamel to form bands that are *less than* 2 mm in vertical height. In this class are included also surfaces where the cuspal rim of facial enamel has been chipped off, and the vertical dimension of the resulting damage is *less than* 2mm.

7 There is a loss of the outermost enamel in irregular areas, and *less than* half the surface is so involved. The remaining intact enamel is opaque.

8 The loss of the outermost enamel involves *more than* half the enamel. The remaining intact enamel is opaque.

9 The loss of the major part of the outer enamel results in a change of the anatomical shape of the surface/tooth. A cervical rim of opaque enamel is often noted.

TABLE 4. 2.: The modified Thylstrup-Fejerskov classification, the TF-index, of the clinical appearance of fluorotic enamel characterizing the single tooth surface.

(Thylstrup and Fejerskov, 1978)

Enamel matrix formation begins at the amelodentinal junction and proceeds outwards. Unfortunately there is no agreement on the amount of fluoride normally present in the blood, but as a fluoride concentration of as low as 1,0 ppm in the drinking water can produce fluorosis, it becomes apparent that only a slight increase in the fluoride content of the blood is enough to affect the function of the ameloblasts.

If the toxic effect of fluoride on the ameloblasts was most marked by the time the last portion of the enamel matrix is laid down, the histologic deficiencies in the interprismatic substance of fluorosed enamel would be most marked in the outer third (Isaac *et al.*,1958).

It is interesting that the surface enamel of normal teeth has an equivalent, or even higher, fluoride content than the subsurface enamel of fluorosed teeth. This suggests that fluorosis is not caused by the actual amounts of fluoride deposited in the enamel but rather by an increase in the amount of fluoride present during enamel formation. (Isaac *et al.*,1958).

Enamel fluorosis can only arise when the fluoride is absorbed prior to eruption, while the tissue is developing, and cannot be caused after the enamel has been formed (Weatherell, Deutsch & Hallsworth, 1977).

Histologically the lesion or defect only stretches from the surface inwards for approximately one third of the enamel thickness, though occasionally the whole enamel thickness may be involved.

Fluorosed enamel consists of areas of diffuse hypomineralisation or porosity below a well-mineralised surface layer. The optical properties of the rods change to give a white stain appearance, and, secondly, the defect allows stains and pigments from the mouth to penetrate the lesion and cause a brown appearance (Palmara, Phakey, Rachinger & Orams, 1980).

Richards (1990), as well as Fejerskov, Manji and Baelum (1990), stated that ....."no useful data on dose (mgF^-/kg b.w.) - response (Fluorosis) relationship are available".

While giving a reasonable indication of water F^-- levels and their likely effect, they make the point that there seems to be no critical level below which fluorosis might not manifest.

(vii) Fluoride and the caries process.

During his early studies, McKay (1916) noted that mottled teeth were not more susceptible to dental decay than normal teeth. Other investigators reported that subjects resident in areas with naturally fluoridated water had significantly less caries than subjects resident in areas with low fluoride in the drinking water (Ainsworth, 1933; Dean, 1938).

Dean, in 1938, in a survey of caries among some 8000 schoolchildren in 21 American cities with fluoride in their water supplies ranging from 0 to 2 ppm, showed a relationship between caries and water-borne fluoride. This was confirmed in a similar survey in Britain by Weaver (1944).

These findings suggested that fluoride acts as an anti-caries agent, and that the addition of fluoride to the drinking water has virtually the same effect on caries as fluoride that occurs naturally. It also proves that fluoride is the substance responsible.

The anti-cariogenic effect of fluoride has since been thoroughly documented and researched worldwide, and it is generally accepted today that, within limits, there is an inverse relationship between the fluoride concentration and the degree of dental decay. Equally well known is the fact that overdosage of fluoride has the effect of fluorosis or mottling of the enamel (figure 3. 2.- page 25).

It has been well established that dental caries is a disease involving dissolution of enamel by acids from bacterial plaque and that this dissolution is inhibited by the presence of fluoride. Several mechanisms have been proposed to explain the cariostatic effect of fluoride. The relative importance of the contributions of each is dependant on the circumstances at the time.

The cariostatic action of fluoride is therefore best described as multifactorial (Mellberg & Ripa,1983; Koulourides,1990), and can broadly be grouped as follows:

-increased enamel resistance

-increased rate of maturation

-remineralisation of incipient lesions

-interference with microorganisms

-improved tooth morphology.

Although investigators are in unanimous agreement about the effectiveness of fluoride against caries, their thinking is divided with regard as to whether the highest fluoride concentration will produce greater cariostasis.

It is remarkable that all clinical evaluations of topical applications of fluoride (including office-applied topicals and fluoride home-use products, toothpastes and mouthrinses), tend to result in caries reduction ranging from 30% to 50%, which is comparable to the effect obtained when the drinking water contains 1 ppm F^-.

However, one important difference between these two applications of fluoride is that the use of F^--containing products requires conscious compliance with procedures, whereas F^- in drinking water does not (Koulourides,1990).

Although each effect is probably partially additive, evidence is not available to quantitate the degree to which it occurs, but, in general, use of higher fluoride concentrations and greater frequency will favour a more pronounced anticaries effect (Mellberg & Ripa,1983).

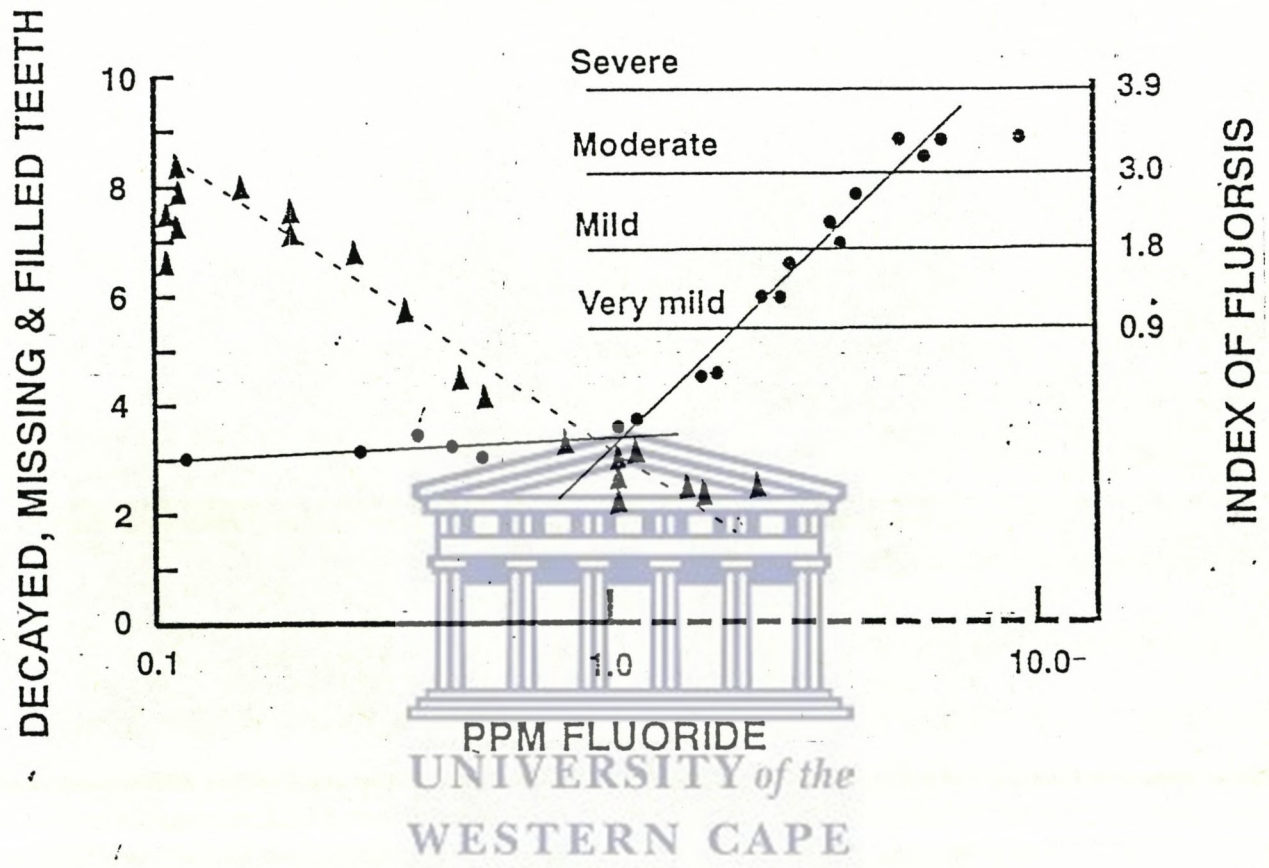

Figure 3. 2.: The relation between the fluoride concentration of the water supply, caries incidence expressed as D.M.F.T. (Decayed Missing Filled Teeth) on the left, and the relation between fluoride content of drinking water and Fluorosis on the right is illustrated.

(Data from D.H.Retief: Institute of Dental Research, University of Alabama).

(viii) **Fluoride on and in enamel.**

In relating the fluoride content of the water supply, ingested F^-, and topical exposure, to the fluoride content of the enamel, it is necessary to distinguish between the fluoride that is deposited during tooth formation pre-eruptively and the uptake of fluoride posteruptively.

An increase in the fluoride content of the water supply results in an increase both in the amount of fluoride deposited in the enamel during tooth formation, and in that acquired posteruptively (Isaac *et al.*,1958; Bernt & Stearns, 1978). The fluoride **"on/in"** enamel on the most outer part of the enamel might contain alkali-soluble fluoride which is loosely bound, mainly in the form of calcium fluoride-like material. The fluoride **"in"** the enamel is the acquired, mainly insoluble fluoride which is firmly bound, mainly in the form of fluoro-apatite (Dijkman, Tak & Arends, 1982).

It is generally accepted today that treatment with fluoride-containing lacquers or other fluoride containing substances will increase the fluoride content in and on the enamel, but that this increase is restricted to the superficial layers (Mellberg, Laakso & Nicholson, 1966; Grobler, Ogaard & Rølla, 1981; Retief *et al.,* 1980; Grobler, Ogaard & Rølla, 1983).

As the tooth erupts into the mouth, it is not yet fully mineralised. Enamel mineralisation is completed during a post eruptive maturation period when calcium, phosphate and trace elements fill the spaces between the hydroxyapatite crystals and decreases the porosity, and also changes the size (larger) of the crystals. This decrease in porosity of matured enamel reduces the amount of fluoride able to diffuse into the enamel and therefore decreases the amount that can be taken up (Mellberg & Ripa,1983).

Resistance of the tooth to dental caries is increased through the interaction of fluoride with the mineral phase. When fluoride is present during the long pre-eruptive phase of enamel,(plasma F^--interstitial fluids), some F^- is incorporated into the hydroxyapatite lattice forming fluoro-apatite or hydroxyfluoro-apatite. Fluoro-apatite crystals are larger, more perfectly formed and less soluble in acid (Mellberg *et al.,* 1966). The intention of topical applications is to convert hydroxy-apatite to fluoro-apatite. When fluoride is applied to an enamel surface it diffuses inward by way of the less dense interprismatic spaces to a depth related to its concentration, the treatment time, pH, and the type of fluoride agent. When the fluoride source is removed and replaced by water or saliva, a large part rapidly diffuses back out as result of the concentration gradient, (Mellberg *et al.,*1966), while another part diffuses out more slowly, probably as a result of absorption into the hydration layer of the crystals (Rølla & Bowen,1978). The fluoride that remains has reacted to form one or more of several compounds, depending on the conditions present.

It was stated by Weatherell *et al.*,(1977) that the most ideal time to apply topical fluoride to the tooth surface is as soon after eruption as possible, when the outer region of the enamel is still fairly permeable, and fluoride uptake by the enamel of an erupting tooth will be maximal when it is exposed to fluoride from the early stages of eruption. Drinking water is the most reliable vehicle for fluoride supplementation during the formation of the tooth, and treatment with fluoride-containing toothpaste, lacquers or other fluoride-containing substances will increase the fluoride content only of the superficial layers of the enamel after eruption (Mellberg *et al.*, 1966; Koch & Peterson 1972; Kirkegaard, 1977; Baijot-Stroobants & Vreven, 1980; Bruun, Givskor & Stoltze, 1980; Retief *et al.*, 1980; Mushanoff, Gedalia & Daphni,1981; Grobler & Joubert, 1988; Grobler & Kotze, 1988; Ten Cate, Exterkate & Rempt, 1988).

Currently the dentist has a choice of topical fluoride agents which may be used in an aqueous solution, a viscous gel, as a paste, or as a dental varnish (Mellberg & Ripa, 1983). Extensive work has been done by various researchers on the effect of various fluoride compounds on the enamel, and thus their effectiveness in increasing the fluoride content of the enamel. Kirkegaard (1977), evaluated four dentifrices and found that after one hour of treatment the fluoride uptake was confined to the outer 5μm of the enamel.

Baijot-Stroobants and Vreven (1980), subjected 74 sound incisors to 4 minutes of topical applications of commercial gels and solutions. The largest amounts of fluoride were acquired by the enamel 30 minutes after an amine fluoride solution application, and it was detected for many weeks after the applications. Mushanoff *et al.*,(1981), investigated fluoride incorporation by the surface enamel following brushing with an amine-fluoride toothpaste. The fluoride uptake did not seem to be stable after discontinuing the toothpaste and the need was expressed for the continuous use of the toothpaste. Bruun *et al.*,(1980), testing a lacquer containing silane fluoride, found that most of the fluoride was lost after 6 months, indicating that a substantial part of the fluoride retained 1 week after treatment was not permanently bound to enamel.

Grobler and Kotze (1988) investigated the fluoride distribution in the enamel of the mesiolingual cusps of pairs of erupted and unerupted third molars of persons with a low fluoride background. Statistically significant differences ($p<0,05$) were found in the mean etch depth between the erupted and unerupted molars, to a depth of ± 5μm. The mean enamel fluoride concentrations of the erupted and unerupted molars also differed significantly to a depth of ± 5μm. There was no influence of fluoride from the oral environment on enamel F^- levels of ± 10μm and deeper. Over an exposure period of 1 to 16 years, sixty percent more enamel fluoride (at a depth of ± 2μm) was found in the

erupted enamel compared to the unerupted enamel (with a low fluoride background) as a result of toothbrushing with fluoride-containing dentifrices.

Fluoride applied to tooth enamel mainly results in the formation of calciumfluoride and fluoro-apatite (McCann 1968; Gron,1977).

At a Conference on Calciumfluoride-like (CaF_2) Minerals on Enamel* in the Netherlands, April 1988, the Arrangement Committee felt that the formation of Calciumfluoride on enamel during topical application of fluoride was a neglected field of study. CaF_2 may well, according to their view, be the key to a more comprehensive understanding of the mechanisms by which fluoride exerts its cariostatic effects.

The following conclusions and suggestions were put forward and agreed upon by the participants:

"1. The CaF_2 that is formed on enamel under clinical conditions contains phosphate and is more soluble than pure CaF_2. The amount of phosphate included is dependant on the circumstances. The presence of HPO_4 and the concentratio of F^- available are probably the most important factors.

2. The dissolution of CaF_2 in water is strongly influenced by the presence of HPO_4 in the aqueous phase. Such ions appear to adsorb to the surface of CaF_2 and provide a dissolution-limiting surface layer. Proteins (albumen and probably pellicle proteins) can also reduce the dissolution of CaF_2 in water.

3. CaF_2 dissolves slowly in saliva owing to adsorption of HPO_4 and proteins.

4. Globular CaF_2-like material is formed in vivo on tooth surfaces after fluoride application at neutral or acidic pH. Its behaviour is most likely influenced by phosphate, bi-carbonate (HCO_3), proteins, and surface-active compounds like the amine part in AmF.

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* Editorial, Acta Odontologica Scandinavica, **46**, 1988.

5. There are indications that phosphate (as HPO_4) controls the F^- release from globules. One can surmise that, at high pH values, the phosphate protects the CaF_2-like globules but at low pH loses its protective value, causing the globules to give off fluoride.

6. CaF_2 -like particles have thermodynamically the tendency to transform into fluoridated apatites(FAP). These apatites are known to be formed. It is feasible, however, that this transformation to FAP is strongly inhibited in in-vivo conditions owing to, for example, protein action (or to unknown inhibitors).

The committee agreed that the preventive effect is most likely condition dependant. The effect of CaF-like globules on the enamel surface may not be the same as the effect of globules formed inside enamel lesions. The CaF_2-like particles formed on enamel surfaces, possibly on depressions in the surface, are thought to be F^-- releasing. The globules presumably act as tiny local reservoirs of fluoride."

In a study done by Grobler and Joubert (1988) on the relative distribution of fluoride in erupted and unerupted enamel of human third molars from a low fluoride area, it was found that there was significantly ($p < 0,05$) more fluoride in erupted than in unerupted third molars up to a depth of about 10μm. No significant differences could be demonstrated at deeper levels, implying that additional fluoride was acquired from the oral environment after eruption, but that penetration of the fluoride to the interior was limited to a depth of about 10μm by the dense enamel.

When fluoride-treated enamel is exposed to a hydroxide solution for 24 hours the calciumfluoride-like material dissolves without affecting the enamel (Caslavska, Moreno & Brudevold, 1975), while at the same time fluoride adsorbed to the enamel surface or attached loosely, for example to protein, will also be removed by the alkali treatment (Rølla & Bowen, 1978).

Grobler and Kotze (1990), determined the amounts of firmly and loosely bound fluoride in sound enamel of erupted and unerupted teeth from a low fluoride area which had been exposed in vivo for 1-16 years to brushing at least once a day with fluoride toothpaste and occasional mouth rinsing and application of sealers.

The results are shown in the graph,(figure 4. 2), which shows that unerupted enamel was etched significantly ($p < 0,05$) deeper than erupted enamel up to a depth of at least 8 μm. Significant differences ($p < 0,05$) were found between the mean enamel fluoride concentrations of unwashed and alkali-washed erupted teeth up to a depth of at least 3 μm and also between unwashed or washed erupted, versus unwashed or washed unerupted teeth.

At a depth of 3 μm, the fluoride treatments of enamel had increased the total amount of fluoride by approximately 78 percent of which approximately 53 percent was loosely bound fluoride like CaF_2 and 47 percent firmly bound (like fluoro-apatite). No increase in sound enamel fluoride as a result of topical treatments over a period of up to 16 years could be found at a level deeper than 20 μm.

However, no significant difference could be found in the etch depth between alkali-washed and unwashed, erupted or unerupted third molars, proving that the removal of loosely bound fluoride does not affect the solubility of the enamel, which is in agreement with the findings of Dijkman *et al.*,(1982).

The curves of the alkali-washed and unwashed erupted enamel intersected each other at a depth of approximately 8μm, and those of the unerupted enamel deeper, at a depth of about 15μm, indicating that although no loosely bound fluoride (like CaF_2) could be found at this depth, fluoride penetrated deeper into the dense enamel to form a firmly bound compound (like fluoro-apatite).

The fact that no significant amount of fluoride was removed from the unerupted enamel as a result of the alkali-wash procedure, (curves 2 and 4, figure 4. 2), shows that there was no important loosely bound fluoride and that all the fluoride gained in the unerupted stage was in a firmly bound form. On the other hand, a significant reduction in the fluoride content of erupted molars (in approximately the outer 3μm) was found as a result of the alkali-washed procedure. Relatively less loosely bound fluoride was formed deeper in the enamel than on the outside.

The trend of this finding is in agreement with those of Retief (1988), where ground enamel was used in an *in vivo* study of two dentifrices. It indicated that fluoride from the fluoride-containing substances penetrated more slowly as one proceeds deeper into the enamel, which facilitated a slower ion exchange reaction (fluoroapatite formation) rather than a precipitate formation (CaF_2).

FIGURE 4. 2

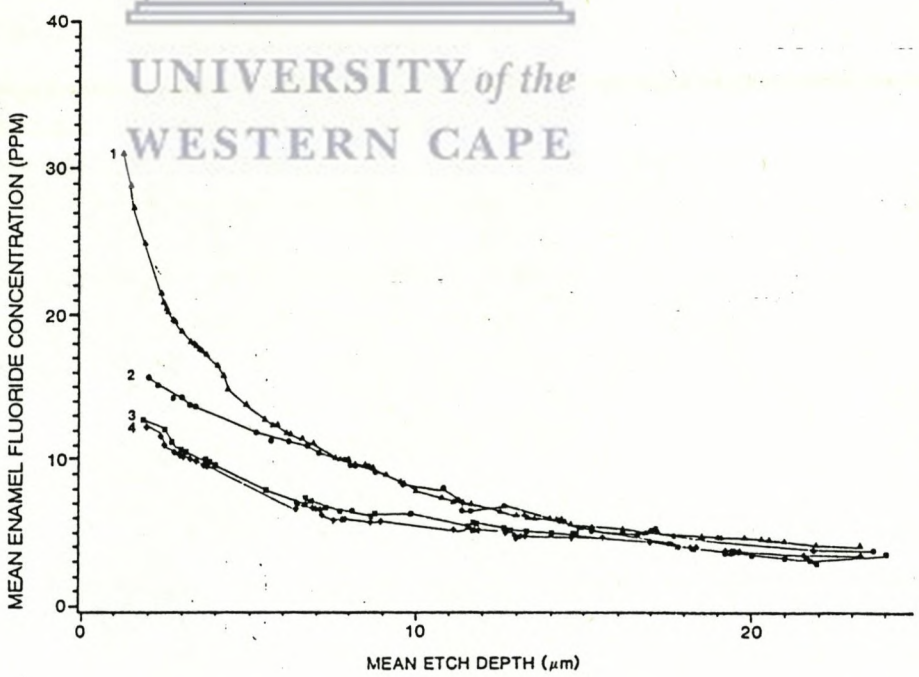


Figure. 4. 2 : A line fitted to the mean enamel fluoride concentration and mean etch depth values of unwashed erupted (1), alkaliwashed erupted (2), unwashed unerupted and alkaliwashed unerupted (4) third molars.
(Grobler & Kotze, 1990, p 797).

CHAPTER III

Materials and Methods

Material.

A total of 25 third molar teeth (erupted [9] as well as unerupted [16]) from subjects who had lived continuously since birth in an area where the water fluoride concentration was more than 1,8 ppm were studied. (Subjects aged 18 - 64 years).

The subjects had no systemic fluoride supplementation since birth. Tooth brushing, once or twice a day with a fluoride-containing dentifrice, and perhaps occasional mouth rinsing, were the only fluoride-containing anti-caries programmes practised singly or in combination for a period of 1 - 16 years.

The teeth were removed for various reasons, eg. lack of space for eruption with associated pericoronitis, unopposed teeth that traumatized soft tissue during mastication, as part of orthodontic treatment, and impacted teeth having a detrimental effect on adjacent erupted teeth. All teeth were donated for the study by the patients concerned.

After removal the teeth were rinsed in distilled water and stored on cotton wool moistened with 0.01% thymol solution in a closed test tube, i.e. kept in a moist atmosphere and not in a solution. The reason for this was to prevent leakage of fluoride from the enamel into the surrounding solution (Grobler *et al.*,1981).

Only teeth without carious lesions or other observable defects after examination under a stereo-microscope (20 x magnification) at the desired sites were selected. No signs of abrasion was detected at this magnification on any of the selected sites on erupted teeth.

As all the teeth examined came from high fluoride areas, (1,8 - 2,64 ppm), some degree of fluorosis was noted on all the enamel surfaces examined under the stereo-microscope, ranging from, according to Dean's classification, very mild to mild, with one or two displaying a possible moderate mottling. According to the TF-index, the scores would vary between 2 and 4.

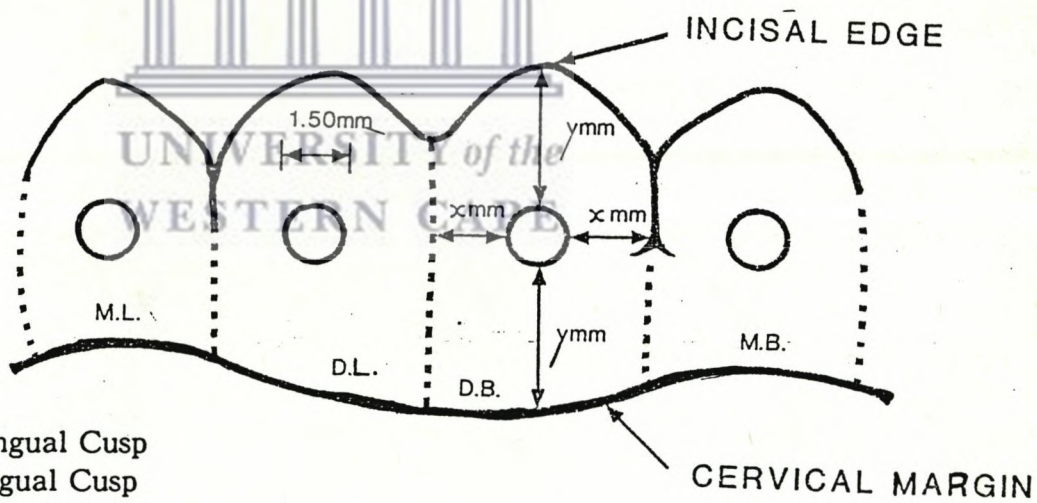
Methods.

The acid-etch biopsy technique as described by Retief, Navia and Lopez (1977) and modified by Vogel, Chow and Brown (1983) was used to determine the fluoride and calcium concentrations at various depths on the enamel surface.

An annular adhesive disc with an inner diameter of 1,5 mm. was punched from adhesive tape, (No. 471, 3M Company) with a selfconstructed punch. The cusps were cleaned by rubbing with a cotton pellet soaked in acetone, to remove all organic debris. (Gibbs, Retief & Bradley, 1981). The selected sites, (the centre of the cusps), was demarcated by the disc and carefully burnished to ensure good marginal adaptation of the disc to the surface to minimize the possibility of leakage of the etching agent. (Figure 1. 3).

Fig. 1. 3.

Selected Sites:



M.L. = Mesio-lingual Cusp
D.L. = Disto-lingual Cusp
D.B. = Disto-buccal Cusp
M.B. = Mesio-buccal Cusp

The biopsy sites were so selected because it was previously reported, (Grobler & Joubert,1988), that the enamel fluoride levels of the mesio-buccal and mesio-lingual sides did not differ from that of the disto-buccal and disto-lingual sides.

(See plate 1 - page 36)

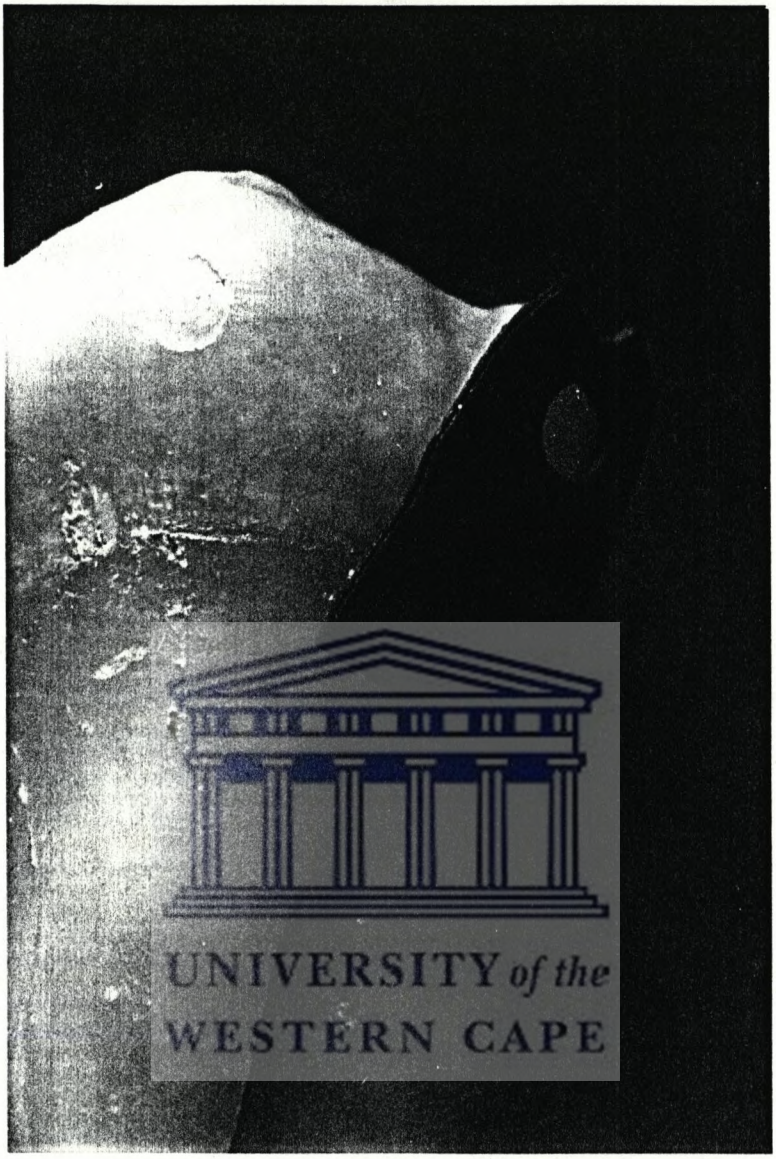


Plate 1: A photograph of one of the teeth investigated showing the annular disc and an etch site after removal of the disc after etching.

The photograph clearly shows a very mild degree of fluorosis or mottling on the surface of the tooth, which came from an area where the water F^- concentration was >1,8 ppm.

The mesiolingual and mesiobuccal cusps were investigated first.

Six consecutive etchings for a duration of 7, 13, 25, 30, 40 and 50 seconds respectively, were done on the selected demarcated site with 2μl of 1,0 M perchloric acid, ($HClO_4$), by depositing the acid with an Eppendorf micropipette of 2μl capacity.

The acid etch solution (2 μl) was withdrawn from the tooth surface after the specified time with the same pipette and transferred to a separate 25 μl polypropylene tube containing 18 μl of adjusted buffer. [The adjusted buffer consisted of a commercial ionic strength-adjusting buffer, (TISAB) to which solid NaOH has been added to give a concentration of 0,25 N.] The etched surface was then washed twice with 2μl distilled water and the washing solutions also transferred to the tube containing the etch solution.

To remove loosely bound fluoride, the teeth were then each placed separately in 20 ml of a 1 M KOH solution, mechanically shaken for 24 hours and rinsed with distilled water until the pH of the rinse water was below 7 (Caslavska *et al.,* 1975).

After the alkali-wash, the same etching procedure as described above was followed in the investigation of the distolingual and distobuccal cusps of each tooth.

Fluoride Determination

The fluoride concentration of the buffered etch solution was determined with an adapted fluoride ion-selective electrode technique reported by Retief *et al.*,(1977), and modified by Vogel, Chow and Brown,(1983).

A fluoride electrode (Model 96-09, Orion Research Inc.) coupled to a digital pH/mv meter (Orion Research Expandable Ion Analyzer EA940) was used for this purpose.

(See Plate 2 - page 38).

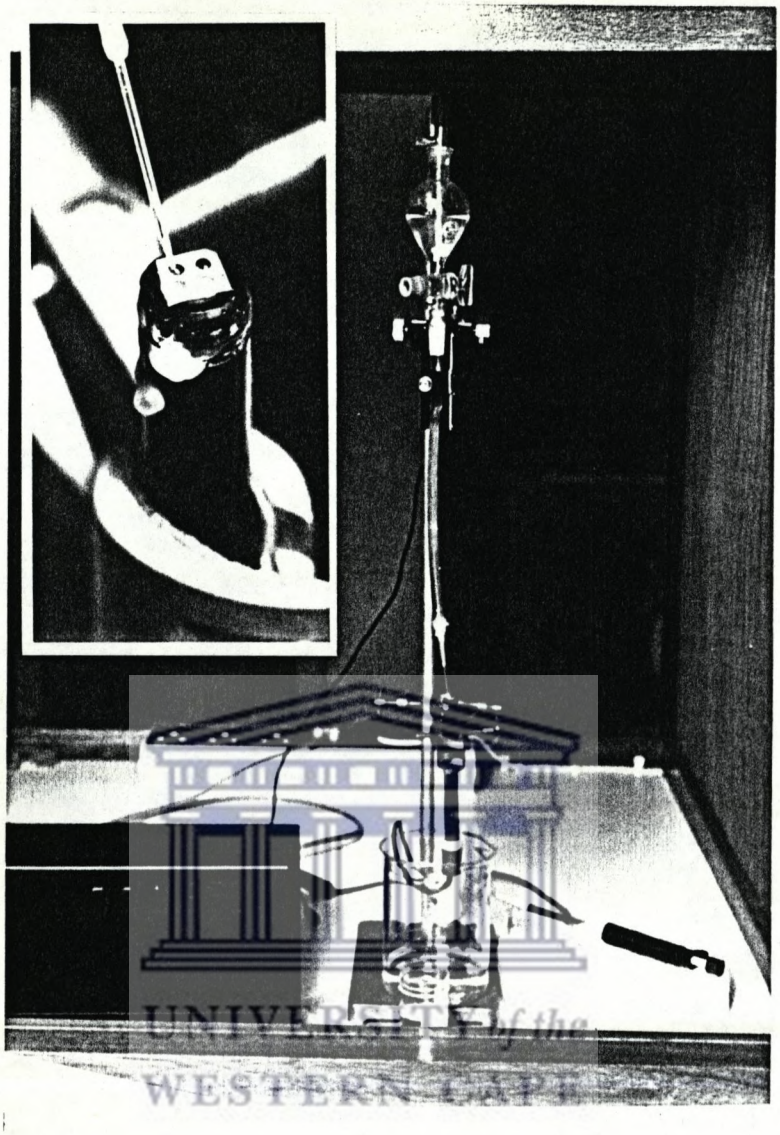


Plate 2 : The apparatus used for the fluoride determination.

Fluoride Standards were so prepared that the standards and the biopsy samples had the same ionic backgrounds. (See Table 1. 3.- page 39)

Table 1. 3.

PREPARATION OF FLUORIDE STANDARDS

Sample solution: TISAB + CDTA in 0,1667M NaOH

18μl Adjusted Buffer + 2μl 1M $HClO_4$ + 4μl Water

Std. No.	Volume of Std.No.to be added	[F] M (*)	$HClO_4$ (1M)(μl)	Adjusted Buffer(μl)	H_2O(μl)	[F] ppm	p M_F
Blank	-		10	70	20		
B	20μl of A	0,001053	10	70	-	20,00	2,978
C	10μl of A	5,26 $x10^{-4}$	10	70	10	10,00	3,279
D	20μl of B	2,11 $x10^{-4}$	10	70	10	4,00	3,677
E	10μl of C	5,263 $x10^{-5}$	10	70	10	1,00	4,279
X	10μl of D	2,11 $x10^{-5}$	10	70	10	0,40	4,676
F	10μl of E	5,263 $x10^{-6}$	10	70	10	0,10	5,279
G	10μl of X	2,11 $x10^{-6}$	10	70	10	0,04	5,676

(*) = mols/litre

TISAB = Total Ionic Strength Activity Buffer

CDTA = Cyclo diamine tetra acetic acid

Calcium Content Determination.

In order to be able to determine how deep each etch was for the different times etched, it was necessary to determine the amount of calcium in each etch sample. The buffered etch solution (1μl) from each propylene tube was diluted (x 1400) in a separate 300μl propylene tube with a solution containing 0,05 mol/l potassium chloride in 0,10 mol/l nitric acid. The potassium ion is needed to act as an ionization depressant (Chakrabarti, 1981.)

The calcium concentration was then determined by flame atomic absorption spectrophotometry (Fricke, 1979). A nitrous oxide (N_2O) and acetylene (C_2H_2) flame was used, because it has the advantage over an air-acetylene flame in that far less disturbances are found on the calcium spectrum, and is also more sensitive.

The apparatus used was a PYE Unicam coupled to a SP 9 computer. The settings used were as follows:

Wavelength	=	422,7nm (nanometers)
Band spread	=	0,2nm
Lamp	±	8,0mA (milliampere)
Integration time	=	1,0 seconds
Burner height	±	5,5 cm
Evaporation	=	2,0 seconds

A D_2 lamp was used with background correction.

Preparation of Calcium Standards.

Use was made of a Calcium-nitrate-standard for atomic absorption analysis. (Supplied by British Drug Houses). Care was taken to ensure that the standards all contained the same amount (concentration) of nitric acid and calcium as that in the solution in the tubes.

The reason for this is that different amounts of nitric acid influences the signal of atomized calcium. It has been found that 24ml concentrated nitric acid per 50ml of calcium standard solution has a significant suppression of the calcium signal when compared to the signal obtained with half of the abovementioned concentration of nitric acid in the solution. Furthermore, it was found that 2.5ml 1M KCl/50ml or 50ml 1M KCl/50ml had no significant effect on the calcium signal.

Determination of the etch depth.

If the amount of Ca in the etch samples are known, the etch-depth can be calculated. The amount of Ca in enamel is constant ie. 37 percent, as enamel has a fixed crystal structure (Retief *et al.,* 1971; Söremark & Samsahl, 1961).

Furthermore, enamel has a density of 2,95g/ml (Manly & Hodge,1939).

To determine the etch depth, the following formula is used for each successive biopsy:

$$\text{Depth of etch}(\mu m) = \frac{\text{Dissolved Enamel Mass } (\mu g)}{\text{Enamel density}(2{,}59 g/ml) \times \text{Biopsy area } (mm^2)}$$

CHAPTER IV

Results and Statistics.

The average value of the etch depth and the fluoride concentration as calculated from the values for the two cusps per tooth was used for the statistical analysis.

The mean etch depths (μm) and mean enamel fluoride concentrations of alkali-washed and unwashed enamel of both erupted and unerupted molars are given in Tables 1. 4, 2. 4, 3. 4 and 4. 4, together with the standard deviations and range for each etch.

Etch No's 1 to 6 were done on the mesiobuccal cusps of each tooth, and Etch No's 7 to 12 on the mesiolingual cusps. After washing the teeth with alkali, Etch No's 13 to 18 were done on the distobuccal cusps and Etch No's 19 to 24 on the distolingual cusps.

Table 1. 4.

ERUPTED UNWASHED (n = 9)

Etch No	Mean Etch Depth(μm)	Stnd Dev	Range	Enamel F^- (ppm)	Stnd Dev	Range
1	6,2	2,6	2,7-10,5	1374,1	645,6	686-2601
2	6,9	1,7	3,6-9,6	1787,1	1142	740-4564
3	10,4	4,6	3,6-18,2	1395,3	1149	639-4066
4	13,0	4,7	6,0-21,4	1252,1	725	411-2667
5	13,4	4,0	6,7-20,7	892	535	453-2127
6	15,1	6,6	4,3-25,1	662	303	284-1197
7	5,8	2,9	1,0-11,5	952	285	686-1410
8	13,1	10,1	4,6-34,6	1180	948	322-3052
9	11,9	4,5	3,4-18,3	1070	740	508-2913
10	12,1	5,6	6,7-24,8	903	365	542-1687
11	12,4	3,9	6,5-19,5	751	347	432-1505
12	13,1	3,9	7,0-17,3	652	347	252-1362

Table 2. 4.

ERUPTED ALKALI-WASHED (n = 9)

Etch No	Mean Etch Depth(μm)	Stnd Dev	Range	Enamel F^- (ppm)	Stnd Dev	Range
13	8,3	4,9	2,7-17,3	1513	1435	274-4648
14	9,1	4,7	3,6-15,8	1800	1513	648-5586
15	11,8	6,8	4,9-26,7	1346	951	488-3639
16	12,9	6	5,3-21,9	1188	914	477-3222
17	14,2	6,7	6,2-23,4	810	536	182-1879
18	11,1	6,2	0-19,9	924	764	366-2685
19	6,8	4,3	2,2-16,8	1089	716	414-2664
20	8,2	3,1	3,8-14	1252	747	445-2617
21	10,8	4,6	3,6-15,9	1158	691	516-2391
22	14,1	4,5	6,7-21	751	471	317-1824
23	15,9	6,9	7,2-24,8	617	325	269-1375
24	15	5,9	7,5-26,6	556	241	335-1111

Table 3. 4.

UNERUPTED UNWASHED (n = 16)

Etch No	Mean Etch Depth(μm)	Stnd Dev	Range	Enamel F^- (ppm)	Stnd Dev	Range
1	7,2	5,4	1,0-23,3	1089	895	178-3408
2	9,5	4,5	2,7-20,7	893	887	212-3742
3	10,8	4,6	3-20,1	861	708	139-2747
4	11,6	4,3	4,1-21	698	677	145-2631
5	11,8	3,3	5,7-16,6	538	412	93-1692
6	11,8	5,1	2,6-19,9	551	634	29-2649
7	5,6	2,7	1,4-12,3	1362	1388	200-5336
8	9,4	5,5	17-25,5	822	575	137-2363
9	12,4	5,6	5-26	630	587	39-2438
10	12,4	5,4	2,7-26,5	693	671	115-2836
11	11,9	4,8	3,9-20,7	553	439	128-1859
12	12,4	4,3	3,8-17,8	532	601	67-2672

Table 4. 4.

UNERUPTED ALKALI-WASHED (n = 16)

Etch No	Mean Etch Depth(μm)	Stnd Dev	Range	Enamel[F^-] (ppm)	Stnd Dev	Range
13	7	4,4	1,5-19,6	1194	1209	251-5728
14	9,6	4,99	3,6-20,2	960	892	186-3363
15	11,8	6,3	3,6-26	984	1034	74-3874
16	12,9	5	5,8-24	552	301	69-1115
14	14,8	6,5	2,6-27	398	195	121-799
18	15,1	5,9	4,8-30	398	344	82-1360
19	7,6	4,6	2,1-22,9	862	513	225-1836
20	8,6	2,6	2,3-12,3	766	454	236-1776
21	11,9	5	4,3-20,2	650	590	213-2266
22	11,2	4	4,8-19	623	556	181-2389
23	12,8	4,4	3,3-21,5	447	384	107-1659
24	14,5	5,5	6,7-26,5	384	307	5-1195

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Statistical analysis of fluoride concentration in erupted and unerupted molars.

The washed and unwashed determinations of the fluoride concentrations of both the erupted and unerupted teeth near to the surface were compared by means of a Wilcoxon signed-rank test, while the fluoride concentration near to the surface in the unerupted and erupted molars were compared by means of a Kruskal-Wallis test.

Lines were fitted to the fluoride concentrations (washed and unwashed determinations) and the corresponding etch depths for the erupted and unerupted molars, using the method of Generalised Additive Models (Hastie & Tibshirani, 1987). These lines provide an estimate of the fluoride concentration over the depth range. The GAIM program of Hastie and Tibshirani(1987) was used to establish the fitted lines.

The Gabriel's test showed no significant differences among the mean non-cumulative etch depths of washed or unwashed erupted teeth versus washed or unwashed, unerupted teeth for the first and second mean etch depths. Also, no significant differences could be demonstrated between washed and unwashed, erupted teeth or between that of unerupted teeth.

In general, the enamel fluoride levels of both erupted and unerupted third molars, whether alkali washed or not, decreased from the outside towards the inside, approaching a plateau value at a depth of approximately 70 μm (figure 1. 4), with a fluoride level of approximately 550 ppm. No significant reduction in the fluoride content of erupted molars (in approximately the outer 4 μm) was found as a result of the alkali-wash procedure.

No significant correlation ($p > 0,05$) in the levels between enamel fluoride concentration and age of the subjects at any one of the etch depths could be demonstrated by the Spearman rank correlation coefficient test.

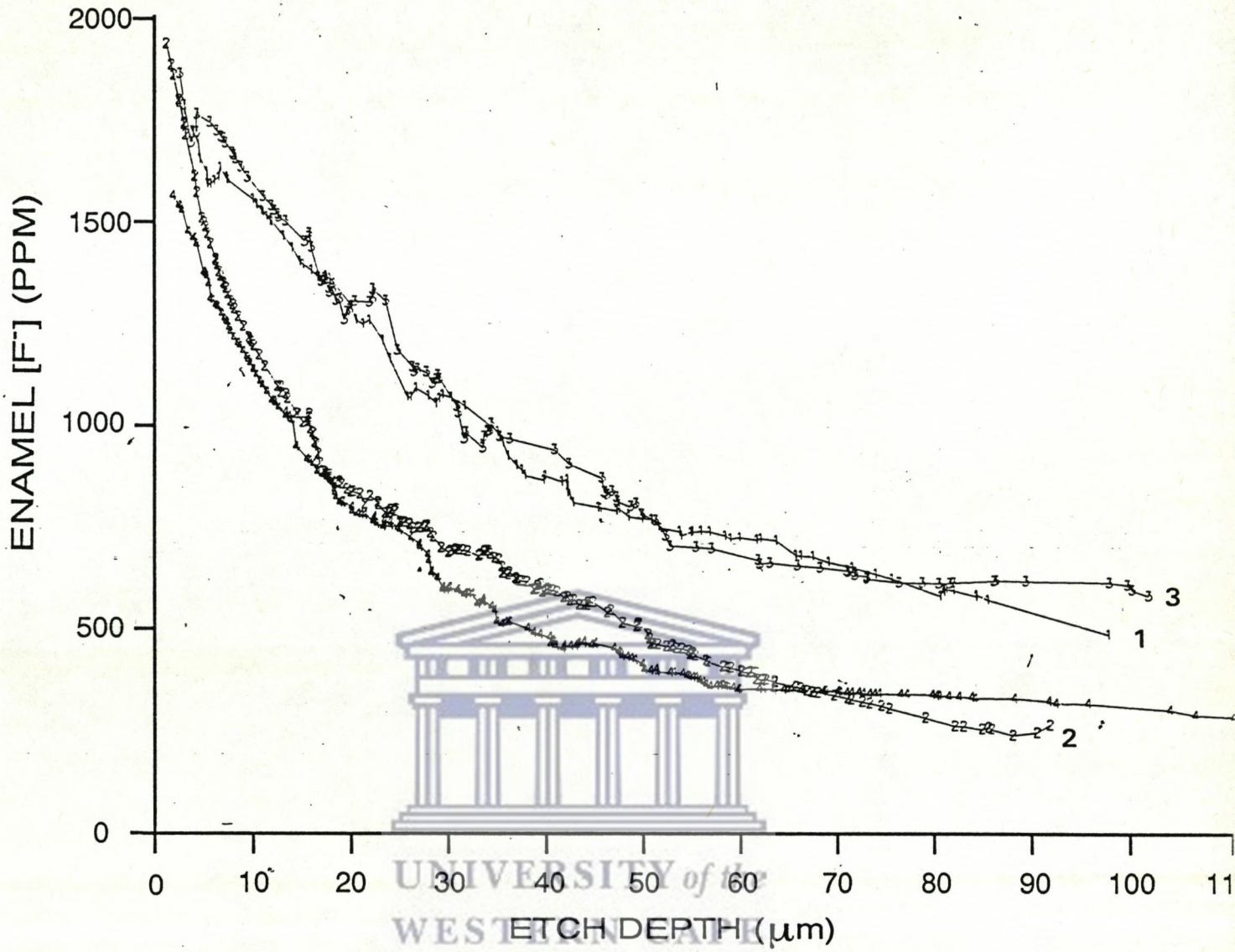

Figure 1. 4

A line fitted to the plotted mean enamel fluoride concentration and mean etch depths values of unwashed erupted (1), unwashed unerupted (2), alkali-washed erupted (3), and alkali-washed unerupted (4) third molar teeth.

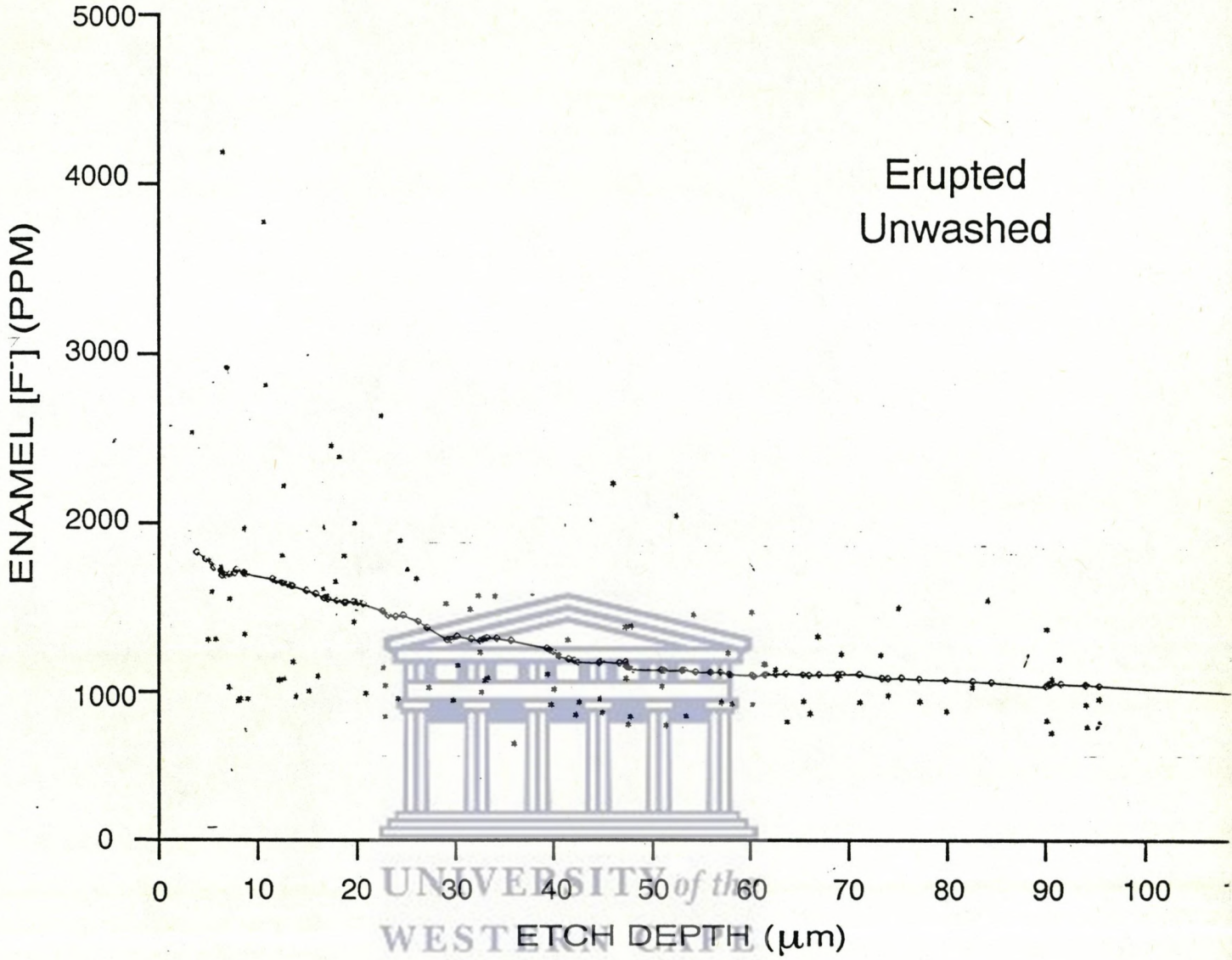

Figure 2. 4.

A line fitted to the plotted mean enamel fluoride concentration and mean etch depths values of unwashed erupted third molar teeth.

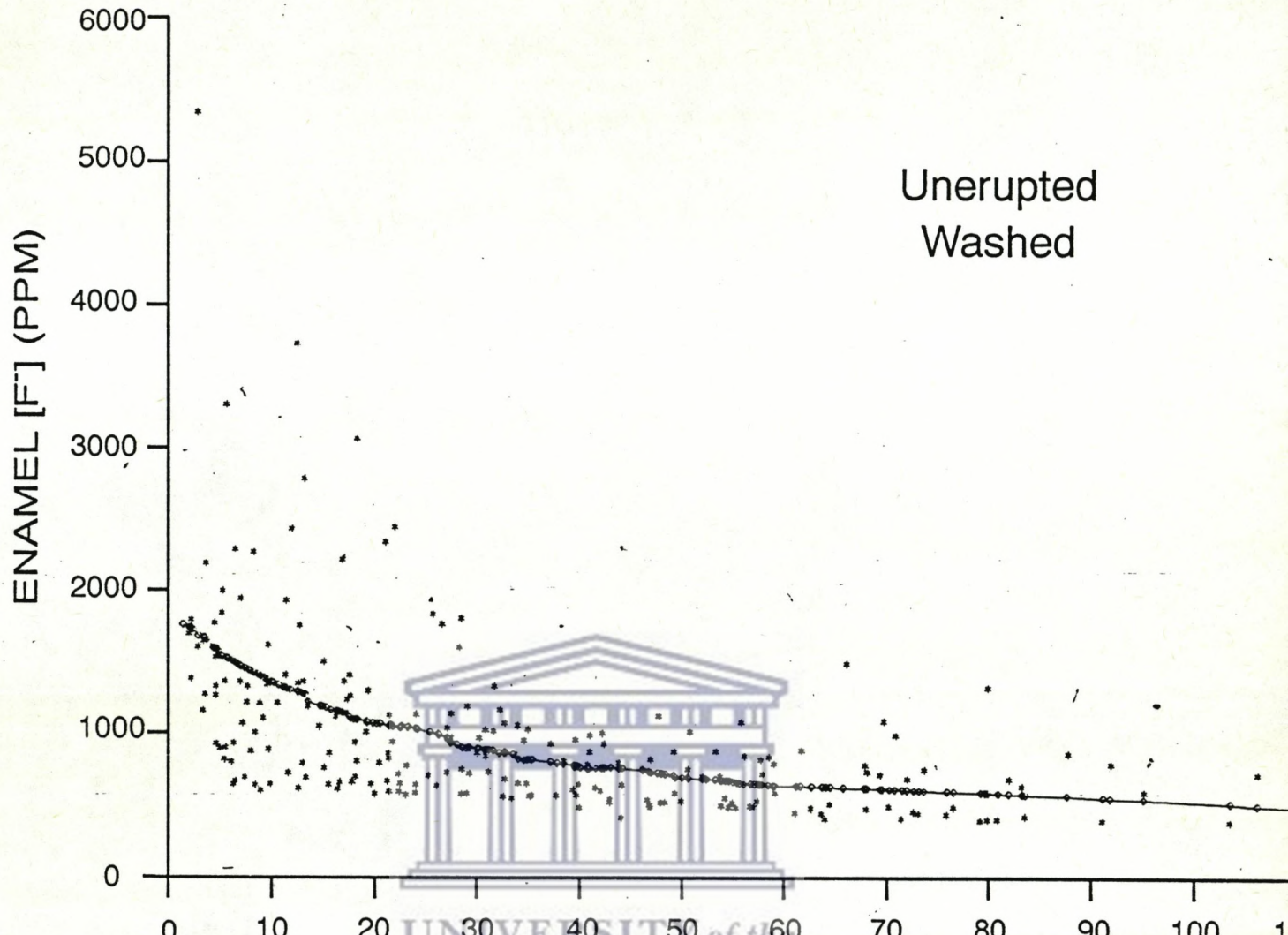

Figure 3. 4.

A line fitted to the plotted mean enamel fluoride concentration and mean etch depths values of alkali-washed unerupted third molar teeth.

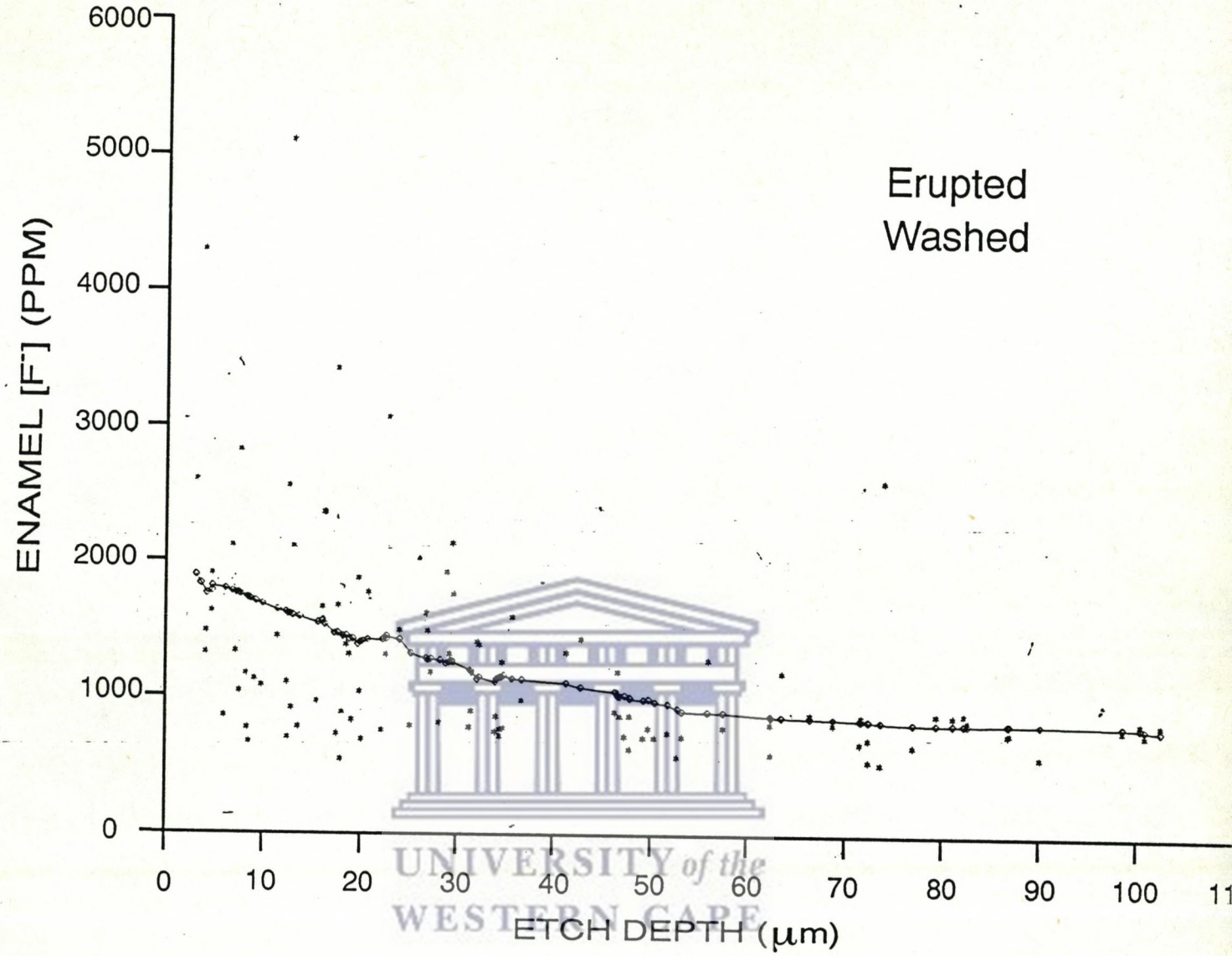

Figure 4. 4.

A line fitted to the plotted mean enamel fluoride concentration and mean etch depths values of alkali-washed erupted third molar teeth.

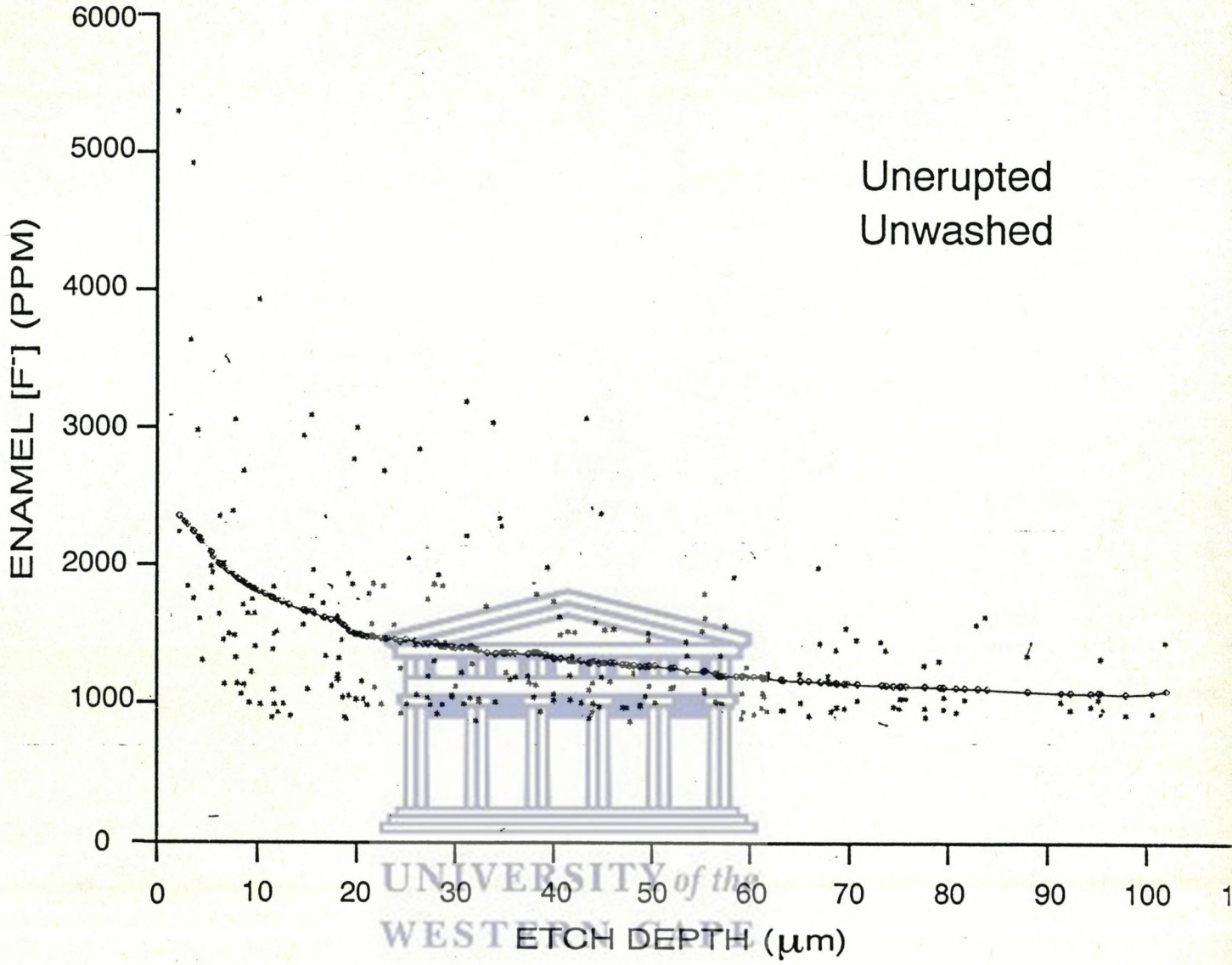

Figure 5. 4.

A line fitted to the plotted mean enamel fluoride concentration and mean etch depths values of unwashed unerupted third molar teeth.

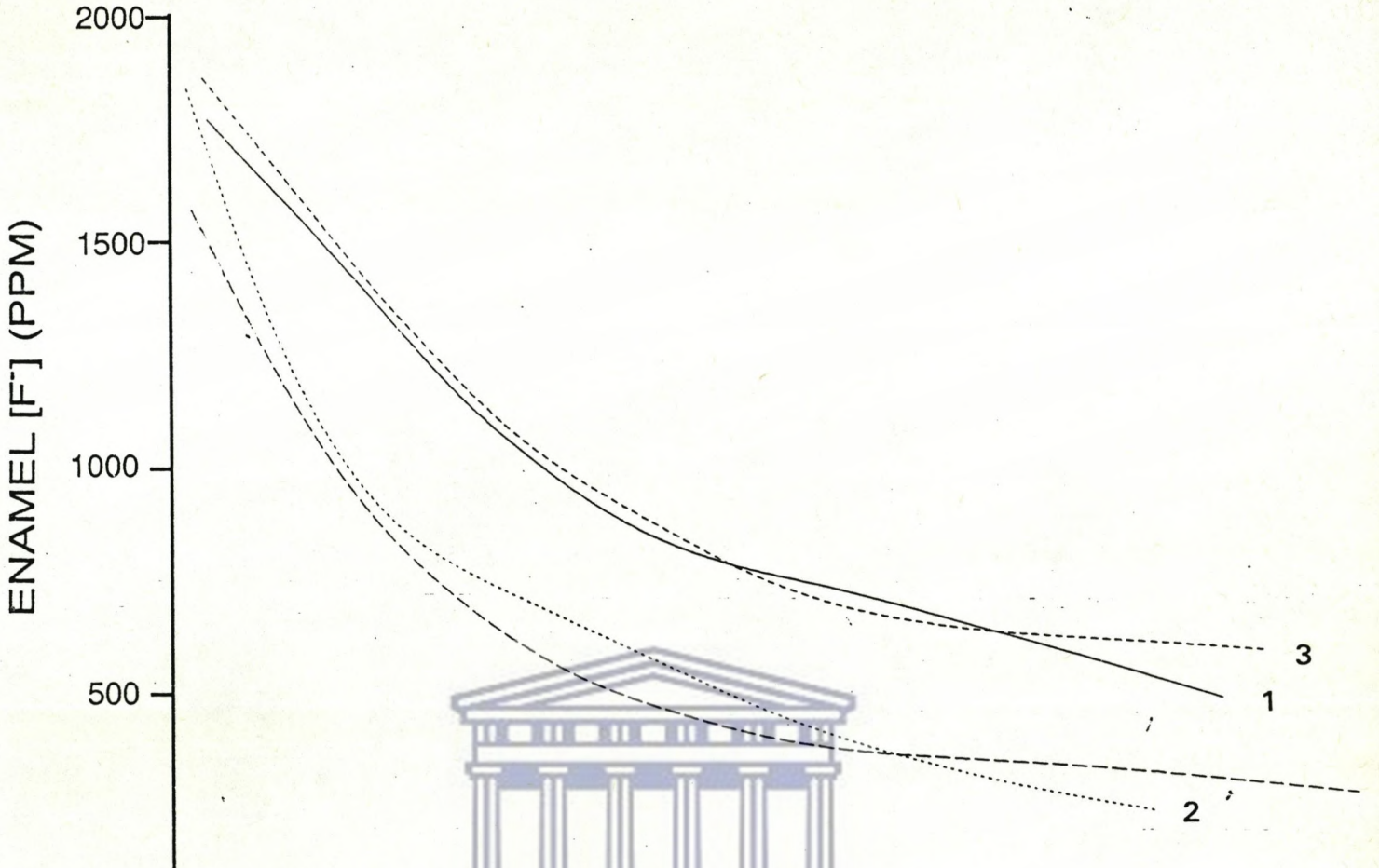

FIGURE 6. 4.

A further graph of Figure 1. 4 [A line fitted to the plotted mean enamel fluoride concentration and the mean etch depths values of unwashed erupted (1), unwashed unerupted (2), alkali-washed erupted (3), and alkali-washed unerupted (4) third molar teeth], after a further spline smoothing was applied to the GAIM lines, to enable easier comparison of the relationship between fluoride concentrations and cumulative etch depths.

CHAPTER V

Discussion.

The enamel fluoride concentration of teeth from a high fluoride area was compared with that of a previously determined enamel fluoride concentration of teeth from a low fluoride area (Grobler & Kotze,1990). As was the case for a low fluoride area, the enamel fluoride concentrations was determined for erupted as well as for unerupted teeth. As the erupted enamel from the high fluoride area was also exposed to dentifrice fluoride during toothbrushing, the effect of both systemic and topical fluoride on the enamel fluoride concentration of human teeth over a long period could be investigated.

The planning for this study was exactly the same as that for the low fluoride area, which is the ideal way to research the relative effect on enamel fluoride concentration in subjects living in a high fluoride area.

For comparative convenience a summary of the results of the two investigations in graphic form are displayed on page 53.

Graph 1. 5., High Fluoride Area

Graph 2. 5., Low Fluoride Area

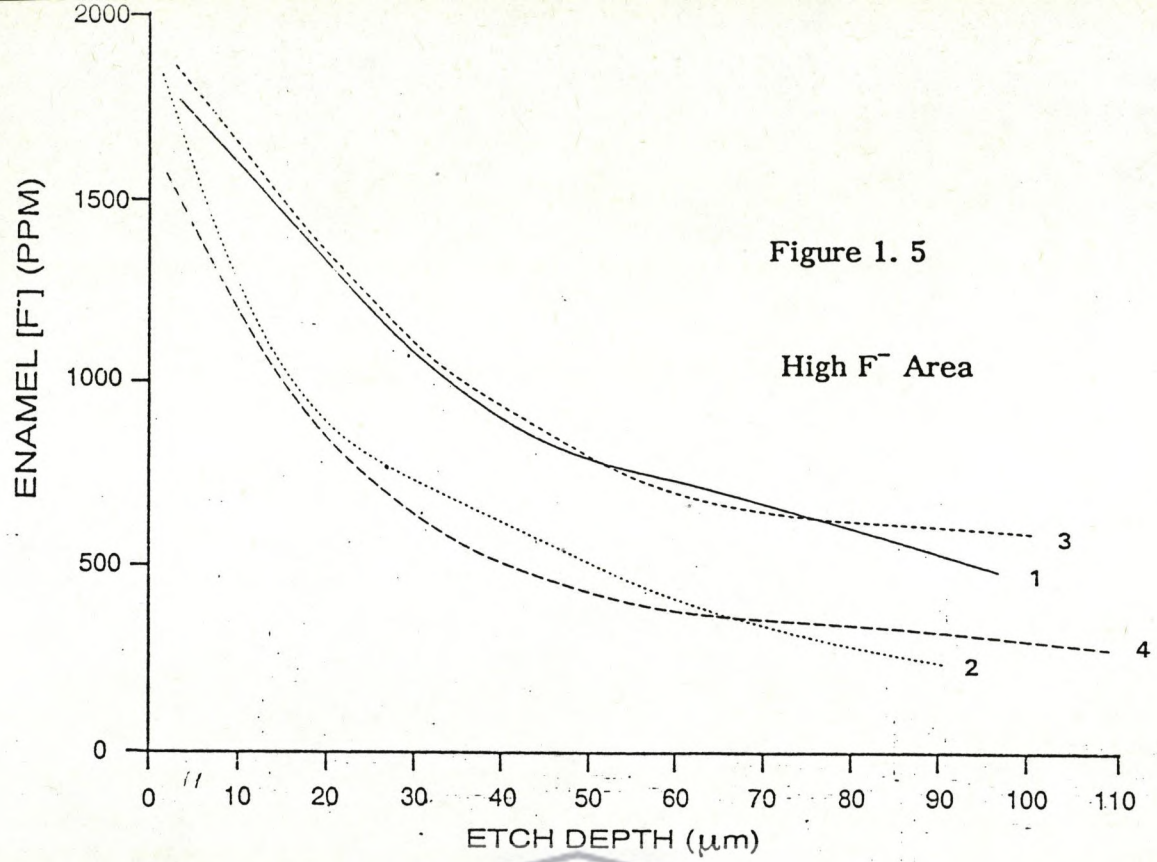

Figure 1. 5

High F⁻ Area

A further graph of Figure 1. 4. (A line fitted to the plotted mean enamel fluoride concentration and the mean etch depths values of unwashed erupted (1), unwashed unerupted (2), alkali-washed erupted (3), and alkali-washed unerupted (4) third molar teeth), after a further spline smoothing was applied to the GAIM lines, to enable easier comparison of the relationship between fluoride concentrations and cumulative etch depths.

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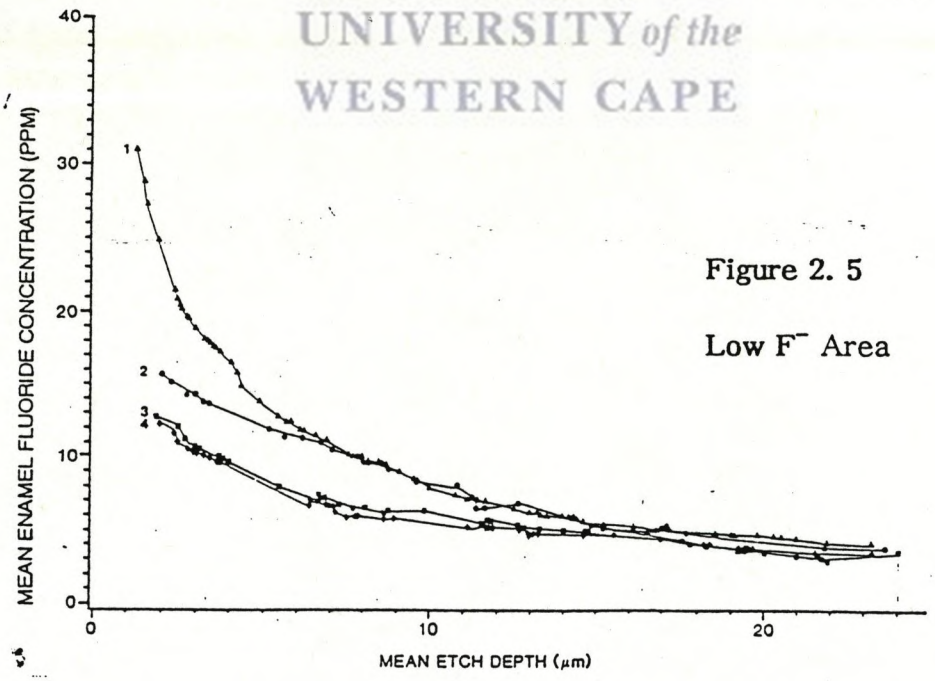

Figure 2. 5

Low F⁻ Area

A line fitted to the plotted mean enamel fluoride concentration and mean etch depth values of unwashed erupted (1), alkali-washed erupted (2), unwashed unerupted (3), and alkali-washed unerupted (4) third molars.

(Grobler and Kotze 1990)

The fluoride concentration in the bulk of the enamel from the low fluoride area (where the subjects brushed with a F^- containing dentifrice at least once a day for a period of 1 - 16 years) is lower than that from an area with F^- > 1.8 ppm in the drinking water. At a depth of ~ 25 μm the enamel F^- is approximately 410 ppm in the low F^- area, while for the high F^- area the value at the same depth for unerupted enamel is approximately 750 ppm (figures 1. 5 & 2. 5).

This indicates that most F^- is built into the deeper enamel prior to eruption as result of a higher F^- content in the drinking water with resulting higher plasma and interstitial fluid levels of F^- during mineralization. To compare the fluoride content of the enamel at 25μm from the surface directly with those in the literature is not possible because of a lack of information on the origin of the teeth, and of the F^- background during tooth development. Investigators do not clearly indicate the fluoride content of the drinking water supply in the area from which their teeth were selected, and inner enamel fluoride levels from approximately 50 ppm (Mellberg,1980) up to approximately 660 ppm (Baijot-Stroobants & Vreven, 1980) have been reported.

The fact that identical curves in the low F^- area (figure 2. 5) as well as in the current study (figure 1. 5) were found for erupted and unerupted third molars demonstrates that enamel has the ability to concentrate fluoride more on the outside surface (from the body fluids), even when the fluoride level of the drinking water is low (F^- < 0,10 ppm). This may be because the last formed, outermost enamel is in contact longer with the extracelluar fluid during the pre-eruptive phase than the inner enamel, which loses contact with tissue fluids after mineralisation is completed (Brudevold *et al.*, 1956).

In contrast to the results for a low F^- area (significant shallower etch depth in erupted than unerupted teeth), no difference in the etch depth between erupted and unerupted enamel was observed in the present study (figure 1. 5). Thus, for the high F^- area (current study) it could be argued that the erupted enamel is just as soluble in strong acid than unerupted enamel because of an exposure to higher plasma F^--levels during tooth development. Furthermore, the erupted enamel did not change significantly regarding its solubility after exposure to the oral environment. Recently however, Lammers, Borggreven, Driessens and van't Hof (1992), reported that remineralised enamel is less soluble than other enamel.

In the case of the low F^- area (Grobler & Kotze,1990), the shallower etching of the erupted enamel may be explained by changes in the enamel composition through the influence of the oral environment rendering the erupted enamel less soluble than the unerupted enamel (Woltgens *et al.*,1981).

After eruption frequent de- and remineralisation dissolves small crystallites and new larger crystallites are formed near the enamel surface (Arends, Jongebloed & Schuthof, 1983). In erupted enamel the crystalline diameter near the anatomical surface (~2.5μm) is larger than the bulk (~150μm) enamel; in unerupted enamel the crystallite diameters near the surface and in the subsurface are similar (Arends *et al.*, 1983). Lenz and Newsley (1964), have suggested that during enamel maturation the minute spaces in immature enamel are reduced.

On the other hand, Palmara *et al.*,(1980), stated that a cuticle with an amorphous structure formed the outermost (0,5 - 1,5 μm) of enamel of unerupted teeth. Enamel underlying this cuticle is composed mainly of loosely packed small crystals (~5nm); the crystals becoming more closely packed with increasing depth.

A graphic representation to explain the relative crystal size is given in figure 3. 5.

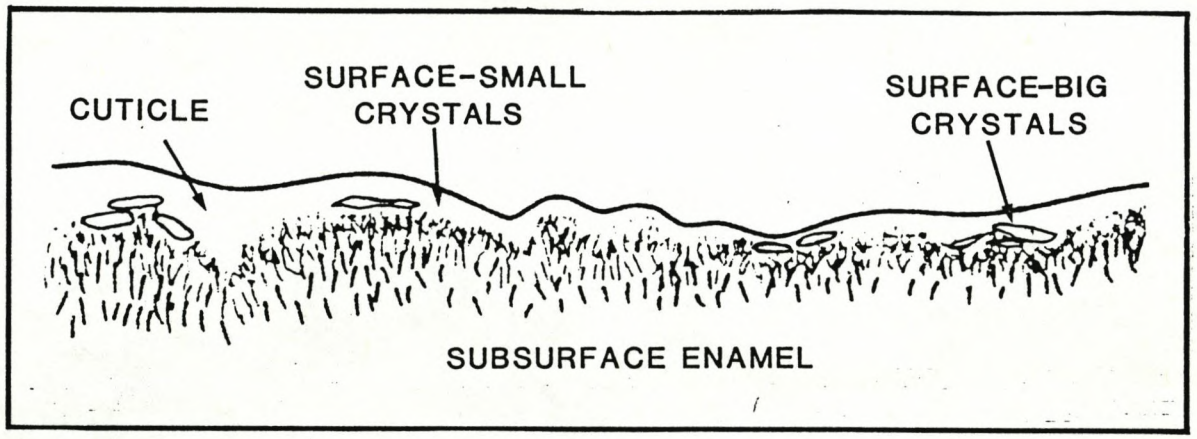

Figure 3. 5. : Diagrammatical representation of the surface enamel of an unerupted human tooth showing the typical variation in crystal size characteristic of all unerupted samples (Palmara *et al.*,1980)

They, (Palmara *et al.*,1980), further found that the surface layers of erupted and partially erupted teeth differed considerably from those of unerupted teeth, and that crystals in the surface of erupted teeth were identical to the subsurface crystals of unerupted teeth.

If crystals in the surface enamel of erupted teeth are small and loosely packed, micropores and interprismatic spaces may provide pathways for acidic agents to dissolve the crystals faster than in unerupted enamel.The presence of the enamel pellicle may also render the surface enamel of erupted molars less soluble than that of unerupted teeth. The pellicle formed from oral fluids is not soluble even in strong acids and cannot be removed by a toothbrush (Jenkins,1978).

The dissolution of CO_3-rich/Mg-rich/F^--poor dental apatite crystals and re-precipitation of CO_3-poor/Mg-poor/F^--rich apatite in the presence of F^--ions in solution contribute to a more acid-resistant surface layer (Le Geros,1990). The above effect could be responsible for the shallower etch depth of erupted enamel from a low F^- area during de-mineralisation. However, in the high F^- area (F^- > 1,8 ppm) this CO_3-poor/ Mg-poor/ F-rich apatite formation could already have happened during the mineralisation process before eruption. Consequently, the effect after eruption could be less pronounced and not reflected in the etch depth as indicated in this study.

There is evidence that F^- could be osteogenic in both embryonic and adult tissues during developing mineralised tissues (Robinson & Kirkham,1990). High plasma F^--levels can however result in the inhibition of proteolysis during enamel maturation and may account for the reported inhibition of enamel crystal growth. It was demonstrated that there is a positive association between fluoride intake and dental fluorosis. Even with very low fluoride intake from water a certain level of dental fluorosis (porosity) will be found in a population (Fejerskov *et al.*,1990).

In its mildest forms, as in this study, (no enamel loss), the "porosity" is to be found in the outermost enamel only, beneath a well mineralised surface layer, but the entire tooth surface is involved. With increasing severity, both the depths of enamel involvement and degree of "porosity" of the enamel increase. Assuming a relatively constant exposure level (water borne fluoride) all surfaces of a given tooth will be equally affected (Thylstrup & Fejerskov, 1978). In the mild form of dental fluorosis however, as was found in this study with a drinking water supply of >1,8 ppm of F^-, mechanical attrition could in time cause an apparent removal of the fluorotic lesions and is most likely due to the removal of the surface enamel (Fejerskov *et al.*,1990).

In this study, this possible effect could have lead to the removal of the outermost enamel with the highest enamel fluoride concentration resulting in a marked smaller difference in the enamel fluoride levels between erupted and unerupted enamel (figure 1. 5), especially at the beginning of the graphs.

The more severe forms of fluorosis result in a diminished physical strength of the enamel, and parts of the superficial enamel may break away exposing the underlying porous enamel. The damage may increase with time. Investigations into the light- and electronmicroscopic changes in human enamel exhibiting various degrees of fluorosis support the concept that fluoride affects the forming enamel and causes porosity of the mature enamel (Fejerskov *et al.*, 1990).

The degree and extent of the porosity depends on the tissue fluid concentration of F^- during tooth development. The structural arrangement of the crystals appear normal but the width of the intercrystalline spaces increases - hence the pores. Likewise the arcade-shaped rod sheaths which partly surrounds the enamel rods during normal development (Fejerskov & Thylstrup, 1987), become widened (Fejerskov *et al.*, 1974).

The pits found in more severe cases of dental fluorosis are a result of post-eruptive breakdown of the surface enamel.

Recently, the F^- content has been examined throughout the enamel in teeth representing the complete range of macroscopically defined degrees of severity of dental fluorosis, classified according to the TF index [page 20],(Richards, Fejerskov & Baelum,1989a). These data show that with increasing severity of fluorosis, the F^- concentration increases not only in the superficial enamel, but throughout the whole tissue. These findings are contradictory to previous observations by Olsen and Johansen (1978), who did not find any association between F^- concentrations in the outer 100μm of enamel and

the surface appearance of the teeth. The authors classified their teeth according to the modification of Dean's index proposed by Møller (1965). As discoloration is included in this classification, (Dean's), this may misleadingly have led to confusion as to the true degree of severity of the fluorosis.

Because of the porous nature of fluorotic enamel, it would be expected that post-eruptive uptake of fluoride might take place to a substantial degree. A rather great variation in fluoride concentration in severely affected teeth (TF scores 7-9), with substantial destruction of the surface enamel, was recorded (Richards *et al.*,1989a).

The observation of increased fluoride concentrations at all enamel depths in teeth exhibiting a TF score of 4, as compared with lower TF categories indicates, however, that the fluoride concentrations represents fluoride incorporated into the enamel prior to eruption.

That this assumption holds true has recently been demonstrated in a study of unerupted human fluorotic teeth by Richards, Fejerskov, Baelum and Likimani,(1989b), who studied the F^- concentrations in unerupted fluorotic teeth to investigate to what degree the increased concentrations previously recorded (the F^- content of fluorosed erupted teeth) were a result of post-eruptive acquisition of F^- by the enamel. In unerupted fluorotic teeth representing TF scores 1-4, the mean F^- concentrations at different depths were similar to concentrations found in erupted fluorotic teeth of comparable severity. It was concluded that up to the stage of lesion severity where posteruptive pitting occurs (TF scores >5) **posteruptive fluoride uptake by fluorotic human enamel seems to be very limited.**

According to the literature (Fejerskov *et al.*, 1990), post-eruptive F^- uptake in fluorotic enamel **without enamel loss** is negligible. This is in agreement with the non-significant

($p>0,05$) difference in F^--content of enamel found in this study between erupted and unerupted teeth (figure 1. 5).

The fact that there was also no difference ($p>0,05$) between the washed and unwashed teeth confirmed this and indicates that no loosely bound F^- was absorbed to the enamel in the mild form of fluorosis.

The curves (figure 2. 5) for the low fluoride area of the alkali-washed and unwashed, erupted enamel intersected each other at a depth of approximately 8 μm, and those of the unerupted enamel at a deeper depth of about 15 μm, indicating that although no loosely bound fluoride (like CaF_2) could be found at these depths, (15μm for unerupted and 8μm for erupted), fluoride did penetrate deeper into the dense enamel to form a firmly bound compound (like fluoropatite).

The fact that no significant amount of fluoride was removed from the unerupted enamel as a result of the alkali-wash procedure, shows that there was no loosely bound fluoride and that all the fluoride gained in the unerupted stage was in a firmly bound form.

On the other hand, a significant reduction in the fluoride content of erupted molars (approximately in the outer 3 μm) was found as a result of the alkali-wash procedure. Relatively less loosely bound fluoride was formed deeper in the enamel than on the outside (figure 2. 5).

The trend of this finding is in agreement with those of Retief (1988), where ground enamel was used in an in vitro study of 2 dentifrices. It indicated that fluoride from the fluoride-containing substances penetrated more slowly as one moves deeper into the enamel, which facilitated a slower ion exchange reaction (fluoroapatite formation) rather than a precipitate formation (CaF_2).

Saxegaard and Rølla (1989) found that during mouth rinsing more calcium fluoride than fluoridated apatite was initially formed on sound enamel. It was suggested that the increased amounts of firmly incorporated fluoride originated from calcium fluoride on enamel and that calcium fluoride is an important and clinically significant source of fluoride ions in enamel. This is because all loosely bound fluoride does not leach off during the first 24 hours (Saxegaard & Rølla,1989; Grobler *et al.*,1983) as previously believed, but dissolves slowly in saliva.

The high variation as illustrated in the standard deviation for the high fluoride content of enamel in the high F^- area (Tables 1. 4, 2. 4, 3. 4 and 4. 4) can be attributed to the fact that all the teeth examined had some degree of fluorosis, resulting in poor control over the variations in the porosity of the enamel, thus the greater variation with technique and consequently the Standard Deviation.

At the level of >1,8ppm fluoride in the water supply, the posteruptive uptake of fluoride seems apparent throughout the enamel, although not significantly. (Compare curves 1 and 3 with curves 2 and 4, fig 1. 5).

This suggests that the average concentration of about 2000ppm fluoride found in the outer 5μm of surface enamel of unerupted teeth from the high F^- (this study) area represents a level of fluoride saturation so that further uptake of fluoride occurs chiefly in the yet unsaturated inner enamel. This would be facilitated by penetration through the mottled enamel due to deficiencies in the interprismatic substance (Isaac *et al.*,1958).

The difference in the fluoride content of mottled and normal teeth warrants some discussion. The finding in this study that high concentrations of fluoride extends further into the subsurface enamel in mottled teeth is probably related to two factors.

One of these is the greater amount of fluoride present in drinking water, food and tissue fluids in areas of endemic fluorosis, thus the systemic uptake.

Secondly, defects are conspicuous in the outer enamel of mottled teeth. This enamel is chalky and soft to grind (Isaac *et al.*,1958), indicating a loosely bound structure. It would seem that penetration of fluoride is facilitated in this type of enamel and that, therefore, the defective enamel contributed to the deeper impregnation of fluoride in these teeth.

It is also clear from figure 1. 5 (alkali-washed vs. unwashed erupted teeth), that F^- from the toothpaste did increase the enamel fluoride concentration throughout the bulk of the enamel, although not significantly. This slight increase was observed even up to a depth of ~100μm. However it seems that the enamel F^- levels tend to equalize at a depth of ~100μm for erupted teeth (figure 1. 5). Unfortunately deeper enamel levels were not analysed for fluoride in this study.

Furthermore, only a slight insignificant decrease in the enamel fluoride concentration was observed in unerupted teeth as a result of the alkali wash procedure. (From 1800 to 1600 ppm F^-).

Likewise, no significant reduction in the enamel fluoride concentration was seen in erupted teeth. This indicates that only a very small insignificant amount of CaF_2-like fluoride could have formed as a result of F^- from dentifrices during the tooth cleaning procedure.

This observation is in contrast to the finding in the low fluoride area, where a significant amount of loosely bound F^- was removed by the alkali-wash procedure, especially from the erupted unwashed teeth.

In conclusion, this study set out to investigate the amounts of fluoride gained in and on enamel of third molars from a high fluoride area (F^->1,8 ppm). The results obtained was compared to that found in a similar study (Grobler & Kotze 1990) on teeth from a low fluoride area (F^-<0,10 ppm).

Firstly it was found that the fluoride concentration in the bulk of the enamel was higher than that from a low fluoride area, and extends further (deeper) into the subsurface enamel. It is thus concluded that this is due to the higher plasma F^- content exposure during the calcification and development of the tooth prior to eruption.

Secondly, it seems that as far as surface enamel fluoride levels are concerned, topical fluoride treatment of enamel from a high F^- area, (drinking water F^- level >1,8ppm), will not have a significant effect on the enamel F^- concentration. This is in contrast to the results from a low F^- area. The assumption can thus be made that posteruptive uptake of F^- by teeth from a high fluoride area is limited.

Thirdly, the amount of alkali-soluble and insoluble F^- on the surface enamel is significantly different to that from a low F^- area where significant amounts of loosely bound F^- was removed by the alkali-wash procedure , especially from erupted teeth, and was due to posteruptive uptake. Thus CaF_2 -like material is not readily gained to a significant level on/in enamel from a high fluoride area.

REFERENCES.

Anon.,Acta Odontologica Scandinavica, **46,** (1988). Ed. Mjör IA, Oslo, Norway.

Arends J, Jongebloed WL & Schuthof J (1983) Crystallite diameters of enamel near the anatomical surface. *Caries Research*, **17**, 97-105.

Ainsworth NJ (1933) Mottled Teeth. *British Dental Journal*, **55**, 233-250.

Baijot-Stroobants J & Vreven J (1980) In vivo uptake of topically applied fluoride by human dental enamel. *Archives of Oral Biology*, **25** ,617-621.

Berndt AF & Stearns RI (1978) **Dental Fluoride Chemistry,** 1st Edition pp 25-70. USA: Charles C Thomas.

Brudevold F, Gardner DE & Smith FA (1956) The distribution of fluoride in human enamel.*Journal of Dental Research*, **35**, 420-429.

Bruun C, Givskor H & Stoltze K (1980) In vivo uptake and retention of fluoride in human surface enamel after application of a fluoride containing lacquer. *Caries Research*,**14**, 103-109.

Carlson CH, Armstrong WD & Singer L (1960) Distribution, migration and binding of whole blood fluoride evaluated with radiofluoride. *American Journal of Physiology*, **199**, 187-189.

Caslavska V, Moreno EC & Brudevold F (1975) Determination of the calcium fluoride formed from in vitro exposure of human enamel in fluoride solutions. *Archives of Oral Biology*, **70** , 333-339.

Chakrabarti CL (1981) Trace element analysis of food and beverages by atomic absorption spectrometry. In: **Progress in Analytical Atomic Spectroscopy,** vol. 2, New York, Pergamon Press, 185-212.

Cutress TW & Suckling GW (1990) Differential diagnosis of dental fluorosis. *Journal of Dental Research*, **69,** (Spec Issue), 714-720.

Dean HT (1934) Classification of mottled enamel diagnosis. *Journal of the American Dental Association,* **20,** 319-333.

Dean HT (1938) Endemic fluorosis and its relation to dental caries. *Public Health Reports*, **53** ,1443-1452.

Dijkman AG, Tak J & Arends J (1982) Fluoride deposited by topical applications in enamel. *Caries Research*, **16**, 147-155.

Driessens FCM (1982) Solubility behaviour of tooth enamel mineral In: **Mineral Aspects of Dentistry**, Vol.10, Chap.6, pp 58-71. S.Krager London.

Ekstrand J (1978) Relationship between fluoride in the drinking water and the plasma fluoride concentration in man. *Caries Research*, **12,** 123-127.

Fejerskov O, Manji F & Baelum V (1990) The nature and mechanisms of dental fluorosis in man. *Journal of Dental Research* **69** (Special Issue) : 692-700.

Fejerskov O & Thylstrup A (1987) Dental Enamel. In: **Human Oral Embryology and Histology.** Eds. IA Mjör and O Fejerskov.pp 50-89 Copenhagen: Munksgaard.

Fejerskov O, Johnson NW & Silverstone LM (1974) : The Ultrasructure of Fluorosed Human Dental Enamel. *Scandinavian Journal of Dental Research,* **82** , 357-372.

Fricke FL (1979) Trace element analysis of food and beverage by atomic absorption spectrometry. In: *Progress in Analytical Atomic Spectroscopy*, ed. Chacrabarti CL, Vol.2, 185-212.

Geddes DAM & Bowen WH (1990) Fluoride in saliva and dental plaque (Summary of Session III at International Symposium on Fluorides) *Journal of Dental Research.* **69,**(Special Issue) 637.

Gibbs M, Retief DH, Bradley EL, Taylor RE & Walker AR (1981) In vivo enamel fluoride uptake from and caries inhibition by topical fluoride agents. *Journal of Dental Research*, **60**, 770-775.

Grobler SR & Joubert JJdeV (1988) The relative distribution of fluoride in erupted and unerupted enamel of human third molars from a low fluoride area. *Archives of Oral Biology*, **33**, 627-630.

Grobler SR, Ogaard B & Rolla G (1981) Uptake and retention of Fluoride in sound dental enamel in vivo after a single application of neutral 2% sodium fluoride. In : **Tooth Surface Interactions and Preventive Dentistry** (Edited by Rolla G, Sonju T & Embery G) 17-25.London: IRL Press Ltd.

Grobler SR & Kotze TJ (1988) Fluoride distribution in the enamel of the mesio-lingual cusps of pairs of erupted and unerupted molars of man with a low fluoride background. *Journal Biology Buccale*, **16**, 89-94.

Grobler SR & Kotze TJ (1990) Alkali soluble and insoluble fluoride in erupted and unerupted sound enamel of human third molars in vivo. *Archives of Oral Biology*, **35,** 795-800.

Grobler SR, Ogaard B & Rølla G (1983) Fluoride uptake and retention by sound enamel after in vivo Duraphat application. *Journal of the Dental Association of South Africa,* **38,** 55-58.

Gron P (1977) Chemistry of topical fluorides. *Caries Research,* **11** , (Suppl.1) 172-204.

Hastie J & Tibshirani R (1987) Generalized additive models: Some applications. *Journal of the American Statistical Association,* **82,** 371-386.

Isaac S, Brudevold F, Smith FA & Gardner DE (1958) The relation of fluoride in the drinking water to the distribution of fluoride in enamel. *Journal of Dental Research,* **37,** 254-318.

Jansen van Rensburg B G In: Mondbiologie. 2nd Edition 1990, 379. Cape Town.

Jenkins GN (1978) Fluoride In: **The Physiology and Biochemistry of the Mouth.** 4th Edition Oxford: Blackwell Scientific Publications.

Jenkins GN & Speirs RL (1953) Distribution of fluorine in human enamel. *Journal of Physiology,* **121,** 21 1953.

Kirkegaard E (1977) In vitro fluoride uptake in human enamel from four different dentifrices. *Caries Research,* 11, 24-29.

Koch G & Petersson L (1972) Fluoride content of enamel surface treated with a varnish containing sodium fluoride. *Odontological Revy,* **23,** 437-446.

Koulourides T (1990) Fluoride and the caries process.(Summary of Session II at International Symposium on Fluorides) *Journal of Dental Research,* **69,**(Special Issue) 558.

Lammers PC, Borggreven JMPM, Driessens FCM & van't Hof MA (1992) Acid susceptibility of lesions in bovine enamel after remineralization in the presence of fluoride and/or carbonate. *Caries Research,* Vol. **26,** No.1, 1 - 7.

LeGeros RZ (1990) Chemical and Crystallographic Events in the Caries Process. *Journal of Dental Research,* **69,** (Special Issue) , 567-574.

Lenz H & Newsley H (1964) Maturation and remineralisation of enamel. In: *Advances in Fluoride Research and Dental Caries* Vol **3** pp 95-98. Oxford:Pergamon Press.

Manley RS & Hodge HC (1939) Dentistry and refractive index studies on dental hard tissues. *Journal of Dental Research,* **18,** 133-141.

McCann HG (1968) Inorganic components of salivary secretion. In: **Art and Science of Dental Research,** Ed. Harris P, 55-73. New York: Academic Press.

McClure FJ & Likins RC (1951) Fluoride in human teeth in relation to drinking water. *Journal of Dental Research*, **30**, 172.

McKay FS (1916) An Investigation of mottled teeth. *Dental Cosmos*, **58**, 477-484.

Mellberg JR (1980) Rate of fluorine uptake by surface and subsurface sound enamel from sodium monofluorophosphate. *Caries Research*, **14**, 50-55.

Mellberg JR, Laakso PV & Nicholson CR (1966) The acquisition and loss of fluoride by topically fluoridated human tooth enamel. *Archives of Oral Biology*, **11**, 1213-1220.

Mellberg RM & Ripa LW. **Fluoride in Preventive Dentistry.** 1st Edition, 1983,Chicago, Illinois: Quintessence Publishing Co.,Inc.

Mellors Modern Inorganic Chemistry. New Edition 1961. Editor GD Parkes Longmans Green & Co. Ltd. Bristol.

Mϕller IJ (1965) Dental Fluorose og Caries. Ph.D.Thesis. Copenhagen: Rhodos Publishers.

Murray JJ(1976) Fluorides in caries prevention. 1st Edition,Bristol: John Wright and Sons.

Mushanoff O, Gedalia I & Daphni L (1981) Fluoride Aquisition by surface enamel of human teeth invivo following tooth brushing with an amine-fluoride toothpaste. *Journal of Dental Research*, **9**, 144-149.

Niibu I, Nakagaki H, Kurosu K & Weatherell JA (1991). Distribution of fluoride across human primary enamel. *Archives of Oral Biology*, **36**, 603-610.

Olsen T & Johansen E (1978) Fluoride content of human fluorosed enamel. *Journal for Dental Research,* **57,** (Special Issue) 281.

Palmara J, Phakey PP, Rachinger WA & Orams HJ (1980) Electron microscopy of surface enamel of human unerupted and erupted teeth. *Archives of Oral Biology*. **25**, 715-725.

Priest ND & Van de Vyver FL (1990) **Trace Metals and Fluoride in Bones and Teeth.** 1st. Edition, Boston:CRC Press.

Retief DH (1988) In vitro enamel fluoride uptake from two Fluoride-containing dentrifices. *Journal of the Dental Association of South Africa,* **43**, 531-535.

Retief DH, Cleaton-Jones PE, Turkstra J & de Wet WJ (1971) The quantitative analysis of sixteen elements in normal human enamel and dentine by neutron activation analysis and high resolution gamma-spectrometry. *Archives of Oral Biology,* **16,** 1257-1267.

Retief DH, Navia JM & Lopez H. (1977) A microanalytical Technique for the estimation fluoride in rat molar enamel. *Archives of Oral Biology,* **22**, 207-211.

Retief DH, Sorvas PG, Bradley EL, Taylor RE & Walker AR (1980) In vitro fluoride uptake, distribution and retention by human enamel after 1- and 24-hours application of various topical fluoride agents. *Journal of Dental Research,* **59,** 573-582.

Retief DH Figure 3. 2. Personal communication.

Richards LF, Fejerskov O & Baelum V (1989a) Enamel Fluoride in Relation to Severity of Human Dental Fluorosis. *Advances in Dental Research,* **3,** 147-153.

Richards A, Fejerskov O, Baelum V & Likimani S (1989b) Enamel fluoride in unerupted fluorotic human teeth . *Caries Research,* **23** , 103.

Richards A . (1990) Nature and Mechanisms of Dental Fluorosis in Animals. *Journal of Dental Research,* **69,** (Special Issue), 701-705.

Richards LF, Fejerskov O, Baelum V & Likimani S . (1989b) Enamel Fluoride in Unerupted Fluorotic Teeth Human Teeth. *Caries Research,* **23** , 103.

Robinson C & Kirkham J (1990) The Effect of Fluoride on the Developing Mineralized Tissues. *Journal for Dental Research,* 69, (Special Issue) , 685-691.

Rϕlla G & Bowen WH (1978) Surface adsorption of fluoride and ionic exchange reactions on hydroxyapatite. *Acta Odontologica Scandinavica,* **36,** 219-224.

Saxegaard E & Rolla G (1989) Kinetics of acquisition and loss of calcium fluoride by enamel *in vivo. Caries Research,* **23,** 406-411.

Söremark R & Samsahl K (1961) Gamma-ray spectrometric analysis of elements in normal human enamel. *Archives of Oral Biology,* **6,** 275-283.

Shannon IL & Edmonds EJ (1978). Fluoride levels in human parotid saliva following ingestion of fluorine compounds of varying solubility. *Journal of Dental Research,* **56,** 1521-1525.

Smith FA & Hodge HC (1959) In: **Fluorine and Dental Health.** Eds. Muhler JC & Hine MK. Bloomington: Indiana University Press.

Smith MC (1932) The cause of mottled enamel. *Journal of Dental Research,* **12** : 149.

Ten Cate JM, Exterkate RAM & Rempt HE (1988) Intra-oral retention of fluoride by bovine enamel from amine fluoride toothpaste and 0,4% amine fluoride liquid application. *Journal of Dental Research,* **67,** 491-495.

Thylstrup A & Fejerskov O (1978) Clinical appearance of dental fluorosis in permanent teeth in relation to histological changes. *Community Dental & Oral Epidemiology,* **6,** 315-328.

Vogel GL, Chow LC & Brown WE (1983) A microanalytical procedure for the determination of calcium, phosphate and fluoride in enamel biopsy samples. *Caries Research*, **17**, 23-31.

Weatherell JA, Deutsch D, Robinson C & Hallsworth AS (1977) Assimilation of Fluoride by enamel throughout the life of the tooth. *Caries Research supplement*, **1, 11** , 85-115.

Weatherell JA, Robinson C, Schaper R & Künzel W (1983) Distribution of fluoride in clinically sound enamel surfaces of permanent upper incisors. *Caries Research*, **17**, 118-124.

Weaver R (1944) Fluorosis and dental caries on Tyneside. *British Dental Journal*, **76** , 185.

Wöltgens JHM, Bervoets JM, Witjes F & Driessens FCM (1981) Changes in the composition of the enamel of human premolar teeth shortly after eruption. *Archives of Oral Biology*, **2**, 717-719.

Whitford GM **The Metabolism and Toxicity of Fluoride** 1st Edition,1989,Basel: Krager Company.

Whitford GM (1990) The physiological and toxicological characteristics of fluoride. *Journal of Dental Research*, **69**, (Special Issue): 539-549.

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