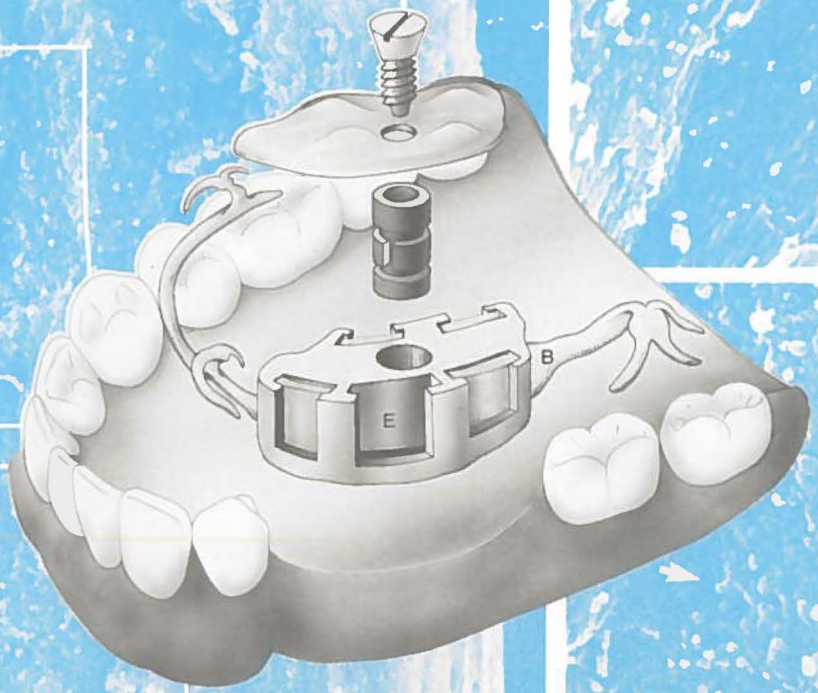
Fluoride varnishes and enamel caries

Bruyn, Hugo de

FLUORIDE VARNISHES AND ENAMEL CARIES



Hugo De Bruyn

FLUORIDE VARNISHES AND ENAMEL CARIES

Cover: Henk Flanderijn

STELLINGEN

behorende bij het proefschrift

"Fluoride varnishes and enamel caries"

Hugo De Bruyn

Groningen, 5 juni 1987

I

De hoeveelheid fluoride in "vaste vorm" aanwezig in het tandglazuur heeft geen voorspellende waarde voor het cariëspreventieve effect van fluoride.

Dit proefschrift

II

Het belang van epidemiologische cariësstudies naar de effectiviteit van fluoride bevattende lakken wordt in het algemeen overschat.

Dit proefschrift

III

Fluoride bevattende lakken bezitten goede eigenschappen om demineralisatie te beperken, dat mag echter nog niet tot de conclusie leiden dat er ten aanzien van de remineralisatie ook gunstige eigenschappen zijn.

Dit proefschrift

IV

De informatie op de bijsluiter van Duraphat betreffende de behandeling van gevoelige tandhalzen is wetenschappelijk onvoldoende onderbouwd.

Clark et al. J. Periodontol. Res. 20: 212-219 (1985)

V

Bij onderzoek van caries met behulp van Scanning electronenmicroscopisch onderzoek moet een onderscheid worden gemaakt tussen initiële cariës en "bleekwatercariës".

Holmen et al. Caries Res. 19: 355-367 (1985)

VI

In de tandheelkundige praktijk is een "tweede mening" geld waard.

VII

De term parodontale aandoening dient uit oogpunt
van voorlichting te worden vervangen
door parodontale infectie.

VIII

Actinobaccilus actinomycetemcomitans zit dieper dan je denkt.

Christersson et al. J. Clinical Periodontol. 12: 465-476 (1985)

IX

Het gebruik van sommige Beta-lytica als
antihypertensiva verhoogt het coronair risico.

X

De Cambodjaanse " Killing Fields " kunnen niet in de
schoenen van de vredesactivisten worden geschoven.

Richard Nixon. No more Vietnams, Avon Books New York (1986).

XI

In België is het geval Happart
geen geval apart.

XII

De Nederlander moet zuiniger zijn op zijn taal dan op zijn geld.

RIJKSUNIVERSITEIT TE GRONINGEN

FLUORIDE VARNISHES AND ENAMEL CARIES

Proefschrift

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. E. Bleumink
in het openbaar te verdedigen op
vrijdag 5 juni 1987 des namiddags te 4.00 uur

door

HUGO DE BRUYN
geboren te Ninove

Promotores: Prof. Dr. J. Arends
Prof. Dr. T. Pilot

Paranimfen: Akkejans van Hoogen
Hans Buskes

I'll remember you
When the wind blows through the piney wood.
It was you who came right through,
It was you who understood.
Though I'd never say
That I've done it the way
That you'd have liked me to.
In the end,
My dear sweet friend,
I'll remember you.

Bob Dylan

Ter nagedachtenis aan Stef

dankwoord

Bij het tot stand komen van dit proefschrift wil ik enkelen bij naam danken:

Prof.Dr. Joop Arends, die dit fluoridelak-onderzoek in goede banen leidde en het risico nam een Belg in zijn promovendigroep op te nemen.

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In het bijzonder Henk Flanderijn die de figuren en de lay-out van dit proefschrift van achteren naar voren en van onder tot boven heeft verzorgd en Gerda Wouwenaar die menig Olivetti-concert heeft gespeeld om dit proefschrift op schijf en papier te brengen.

Mijn ouders, Liliane, Erik, Ina en Ken die tijdens mijn korte bezoeken aan Vlaanderen mij weer echt thuis lieten voelen.

Hugo

contents

The fluoride varnishes used in the experiments described in this thesis are

* Duraphat® further denoted Duraphat.
  Woelm and Pharma Co.
  Eschewege, FRG.

** Fluor Protector® further denoted Fluor Protector.
  Vivadent.
  Schaan, Liechtenstein.

The printing of this thesis was financially supported by Ivoclar (Schaan, Liechtenstein).

aim of the investigation 1

Fluoride is regarded as the most important factor responsible for the decline in enamel caries incidence in the industrialized countries (Murray and Rugg-Gunn 1982).

Fluoride has been administered in several ways, with the object of increasing the fluoride content in enamel and as a consequence reducing dental caries. Water fluoridation has proven to be highly effective mainly because fluoride is, at low doses, constantly available in the oral cavity. The effect of **systemic administration** is most likely important during the pre-eruptive stages of tooth formation and mineralization (Weatherell et al. 1984).
Topical applications on the other hand, such as toothpastes, gels, rinses, etc., deliver fluoride **in** and **on** the enamel in large amounts. They have a local and temporary effect and re-application is a prerequisite for an optimal caries preventive effect.

Fluoride varnishes were introduced in clinical practice in the mid-sixties in order to achieve a higher fluoride uptake after application. They have a longer contact time and contain large amounts of fluoride. The use of varnishes is now widespread in Northern Europe and they are generally considered to possess good caries preventive properties (Clark 1982).

Recently, the importance of **small amounts** of fluoride in the de- and remineralization processes of enamel have been stressed, as has the importance of fluoride around the enamel crystallites, in "the liquid state" (Arends et al. 1983b). For topical applications the fluoride concentration for optimal caries prevention is not yet known. The experiments described in this thesis are part of an investigation of fluoride varnishes with a relatively small fluoride content on human enamel.

Two varnishes have been used extensively in clinical practice: Duraphat and Fluor Protector. In the literature there are contradictory results on the effects of Fluor Protector, a polyurethane based varnish system. In most clinical demineralization studies, however, additional fluoride administration has been used for ethical reasons. To avoid this complicating factor, the present clinical experiments were carried out by using an intra-oral caries model and fluoride administration was avoided.

The aims of this study were to determine, for polyurethane based varnishes with differing F^- concentrations:

1. The fluoride uptake in enamel after varnish application.

2. The caries preventive properties of the varnishes in vivo as well as in vitro.

3. The remineralization of varnish-treated artificial caries lesions.

4. Possible relations between F [uptake] and caries preventive effect.

introduction on the cariostatic action of fluoride 2

THE CARIES PROCESS

Of all human diseases tooth decay is the most ubiquituous, certainly in the industrialized countries. **Caries** is the process of dissolution of the mineral phase of enamel by acids formed in the plaque. In the oral environment, mineral loss occurs several times a day when micro-organisms, food substrates and tooth material interact.
An enamel surface, partially covered by dental plaque, may come under acid attack if nutrients suitable for the plaques' metabolism are present. Depending on the type and duration of the acid attack, the presence of inhibitors (e.g. fluoride) as well as the acid resistance of enamel, mineral will dissolve and an outward diffusion of the mineral components calcium and phosphate will take place.
In general practice the first detectable clinical sign of an early caries lesion is "the white spot". No cavitation is initially evident but the enamel is already softer than the surrounding sound enamel; a cross section through such a spot ("the lesion") reveals a subsurface defect with a relatively intact surface layer. In a clinical white spot the enamel has been attacked for several months or years. The surface layer is porous but still mineral-rich compared with the underlying area with low mineral content. For a review of lesion formation see Arends and Christoffersen (1986).
In Figure 2.1., the various tooth structures, enamel (E), dentine (D) and pulp (P) are shown in cross-section and in detail in SEM-micrographs taken from a human premolar.

A variety of measures can be taken, at various stages of demineralization, to **prevent mineral loss** from enamel lesions.
Oral hygiene measures such as sufficient and regular plaque removal, availability of fluoride and proper food habits can prevent demineralization.

Preventive measures when the initial caries lesion has been formed, may arrest or remineralize the enamel defect. If caries control is completely lacking further demineralization will finally lead to cavitation and professional restoration of the tooth is inevitable and necessary.

The central role of fluoride in caries prevention is widely accepted; the prevention mechanism, however, is multicausal, complex and not understood in all details. In this chapter the cariostatic action of fluoride will be briefly reviewed.

TOOTH ENAMEL

Sound mature enamel consists of approximately 95 wt% mineral, 0.5 wt% organic material and 4.5 wt% water (Jenkins 1978). The mineral phase is apatite-like, denoted in a **simplified** way as $Ca_{10}(PO_4)_6(OH)_2$. It contains numerous elements (of which carbonate and fluoride are the most important) as impurities, and vacancies in the crystal lattice. Due to the presence of vacancies the mineral phase in enamel is better described as a non-stoichiometric apatite, denoted as $Ca_{10-x}(PO_4)_{6-x}(HPO_4)_x(OH)_{2-x}$.

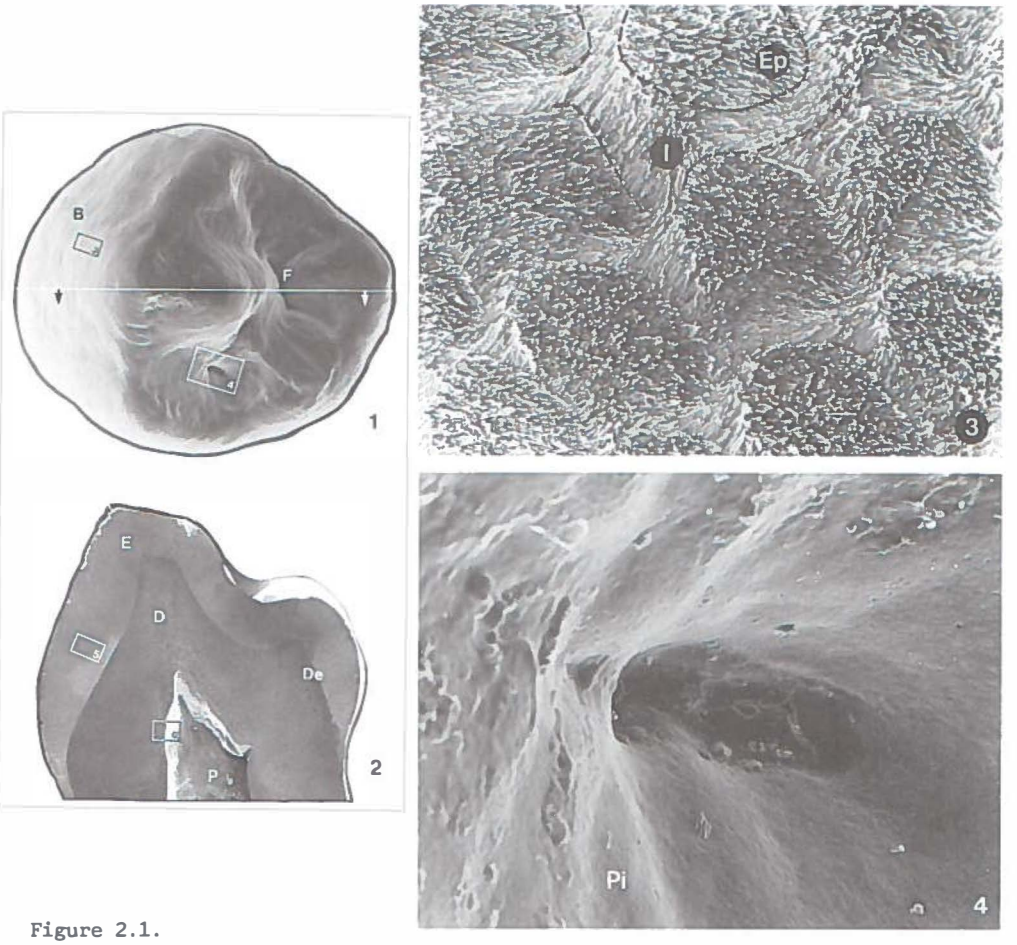
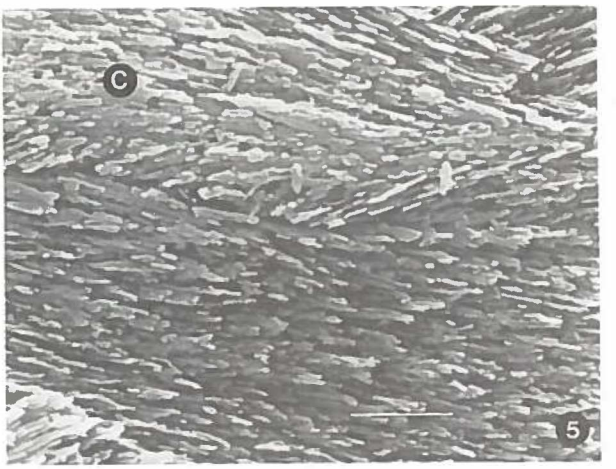
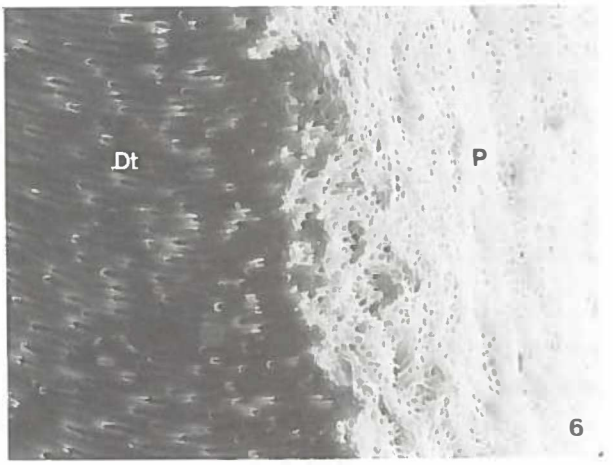


Figure 2.1.
A collage of SEM-micrographs of a human premolar showing the various tooth structures.
1. Occlusal top view of the premolar; the tooth has subsequently been cut in the axial direction, indicated by the dotted line; 2. Cross section of the tooth; 3. Prism structure of the surface etched 10 sec. with 0.001 M HCl; 4. Detail of an occlusal pit; 5./6. Micrograph of the cut surface (not etched).
B: enamel surface; F: fissure; Pi: detail of an occlusal pit; Ep: enamel prism; I: interprismatic area; E: enamel; D: dentine; De: dentino-enamel junction; P: pulp chamber; C: enamel crystallites; Dt: dentine tubules.

It should be stressed that the impurities and trace elements may also contribute to the non-stoichiometry. Please note that the above formula can account for the calcium deficiency found at the enamel surface [Ca/P = 1.4, take $x \approx 1.5$ (Arends and Jongebloed 1977)]; for the Ca/P ratios found in bulk enamel [Ca/P = 1.5-1.6 ,take $0.5 < x < 1$ (Weatherell and Robinson 1973)] as well as for the HPO_4 content in carious enamel [≈ 15% (Arends and Davidson 1975)].





Morphologically, enamel consists of apatite crystals, called "crystallites" (Figure 2.1.c). These are arranged in structures called "prisms" or rods running from the dentino-enamel junction (Figure 2.1.de) to the tooth surface. In cross section the prisms are often keyhole shaped (Figure 2.1.I.e). The crystallites are densely packed and oriented roughly parallel to the axis of the prism in the center of the prism but are arranged less densely and randomly at prism borders. Arends et al. (1983a) showed, using SEM, that the average crystallite diameter at the outer enamel surface (≈ 2.5 µm from the outer surface) is about 59 nm and in the enamel (≈ 150 µm from the outer surface) 38 nm. These differences are due to reprecipitation and crystal growth at the surface, induced by very frequent in vivo de- and remineralization cycles.

CARIOSTATIC ACTION OF FLUORIDE

Introduction.

In the oral environment fluoride is present in the enamel, in large amounts in dental plaque and in small concentrations in saliva.

There is a concensus in the literature that fluoride acts in several ways to prevent caries. The role of fluoride at various levels and stages of the demineralization/remineralization process will be discussed in the following sections. In practice the cariostatic action of fluoride is, of course, a combination of the various effects.

Fluoride and (sound) enamel solubility.

A. Fluoride in the enamel lattice (Fs).

An important aspect of the cariostatic action of fluoride on enamel is that it affects mineral solubility directly. From apatite studies (Christoffersen et al. 1984) it is well known that the solubility product of apatite can be changed by the incorporation of fluoride in calcium hydroxyapatite. Figure 2.2.a. shows, schematically, a crystallite with incorporated fluoride (denoted Fs). This is expressed in the (simplified) formula:

$$Ca_{10}(PO_4)_6(OH)_2 + xF^- \longrightarrow Ca_{10}(PO_4)_6(OH)_{2-x}F_x + xOH^-.$$

Please note that for $x = 2$ fluorapatite containing more than 3 wt% F^- (FAP) is obtained; for $x < 2$ a partially and inhomogeneously fluoridated hydroxyapatite is obtained; for $x = 0$ calcium-hydroxyapatite without fluoride is obtained (HAP). The solubility of FAP in the acidic range is lower than the solubility of HAP.

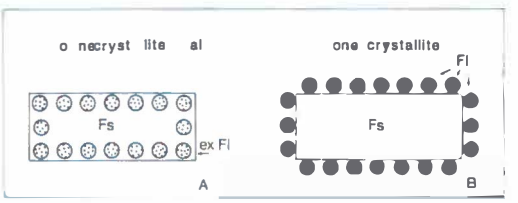


Figure 2.2.
Schematic representation of an enamel crystallite with a: fluoride (Fs) incorporated in the crystallite lattice and b: with adsorbed fluoride on the surface delivered by fluoride in the liquid state (Fl).

In vivo there is no clear relationship between fluoride content in enamel and caries prevalence (Englander et al. 1967; Fejerskov et al. 1981; Weatherell et al. 1984; Nasir et al. 1985). Fejerskov et al. (1981) showed that in populations with 1 ppm F^- in the drinking water, only 10 % of the totally available OH-groups were substituted by F. The solubility difference between a partially substituted hydroxyapatite (10 % OH by F) and pure HAP is too small to account for the large cariostatic effect of water fluoridation (it is however in agreement with section B, because all surface OH sites could be covered by F^-).

Both in vitro and in vivo research results proved that the substitution of fluoride IN the crystal lattice influences the dissolution of the mineral. The prevention of dissolution by Fs in the oral environment is most likely one of the minor effects of fluoride action.

B. Fluoride in the liquid phase (Fl).

For many years, the structurally incorporated fluoride in the enamel was believed to be the main reason for caries prevention. A common view has been "more fluoride in the enamel, gives even better protection". Recently, however, the importance of fluoride in the liquid phase (denoted Fl in Figure 2.2.b.), around the enamel crystallites, has been stressed (Arends et al. 1983b). Nelson and co-workers (1983a) described that the initial dissolution behaviour of hydroxyapatite pellets with or without 1000 ppm fluoride as Fs was almost identical. However, 1 ppm fluoride in the demineralization solution as Fl decreased the kinetic dissolution substantially. Ten Cate and Duijsters (1983) showed that 2 ppm F^- in the demineralization solution could block enamel dissolution completely at pH 4.5.

According to Arends et al. (1984) and Arends and Christoffersen (1986) Fl is much more active in decreasing dissolution of crystallites than Fs incorporated in the crystal lattice. Fl is continuously delivered by fluoride in saliva and plaque from toothbrushing, mouthrinsing and leakage from topical fluoride applications. This is schematically depicted in Figure 2.3..

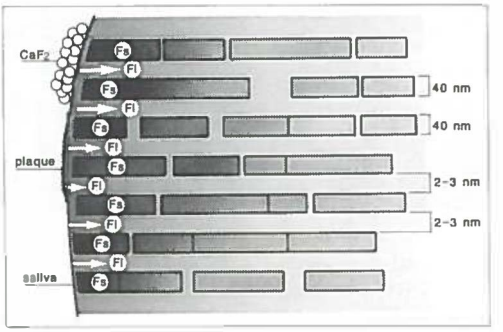


Figure 2.3.
Schematic representation of the diffusion of fluoride in the interprismatic area. Fl is delivered by fluoride in plaque, saliva or topical applications. Fs is the fluoride incorporated in the crystallite lattice.

C. Fluoride deposited on the tooth surface (F [on]).

Topical fluoride applications, with a high fluoride content, deposit mainly calcium fluoride (CaF_2)*. The general reactions taking place at the surface are:

$$Ca_{10}(PO_4)_6(OH)_2 + H^+ \longrightarrow 6CaHPO_4 + 4Ca^{2+} + H_2O.$$

$$Ca^{2+} + 2F^- \longrightarrow CaF_2.$$

After an acidic topical fluoride application, calcium is partially removed from the enamel by the acid interaction and CaF_2 and some FAP are precipitated on the surface. This can be seen by SEM after topical fluoride appli-

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* Please note that CaF_2 is used as a simplification in the sense of "CaF_2-like" material. The deposited material contains many more ions than F and Ca (especially HPO_4).

cation (Figure 2.4.). Non-acidic applications with a high F^- content need some dissolution to react with enamel or form CaF_2 directly.

Nelson et al. (1984) explained in detail that the above mentioned fluoride interactions determine the crystallographic properties of the coating on the enamel; the amount of coating material is influenced by the duration of contact with the enamel.

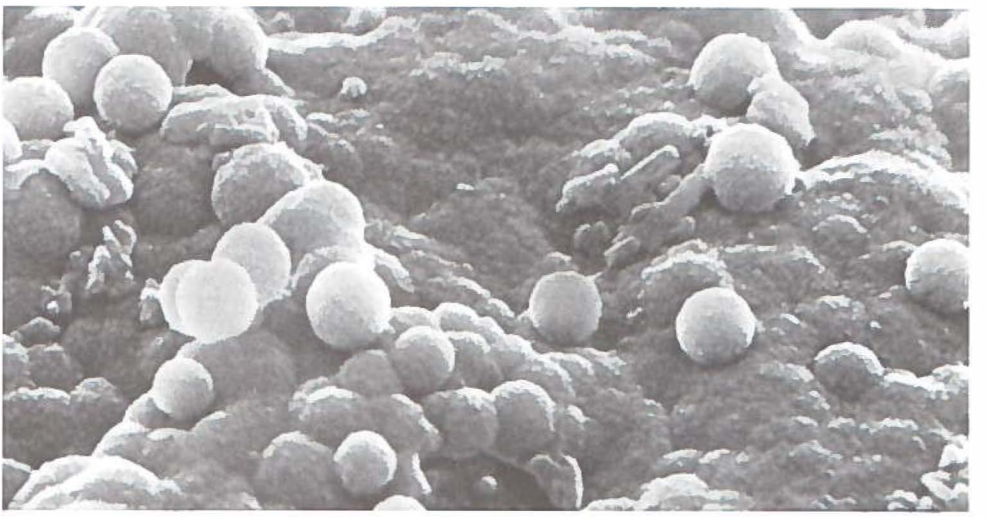

Figure 2.4.
SEM-micrographs showing CaF_2-like globules on the enamel surface after Fluor Protector varnish application. (From Nelson et al. 1983b).

Although some authors state that CaF_2-layer on the enamel surface dissolves quite quickly, because of its supersaturation with respect to saliva (Brudevold et al. 1956), it is now generally accepted that the CaF_2-like globules dissolve in periods of weeks or months (Dijkman 1982; Øgaard et al. 1983).
The significance of the CaF_2-like layer on the enamel in caries prevention is still controversial. Ten Cate and Duijsters (1983) showed that a demineralization solution supersaturated to CaF_2 has a strong inhibiting effect on demineralization. They suggested that CaF_2 may act as a diffusion barrier at the surface enamel pores. The same suggestion was also made by Nelson et al. (1983b, 1984), who investigated the precipitation of various fluoridated agents. The latter authors also stressed that the surface morphology was altered by the **CaF_2-like globules filling** up enamel **pits or defects**, normally the most **dissolution prone** sites.

The most important role of CaF_2 is probably the fluoride "depot" function. Fluoride leaches away by various mechanisms with the dissolution of the globules and consequently increases the fluoride level in saliva (Lambrou et al. 1981). Fluoride may also diffuse through the underlying enamel and increase the fluoride concentration in the liquid phase (Fl) around the enamel crystallites (see previous section). This phenomenon has been described by Dijkman et al. (1983) after in vivo fluoride varnish applications.

Recently, Rølla and Øgaard (1986) suggested that the CaF_2-layer on the enamel in contact with saliva, is coated by phosphates and proteins. They tentatively suggested that the CaF_2 coating may act as a pH controlled fluoride reservoir. During a pH drop, CaF_2 is partially dissolved and fluoride can thus become useful in periods of remineralization as the pH increases.

The role of calcium fluoride as F [on] is probably threefold
i) a coating layer on enamel affecting dissolution,
ii) a fluoride reservoir contributing to the liquid fluoride in enamel and
iii) a remineralization enhancing source of fluoride.

Fluoride and de- and remineralization processes.

Remineralization of early caries lesions has been described as early as 1912 (Head 1912). Backer Dirks (1966) convincingly proved that white spots, detected clinically and by bitewing examination, could visibly disappear as a result of repair mechanisms in saliva combined with good oral hygiene and the presence of fluoride. Extensive research, both in vitro and in vivo, on remineralization phenomena has been carried out in the last decades (for reviews see Silverstone 1977; Ten Cate 1979 and Gelhard 1982; Arends and Ten Bosch 1986).

A considerable contribution to the understanding of in vivo remineralization was made by Koulourides and his group (1974; 1980). From their investigations it became clear that demineralized enamel, created in an intraoral device, could be rehardened by saliva and that 1 ppm fluoride enhanced the rehardening. They suggested that the cyclic alternation of de- and remineralization in the oral environment made teeth more resistant to a subsequent acid attack when fluoride was available. This increased resistance is caused by the formation of a fluoridated hydroxyapatite, when mineral is redeposited in presence of fluoride (Feagin et al. 1972). Hallsworth et al. (1973) described that, during an acid attack, carbonate magnesium, calcium etc. are preferentially lost from the enamel, followed by a preferential uptake of fluoride during recrystallization of mineral.
Arends and Ten Cate (1981) stressed the importance of low but constantly available fluoride levels to enhance remineralization.

Using a pH-cycling model, Ten Cate and Duijsters (1982) showed that if de- and remineralization alternated, fluoride in the solution could not only stimulate remineralization but even lead to the formation of arrested lesions presumably protected against any future acid attack.

In vitro research has clearly demonstrated that depending on the test conditions used, remineralization of artificial caries lesions is possible (Ten Cate and Arends 1977, 1978, 1980; Silverstone et al. 1981; Ten Cate et al. 1981).
In general enamel remineralization is enhanced by fluoride if it is added to the solution while the supersaturation is low.
Complete remineralization is even possible in vitro (Chen et al. 1985; Buskes et al. 1985). Sometimes arrested lesions are formed because of the high fluoride levels in the remineralization solution (Ten Cate and Duijsters 1982).
In vitro it has been shown that multiple applications of low concentrations of fluoride are preferable to single high concentrations (Ten Cate et al. 1981).

In vivo, remineralization is always caused by the Ca and phosphate from the saliva. Larsen (1973) showed that saliva is supersaturated with respect to both HAP and FAP at pH 6. Saliva is undersaturated with respect to FAP at pH values between 5.5 and 4.5. At pH values less than 4.0 saliva is undersaturated to both HAP and FAP. Natural repair is thus likely to occur at normal pH values. Featherstone et al. (1982); Gelhard and Arends (1984a,b); Dijkman et al. (1986) and Ten Cate and Rempt (1986) have shown that saliva has indeed remineralizing properties when good oral hygiene is performed, even if no fluoride is available. Fluoride is, however, a remineralization promoting agent.

Gelhard and Arends (1984a,b) showed that the remineralization (repair) rate of lesions was much lower in vivo than in vitro. In the presence of fluoride a 31% mineral gain was found, compared with 20% mineral gain in the control.
Øgaard et al. (1986) investigated the effect of an 0.02% sodium fluoride mouthrinse on initial lesion formation. Fluoride could retard lesion development substantially and 80% reduction in mineral loss was found compared with the control group.
Mellberg et al. (1985) tested a MFP/DCPD (monofluorophosphate-dicalciumphosphate dihydrate) dentifrice and found a substantial reduction in lesion depth compared with a placebo group.
Although several investigations have shown that fluoride is an important factor in the de- and remineralization process, it is most important to realize that the effect is strongly dependent on the oral environmental conditions of the patient (Dijkman et al. 1986).

In the oral environment de- and remineralization alternate several times a day. The effect of fluoride, tested in vivo, is therefore always a combined effect on both de- and remineralization (Ten Cate and Duijsters 1982; Arends and Ten Bosch 1986).
Featherstone et al. (1986) investigated the effect of several fluoride regimens on remineralization both in vivo and in vitro. A pH-cycling model was used to simulate the de- and remineralization cycles in vitro. A comparison with in vivo results obtained under orthodontic banded plaque covered enamel show that frequent application of relatively low fluoride concentrations is effective in inhibiting demineralization and enhancing remineralization.

Caries lesions formed in vivo are mainly subsurface lesions. After several months of formation they have the classical mineral-rich surface layer covering the lesion body with a low mineral content. Using microradiography, one can visualize the mineral distribution as a function of the distance from the enamel surface to the dentino-enamel junction. In Figure 2.5. the mineral distributions of sound enamel, surface softened enamel and for lesions with a surface layer are depicted.
Recently, Øgaard et al. (1986), showed that initial caries lesions in vivo mainly had a surface softening type of mineral distribution.
In vitro it has also been shown that the initial caries lesion has a surface softening type of mineral distribution (Borsboom et al. 1985, Buskes et al. 1987). In the latter study mineral loss was followed in time at the same area of enamel. The first stage of demineralization is surface softening, followed by subsurface lesion formation.

From in vitro investigations (Ten Cate and Duijsters 1983a,b; Borsboom et al. 1985 and Margolis et al. 1986) it is obvious that fluoride ions often

cause surface layer formation. When no fluoride is present in the demineralization systems, surface softening mineral distributions are observed. The presence of small amounts of fluoride in the **liquid**, however, result in surface layer formation. There are also indications that proteins may induce surface layer formation (Arends and Christoffersen 1986; Van der Linden 1986).

For a complete review of de- and remineralization phenomena is referred to the proceedings of the 'Research group on surface and colloidal phenomena in the oral cavity' (Leach and Edgar 1983; Leach 1986).

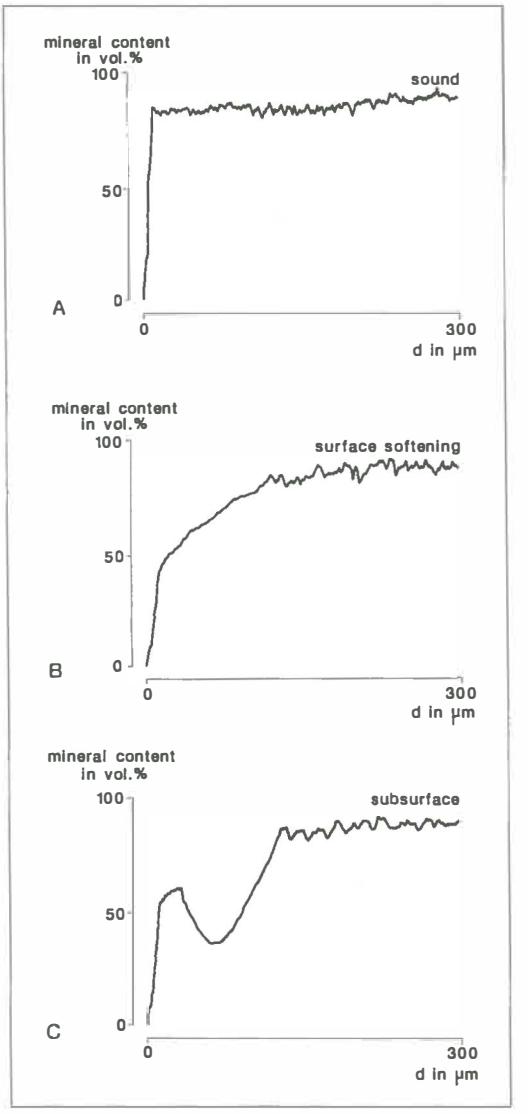

Figure 2.5.
The mineral distributions of sound enamel, surface softened enamel and a subsurface lesion.

Fluoride and plaque metabolism.

The normal level of fluoride in human saliva is too low (≈ 0.02 ppm) to affect bacterial metabolism (Shannon et al. 1973). Fluoride concentrations in dental plaque are in the 0-60 ppm range (Hardwick and Leach 1962). It seems that only 2-3% (or about ≈ 0.08-0.8 ppm) of the fluoride is in the active ionized form and at these levels is of minor importance (Jenkins et al. 1969). The rest of the fluoride in plaque is thought to be bound to inorganic components (Birkeland and Rølla 1972) and/or to bacteria (Jenkins et al. 1969).

When the plaque pH drops, during acid formation, fluoride ions are assumed to be liberated and bacteria may become more sensitive to fluoride (Birkeland and Charlton 1976). The increased fluoride ion activity can thus affect the carbohydrate metabolism of the micro-organisms and even at a 1 ppm F^- level acid production can be inhibited. Ferretti et al. (1982) tested the effect of several 10 ppm F^- products on bacterial viability, growth, acid and glucan production and adherence. It was found that fluoride could reduce acid production of bacteria as well as bacterial growth. SnF_2 resulted in a greater growth reduction of streptococcus mutans which is explained by the presence of tin. Tin is a heavy metal and able to precipitate proteins and may have a germicidal effect.

In vivo, however, there is no substantial evidence for the effects of fluoride on plaque metabolism or plaque growth. Very contradictory results have been published.
Myers and Handelman (1971) could not show any changes in the **proportions** of bacterial numbers after regular fluoride applications. Birkeland and Rølla (1972) claimed on the other hand that topical fluoride applications reduce **plaque growth** and **plaque thickness.**
Loesche et al. (1975) showed that acid APF applications had specific **antimicrobial** effects against streptococcus mutans in occlusal plaque and reduced plaque and gingivitis scores. Kilian et al. (1979) on the other hand did not find any difference in plaque composition between two populations exposed to low and high water fluoride concentrations, respectively. Zickert and Emilson (1982) investigated the level of streptococcus mutans in saliva and plaque after Duraphat application and found that the high fluoride levels had no significant effect on bacteria.
Renggli (1983) described that a 100 ppm fluoride mouthrinse could inhibit **plaque formation,** although not to the same extend as a 0.2% chlorhexidine solution. A 200 ppm solution could destroy plaque completely. Dijkman et al. (1985) suggested that CaF_2 formation on the tooth surface could influence and inhibit dental **plaque accumulation.**

The controversial clinical data on the influence of fluoride on plaque formation, plaque accumulation and plaque growth inhibition are extremely difficult to interpret due to the large variations between subjects as well as within the oral environment.
The controversial data on plaque inhibition by fluoride make it obvious that the cariostatic effect of fluoride on plaque inhibition is not yet understood.

CONCLUSIONS

The cariostatic action of fluoride in the oral cavity is a combination of several mechanisms.
Fluoride in the liquid phase, around the crystallites inhibits enamel demineralization most significantly. Fluoride also acts as a catalyst in the transformation of unstable mineral phases of apatite into the more stable and less soluble phases such as HAP or FAP.
Fluoride enhances enamel remineralization; it is an important ion during de- and remineralization cycles and promotes the formation of acid resistant enamel.
Fluoride may also have an effect on bacteria and plaque metabolism; the last two factors are however not well established.

fluoride varnishes
a review

3

INTRODUCTION

Caries reduction was demonstrated in the early 1940's in areas with fluoridated drinking water (Dean et al. 1942). Numerous dental research projects have been carried out since then to explain the mechanism of fluoride action.

Various oral hygiene and topical fluoride regimens have been employed in practice to achieve caries protection. For reviews see Goorhuis et al. (1985) and Murray and Rugg-Gunn (1982).
Acidulated fluoride gels and solutions were introduced in 1963 (Pameijer et al.); clinical trials revealed a remarkable caries decrease using these systems. It was found, however that considerable amounts of fluoride "leached away" in the first 24 hours after application (Mellberg et al. 1966). Brudevold et al. (1967) observed that the efficiency of topical applications was strongly related to the contact period to dental enamel. A longer exposure time increases the amount of fluoride retained permanently in enamel, enhances the formation of fluoridated hydroxyapatite and reduces the enamel acid solubility (Mellberg et al. 1967).

In the light of these results, researchers have developed alternatives to the conventional topical application methods in order to prolong contact periods.
Richardson (1967) demonstrated 'in vitro` that fluoride uptake was increased by prolonging the time interval between application and washing in saliva. He suggested the application of a waterproof coating on enamel following fluoride treatment. Mellberg et al. (1967) used shellac, polyvinyl pyrrolidone vinylacetate and nonylphenol in 20% ethanol as an effective coating after fluoride application. Schmidt (1964) introduced sodium fluoride in a natural colophonium base. This led to the commercial development of fluoride varnishes.

TYPES OF VARNISHES

The product used by Schmidt (1964) was later marketed as Duraphat (Woelm and Pharma Co., Eschwege, FRG). It contains 5 wt% sodium fluoride or 2.26 wt% F^- in a neutral colophonium base. The material is commercially available as a 10 ml tube of 50 mg NaF/ml and recently also in cartridges of 1.6 ml. The latter is ideal to apply the varnish directly onto the tooth surface with a syringe (Schienbein 1986). Duraphat hardens into a yellowish-brown coating.

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De Bruyn, H. and Arends, J.: Fluoride varnishes - a review-. Jour. Biol. Buccale (accepted 1987).

Table 3.I *In vitro studies of fluoride uptake in enamel after topical application.*

Study	Application type	Application period	Evaluation period	Biopsy depth (μm)	Fluoride acquired (ppm)
Koch & Petersson (1972)	Duraphat	1 h	1 h	9.8	1109
		3 h	3 h		1093
		6 h	6 h		2475
		12 h	12 h		2617
Arends & Schuthof (1975)	APF	3 min	3 min	9.0	10973
			1 day		1054
			1 week		534
	Fluor Protector	3 min	3 min		1735
			1 day		937
			1 week		334
		1 day	1 day		1635
			1 week		1650
Edenholm et al. (1977)	Duraphat	1 day	1 day	4.2	4603
			1 week	2.1	2325
	Fluor Protector	1 day	1 day	2.7	6784
			1 week	3.0	4039
Retief et al. (1980)	APF	1 h	1 h	7.5	772
		1 day	1 day		402
			1 week		344
	Duraphat	1 h	1 h		949
		1 day	1 day		3292
			1 week		1704
	Fluor Protector	1 h	1 h		2081
		1 day	1 day		3113
			1 week		2292
Dijkman et al. (1983)	Duraphat	1 day	1 day	9.0	3506
			1 week		2345
			4 weeks		1991
			12 weeks		2401
	Fluor Protector	1 day	1 day	8.7	4285
			1 week		3695
			4 weeks		5850
			12 weeks		4685
	APF	5 min	5 min	9.8	2830
			1 week		1296
			4 weeks		1445
Retief et al. (1983)	Duraphat	4 h	4 h	7.5	823
		24 h	24 h		1225
	Fluor Protector	4 h	4 h		1368
		24 h	24 h		1225
	APF	4 h	4 h		323
		24 h	24 h		200
Bryant et al. (1985)	Duraphat	1 day	1 day	2.5	3006
	Fluor Protector	1 day	1 day		5276
	APF	1 day	1 day		2110
Retief et al. (1985)	Duraphat	2 h	4 weeks	3.1	780
		4 h	4 weeks		1800
	Fluor Protector	2 h	4 weeks		1360
		4 h	4 weeks		2000
	APF	2 h	4 weeks		600
		4 h	4 weeks		1200

A second varnish system Fluor Protector (Vivadent, Schaan, Liechtenstein) was first introduced by Arends and Schuthof in 1975. It is a polyurethane based lacquer containing 0.7 wt% fluoride in the form of difluorosilane (5 wt%). Fluor Protector has acidic properties and a lower fluoride content than Duraphat. It has a clear transparant color after hardening in contact with air and is packed in 1 ml ampules. Fluor Protector is applied with a small brush.

Both varnishes are commonly used in European dental practices and have been tested in many laboratory and clinical studies.
In this chapter the use and advantages of varnish applications will be discussed. A review of in vitro and in vivo research as well as animal studies will be given with respect to both varnishes.

FLUORIDE UPTAKE

Fluoride uptake in vitro.

Although no strong direct relationships between fluoride uptake and caries prevention has as yet been demonstrated, the working action of varnishes may be the enhanced fluoride deposition in enamel.
Table 3.I. summarizes the amount of fluoride acquired in an outer enamel layer, at depths varying from 2.5 to 9.8 μm, as presented in several in vitro studies. Koch and Petersson (1972) demonstrated that a longer exposure period of Duraphat delivered more fluoride in enamel. This was also demonstrated by Retief et al. (1980, 1985) for Fluor Protector.

When compared with APF-gel and amine fluoride applications both varnishes induce a significantly greater amount of fluoride in enamel. In general Fluor Protector demonstrates a greater affinity for enamel and fluoride remains more permanently bound than Duraphat (Arends and Schuthof 1975; Kolehmainen et al. 1978; Retief et al. 1980, 1983, 1985; Dijkman et al. 1983; Bryant et al. 1985). Varnishes are also superior to NaF and MFP containing dentifrices as far as fluoride uptake is concerned (Arends et al. 1980).

Arends et al. (1980) used secondary ion mass spectrometry (SIMS) to compare fluoride acquired after various fluoride regimens near the enamel surface. Treatment with Fluor Protector or Duraphat resulted in gains of 1000-10000 ppm F^- at 1.5 μm depth and they found a reactive layer of CaF_2 precipitated on the outer enamel surface.

Fluoride loss after varnish application has also been investigated as a function of time. Arends and Schuthof (1975) showed that after 1 week of washing, the Fluor Protector varnished specimens had not lost substantial amounts of fluoride. Dijkman et al. (1983) even found a netto fluoride gain 3 months after Fluor Protector application in vivo. Compared with APF both varnishes have a long lasting fluoride effect.

Retief et al. (1985) found that an increase in the exposure period from 2 to 4 hours had a positive effect on the amount of fluoride acquired; prolongation from 4 to 24 hours had, in contrast, no further effect (Retief et al. 1983). This finding is important in dental practice because it implies that a clinical application of about 4 hours is required to maximize the fluoride uptake.

Fluoride uptake in vivo.

Table 3.II. summarizes fluoride uptake studies in vivo.
Bang and Kim (1973) used electron microprobe analysis to assess fluoride uptake in human teeth after Duraphat application. Stamm (1974) and Petersson (1975) investigated fluoride uptake 5 weeks after a single application of Duraphat in vivo and still found substantial amounts of fluoride. Petersson (1976) reported that Duraphat provided the highest amount of fluoride, when compared with eight conventional topical fluoride applications. Fluor Protector has been investigated in vivo by Bruun et al. (1980). The best results were found when application was repeated within one week. Increased fluoride levels could still be measured in deeper enamel layers 6 months after application.

Table 3.II *In vivo studies of fluoride uptake in enamel after topical applications.*

Study	Application type	Application period	Evaluation period	Biopsy depth (μm)	Fluoride acquired (ppm)
Bang & Kim (1973)	Duraphat	unknown	1 week	15-30	500-750
Stamm (1974)	Duraphat	unknown	5 weeks	11.0	591
Petersson (1975)	Duraphat	unknown	1 week	9.2	848
			5 weeks	10.8	888
Petersson (1976)	Duraphat weekly	unknown	3 weeks	9.9	1244
	2% NaF gel weekly	4 min	3 weeks	13.4	287
	2% APF gel weekly	4 min	3 weeks	10.6	379
	0.025% NaF rinse daily	4 min	3 weeks	7.9	851
Bruun et al. (1980)	Fluor Protector 1x	unknown	1 week	2.0	1100
	Fluor Protector 2x	unknown	2 weeks	2.0	1825
	Fluor Protector 2x	unknown	24 weeks	2.0	950
Koch et al. (1982)	Duraphat	unknown	1 day	5.2	854
			1 week	5.5	959
			4 weeks	5.1	227
			12 weeks	4.6	229
			24 weeks	4.6	304
Seppä et al. (1982a)	Duraphat 6x	unknown	3 years	2.9	1328
	Fluor Protector 6x	unknown	3 years	2.9	2143
Dijkman et al. (1983)	APF	5 min	5 min	2.3	1245
			1 week	2.2	730
			4 weeks	2.2	469
	Duraphat	1 day	1 day	2.2	1440
			1 week	2.1	614
			4 weeks	2.1	838
			12 weeks	2.1	660
	Fluor Protector	1 day	1 day	2.1	1585
			1 week	1.9	2890
			4 weeks	1.9	4860
			12 weeks	1.9	6650
Seppä (1983)	Duraphat	unknown	3 weeks	3.0	1620

The long term effect of both varnishes was also reported by Koch et al. (1982) and Dijkman et al. (1983). The latter used an intra-oral device to measure both alkali-soluble fluoride (CaF_2-like) on the enamel surface as well as fluoride acquired in the enamel. Fluor Protector was reported to be superior in both cases. Nelson et al. (1983b, 1984) confirmed the CaF_2-like precipitation after varnish applications in vitro, using X-ray diffraction and electron diffraction combined with scanning electron microscopy. The CaF_2-like material is shown in the SEM-micrographs presented in Figure 3.1 a,b,c. Fluor Protector deposits more CaF_2-like material on the enamel surface than e.g. AFP, while fluoride loss in time is slower.

Table 3.III *Clinical trials testing Duraphat.*
** measured values*
+ additional 0.2% NaF rinse
(NS) not significant

Study	Application type	Application number	Study length (months)	Subjects' age (year)	Subjects' number	Caries reduction		Study design Split mouth	Study design Control
Heuser & Schmidt (1968)	Duraphat	1	15	11-13	224	DMFT	30%		208
Hetzer & Irmisch (1973)	Duraphat	6	36	9.5	72	DMFS	18%		137
		6	36	10.5	67	DMFS	43%		
Maiwald & Geiger (1973)	Duraphat	6	23	11.5	97	DMFS	46%		64
		2	23	11	82	DMFS	6% (NS)		110
Maiwald (1974)	Duraphat	9	36	11.5	65	DMFS	38%		65
Hochstein et al. (1975)	Duraphat	2	12	3	94	DMFT	37%		183
		3	24	3	94	DMFT	16%		270
		4	36	3	94	DMFT	17%		203
Koch & Petersson (1975)	Duraphat	2	12	15	60	DMFS	77% +		61
Wegner (1976)	Duraphat	4	24	10-12	43	DMFT	54%		44
Murray et al. (1977)	Duraphat	4	24	5	302	DMFS	36.6% permanent	X	
						DMFS	7.5% (NS) primary		
Lagutina et al. (1978)	2.9% F^-	6	36	children	450	DMFS	54%		482
Holm (1979)	Duraphat	4	24	3	112	DEFS	44%		113
Grodzka et al. (1982)	Duraphat	4	24	3.5	148	DMFS	9.4% (NS)		100
Holm et al. (1984)	Duraphat	4	24	6	50	DMFS	56%		59
Modéer et al. (1984)	Duraphat	12	36	14	87	DMFS	24%		107
Kirkegaard et al. (1986)	Duraphat	10	60	10	129	DMFS	2.96*		no
	NaF 0.2%	fortnight	60	10	119	DMFS	2.77*		no

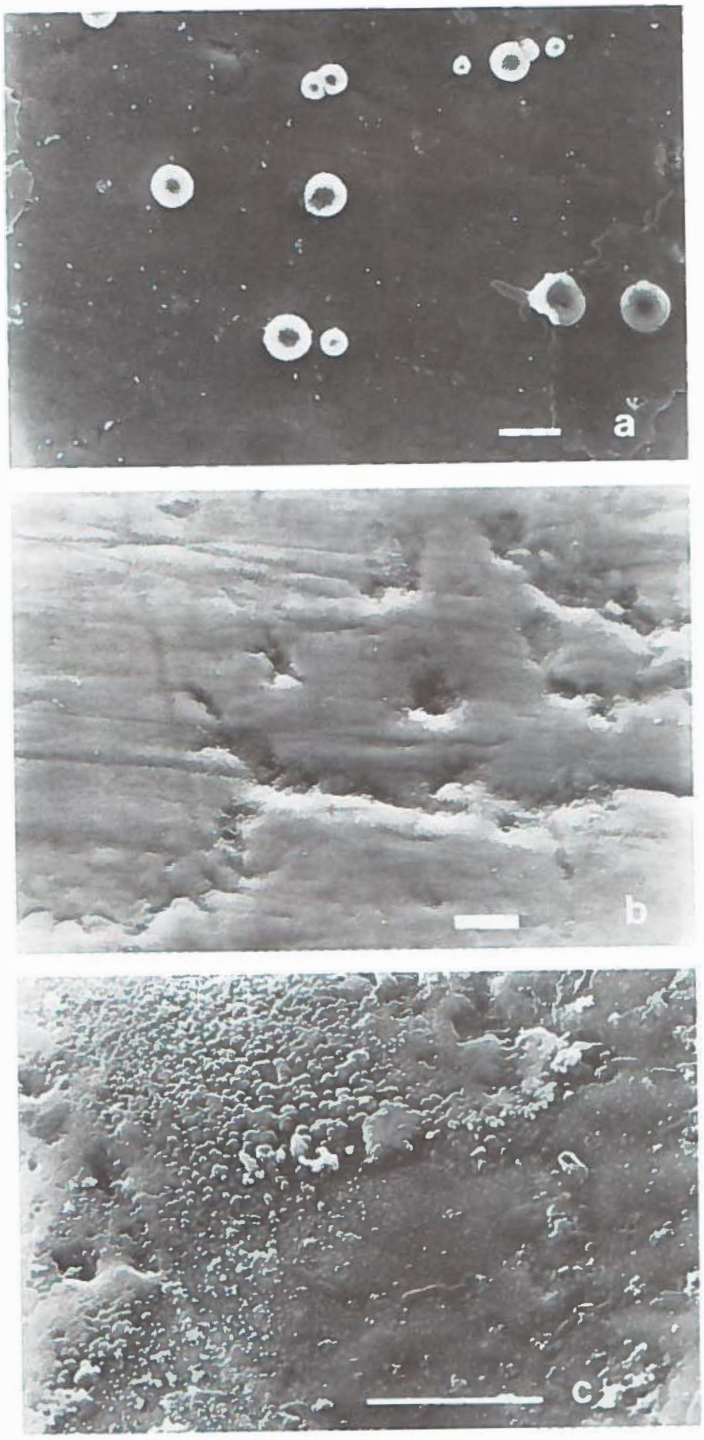

Figure 3.1.
Scanning electron micrograph of human enamel (≈ 1000x magnification; the bar denotes 10 μm).
a: 5 minutes after APF-gel application; b: 1 day after APF-gel application; c: 1 day after application of Fluor Protector 0.7%.
(courtesy by Dr. W.L. Jongebloed.)

Seppä et al. (1982a) investigated varnishes in a community with fluoridated water. After three years, with semi-annual applications, a significant fluoride uptake was reported for both Duraphat and Fluor Protector. Two years after discontinuation of the regular applications, the fluoride acquired had not been lost, which suggests that fluoride is deposited in a stable form (Seppä 1984). Most likely F^- is present as Fs (see Chapter 2).

The enhanced fluoride uptake after application of fluoride varnishes is evident from both in vitro and in vivo studies. It is related to the adhesive properties of the varnishes to enamel, which leads to a long effective contact period.

CARIES PREVENTION

Clinical trials.

Many clinical trials using fluoride varnishes have been carried out over the last 20 years. Results of trials testing Duraphat, Fluor Protector or both varnish systems are given in Tables 3.III., 3.IV. and 3.V., respectively.

Duraphat.

Heuser and Schmidt (1968) reported significant caries reductions of 30% DMFT after a single Duraphat application in 224 children, aged 11-13 years. With the exception of Maiwald and Geiger (1973) all trials on **permanent teeth** have shown caries reductions of 18-56%. Caries reductions of 37% (Murray et al. 1977) and 56% (Holm et al. 1984) were found when Duraphat was applied on newly erupted permanent molars. Koch and Petersson (1975) found a DMFS reduction as high as 77% but an additional fluoride rinse by the subjects may have influenced the caries progression.

Inconclusive results have been reported from various studies when the varnishes are applied on the **primary dentition**. Murray et al. (1977); Grodzka et al. (1982) and Clark et al. (1985a) reported insignificant caries reductions after semi-annual applications of Duraphat in children aged 3-7 years. This however is in disagreement with a number of studies in which Duraphat was shown to have a strong caries preventive effect (Holm 1979; Hochstein et al. 1975).
Compared with a weekly rinse of sodium fluoride, Duraphat pretreated subjects developed 30% less lesions in their permanent dentition (Koch et al. 1979). Kirkegaard et al. (1986) did not confirm this point but reported that both fluoride administrations were equally effective. It became obvious from the work of Maiwald and Geiger (1973) that an annual application of Duraphat has no significant effect on caries reduction. Modéer and coworkers (1984) applied Duraphat every 3 months and obtained a reduction of 24%. The re-application of the varnish every 3 months seems impractical; semi-annual application appears to be a more reasonable choice in dental practice.

Fluor Protector.

There have been fewer clinical trials with Fluor Protector then with Duraphat; the results are somewhat confusing (Table 3.IV.) and it is difficult to draw an overall conclusion.

Table 3.IV *Clinical trials testing Fluor Protector.*
* *measured values*
** *low caries incidence*
++ *extra fluoride administration*
(NS) not significant

Study	Application type	number	Study length (months)	Subjects' age (year)	number	Caries reduction		Study design Split mouth	Control
Riethe et al. (1977)	Fluor Protector	1	12	children	44	DMFT	72%	X	
	Nuva Seal	1	12	children	49	DMFT	89%	X	
	Epoxylite 9070	1	12	children	47	DMFT	38%	X	
Kolehmainen (1979)	Fluor Protector	2	12	13	92	DMFS	15% (NS)**	X	
Salem et al. (1979)	Fluor Protector	4	24	children	200	DMFS	45%	X	
Kolehmainen (1981)	Fluor Protector	4	24	13	163	DMFS	5% (NS)**	X	
Bruun et al. (1985)	Fluor Protector	6	36	9-12	125	DMFS	3.3* (NS)		no
	NaF 0.2% rinse	fortnight	36	9-12	125	DMFS	3.5*		no
Eck et al. (1985)	Fluor Protector	3	36	10	126	DMFS	15% (NS)++		131
Theuns et al. (1985)	Fluor Protector	3	36	12	154	DMFS	6% (NS)++		149

Table 3.V *Clinical trials testing Duraphat and Fluor Protector under comparable test-conditions.*
* *measured values*
** *readjusted values (test Fluor Protector vs. control Duraphat)*
\+ *water fluoridated area*
++ *extra fluoride administration*
(NS) not significant

Study	Application type	number	Study length (months)	Subjects' age (year)	number	Caries reduction		Study design Split mouth	Control
Borutta (1981)	Duraphat	3	12	11-14	88	DMFS	4.5%* (NS)		no
	Fluor Protector	3				DMFS	4.7*		
	Duraphat	6	24	11-14	88	DMFS	5.1* (NS)		no
	Fluor Protector	4				DMFS	5.3*		
Seppä et al. (1981)	Duraphat	4	24	11-13	67	DMFS	24% +	X	
	Fluor Protector	4			71	DMFS	12% (NS) 35%**		
Seppä et al. (1982b)	Duraphat	5	30	13-15	51	DMFS	37% +	X	
	Fluor Protector	5			60	DMFS	16% (NS) 28%**		
Seppä et al. (1982d)	Duraphat	6	36	11-13	62	DMFS	30% +	X	
	Fluor Protector	6			70	DMFS	11% (NS) 29%**		
Clark et al. (1985a)	Duraphat	2	20	6-7	255	DMFS	14.4% (NS)++		247
	Fluor Protector	2			201	DMFS	15.8% (NS) ++		
Clark et al. (1985b)	Duraphat	4	32	6-7	245	DMFS	21.9% ++		234
	Fluor Protector	4			197	DMFS	17% ++		

Von Riethe et al. (1977) reported a reduction of 72% DMFT in a study on only 44 teeth. Kolehmainen (1979, 1981) did not find any caries reduction in a population of 13 year old Finnish children with a low caries incidence. Salem et al. (1979) on the other hand reported a 45% DMFS reduction after 4 semi-annual applications in a caries active population. Van Eck et al. (1984); Clark et al. (1985a), Theuns et al. (1985) did not find any caries reduction compared to the age-matched control group. These trials investigated the effect of one annual application and the additional fluoride administration in the control group (comprised of pupils from a school in a different health service area) could have affected the caries increments. Bruun et al. (1985) compared an application of Fluor Protector with a fortnightly NaF mouthrinse and reported that there was no significant difference, indicating that varnish application offers a good alternative to mouthrinsing.

Studies with Fluor Protector are confusing for various reasons:

i) Several investigators have applied the varnish only once a year. From the Duraphat studies however, it is well known that a semi-annual application is a prerequisite (Maiwald and Geiger 1973).

ii) In most clinical trials no attempts have been made to exclude additional fluoride administration. The participants brushed their teeth with a fluoride dentifrice and regularly used fluoride mouthrinses or recieved topical applications. As a consequence low caries susceptibility was present (Clark et al. 1985a,b; Kolehmainen and Kerosuo 1979; Kolehmainen 1981; Van Eck et al. 1984; Theuns et al. 1985). Seppä's studies have even been carried out in areas with optimal fluoridation.

iii) In split-mouth studies control sites are often unreliable because a fluoride cross-over from the treated sites could have an effect on demineralization inhibition (Seppä et al. 1981, 1982b, 1982d).

Duraphat and Fluor Protector.

Borutta (1981) designed a half-mouth study to compare bi-annual Fluor Protector and tri-annual Duraphat application (Table 3.V.). No difference between both treatments was found. Continuing the applications for a further year led to a further caries decrease. Clark et al (1985a,b) confirmed these results. Seppä et al (1981, 1982b, 1982d) reported DMFS reductions of 24-37% for Duraphat in a split-mouth study design; insignificant reductions were found for Fluor Protector. The contralateral control teeth, however, showed an extremely low caries incidence. The authors suggested that fluoride leakage may have enhanced fluoridation of untreated sites, thus affecting the number of caries lesions. Fluor Protector data were therefore compared with the Duraphat control sites and this revealed a reduction of 28-35%, as shown in Figure 3.2. (Seppä et al. 1981).

In vitro/animal studies.

The effect of varnish applications on enamel acid solubility and the influence of the varnishes on artificial caries inhibition has been investigated in both laboratory and animal studies.

The results of animal studies are summarized in Table 3.VI..
Although the study design of various experiments is not comparable, it can be concluded that, in animal studies, Duraphat application leads to a caries reduction of 48-70% in fissures and 79-94% on approximal surfaces.

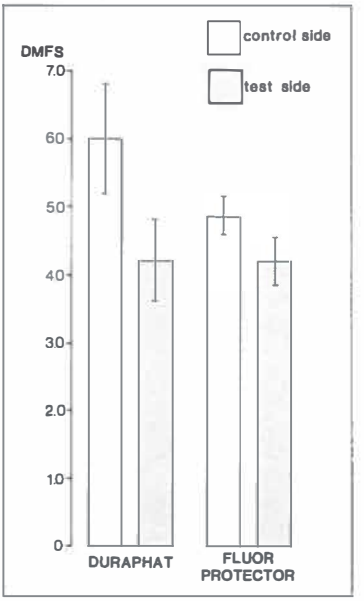

Figure 3.2.
Mean overall DMFS increments three years after bi-annual application of Duraphat and Fluor Protector. The caries progression of the untreated control sites is influenced by Fluor Protector treatment and is thus lower than in Duraphat control sites (Seppä et al. 1981).

Fluor Protector and Duraphat were compared by Gibbs et al. (1981) and Seppä et al. (1982c) and their results are in the same order for both varnishes. When compared with APF, only the fluoride gel reduced caries at all sites equally, while varnishes had a site dependent effect (Gibbs et al. 1981). Laboratory investigations by Arends and Schuthof (1977) did not confirm these results. They showed that the acid resistance of flat enamel surfaces pretreated with APF, Duraphat and Fluor Protector was 30, 63 and 67%, respectively.

Table 3.VI *Animal studies investigating the demineralization inhibition after varnish applications.*

Study	Application type	number	Caries Reduction	%
Heuser & Schmidt	Duraphat	1	fissures	67
(1966)		1	approximal	94
		2	fissures	70
Riethe & Weinmann	Duraphat	4		26
(1970)	APF	4		23
Gibbs et al.	Duraphat	1		reduction at some sites
(1981)	Fluor Protector	1		reduction at some sites
	APF	1		reduction at all sites
Seppä et al.	Duraphat	3	fissures	61
(1982c)			approximal	79
	Fluor Protector	3	fissures	53
			approximal	76
Seppä et al.	Duraphat	2	fissures	48
(1984a)			approximal	82
	Duraphat+F food	2	fissures	75
			approximal	100

Petersson and Dérand (1981) reported that a single Duraphat application could retard initial lesion progress at pH 4.5. Dérand and Petersson (1981) compared the demineralization inhibition of Duraphat and Fluor Protector; Fluor Protector was able to inhibit lesion formation completely, Duraphat reduced lesion formation.

SAFETY

A disadvantage of fluoride applications in young children is the possible risk of fluoride ingestion (Ekstrand et al. 1981), which may lead to minor toxic symptoms (Duxburry et al. 1982). Complaints from some children (such as vomiting and nausea) after topical fluoride gel applications are often reported. According to Ericsson and Forsman (1969), 10-30% of the fluoride from toothpastes and rinsing solutions is swallowed. Higher amounts of fluoride are ingested after 1.23% fluoride gel application (Ekstrand et al. 1981; Heeres and Purdell-Lewis 1984; Heeres et al. 1984). They reported ingestion of 0.48-1.80 and 0.2-1.16 mg F^-/kg body weight, respectively. The toxicological studies from Ekstrand et al., investigating fluoride in plasma after administration of various fluoride regimens are compiled in Table 3.VII. The range of F^- in plasma after application of fluoride gel or fluoride varnish (from Ekstrand et al. 1980, 1981) is shown in Figure 3.3.. It is interesting to note that the nephrotoxic level of fluoride (850 ng/ml Cousins and Masze 1973) is exceeded in some patients after gel application. Fluoride varnishes on the other hand have a much higher fluoride content (7000-22500 ppm F^-) but induce the lowest plasma levels ranging from 60 to 180 ng/ml (Ekstrand et al. 1980; Seppä and Hankijärvi 1983).

It is beyond doubt that if varnishes are applied according to the manufacturers' instructions, no side effects with respect to renal and other functions will occur because plasma levels will be far below those considered toxic (Ekstrand et al. 1980).

Table 3.VII *Plasma fluoride peak concentrations after various topical fluoride applications. The nephrotoxic level in plasma is 850 ng/ml (Cousins & Masze 1973).*

Study	Application type	F-dose applied (mg)	Plasma Peak time (hours)	Plasma Peak value (ng/ml)
Ekstrand et al. (1980)	Duraphat	2.3-5.2	2	60-120
Ekstrand et al. (1981)	1.23% NaF gel	61.5	0.5-1	300-1443
Ekstrand et al. (1983)	NaF tablets	0.5	0.5	76-94
	1000 ppm NaF	0.6	0.5	60-78
	250 ppm NaF	0.15	0.5	25-33
Seppä & Hankijärvi (1983)	Duraphat	11.3	0.5-1	180
	Fluor Protector	3.1	0.5-1	140
Heeres et al. (1984)	0.45 % NaF gel	2.4-9.4	0.5-1	342

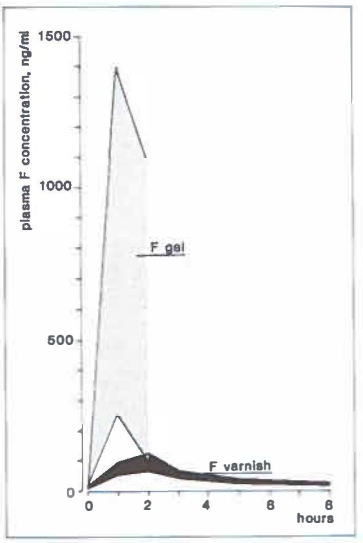

Figure 3.3.
Plasma F-concentration after F-gel and F-varnish application. The dotted area shows the range found in 8 children after F-gel application; the black area shows the range over 4 children after Duraphat application (Ekstrand et al. 1980; 1981).

This clearly demonstrates that varnishes are safe to apply. This is explained by the stickiness and the fast setting period (≈ 2 minutes) of the varnish base; the slow release of fluoride in time and the small amounts of varnish and thus of fluoride needed for treatment of the whole dentition. Seppä and Hankijärvi (1983) reported that 0.3 ml varnish was sufficient for preschool children and 0.5 ml for school aged children. This is equal to a maximum fluoride content of 11.3 mg and 3.1 mg F for Duraphat and Fluor Protector, respectively.

Seppä et al. (1984a) investigated histological changes in the oral mucosa of rats after Duraphat application. No side effects were observed in spite of the high fluoride concentration.

COMFORT

The quick setting period of the varnishes and the application technique make these materials an ideal vehicle for applying fluoride under general anaesthesia, because it cannot compromise the trachea.
A study by Bennett and Murray (1973) reported that, in comparison with eight other topical fluoride applications, Duraphat was the easiest and quickest to apply, with a minimum of chair-time and much less discomfort to the patient. Last but not least it is obvious that acceptance of the varnish is widespread among children who receive it (Murray et al. 1977).

DESENSITIZING PROPERTIES

The use of fluoride varnishes as desensitizing agents has recently been indicated in a small pilot study (Clark et al. 1985c).
Dentinal hypersensitivity can be classified as an adverse reaction or pain resulting from either a thermal, chemical or mechanical stimulus. The exact mechanism of dentine sensitivity is not understood yet, but the hydrodynamic theory described by Brännström (1966) is well accepted. Osmotic changes

in the dentine tubuli could stimulate the nerves and result in pain reactions.
Because varnishes enhance fluoride uptake and are also retained on the surface for hours, they may provide a temporary coating on the affected surfaces and be an effective and practical home treatment for dentinal hypersensitivity. The application procedure is painless and surfaces do not have to be dried (which normally causes pain).

In a small study with 21 patients beneficial effects on dentine sensitivity could be demonstrated with Duraphat. After a few treatments all patients experienced some improvement. Sensodyne, promoted as a desensitizing toothpaste, failed to show any effect (Clark et al. 1985c). This study dealt with small numbers of participants however, and definite conclusions should not be drawn. It is nevertheless interesting to keep the possible desensitizing properties of fluoride varnishes in mind.

CONCLUSIONS

Fluoride varnishes are **safe** and **efficient** topical agents with good **long lasting caries preventive** properties; they may have **desensitizing** properties.
Compared with other types of fluoride applications they are **easy** to use and cause **no discomfort** to the patients.

fluoride uptake in sound human enamel after varnish applications

a study on varnishes with various fluoride contents

4

INTRODUCTION

In the last decade several papers concerning fluoride uptake in enamel and caries preventive effects of fluoridating varnishes have been published (Stamm 1974; Arends and Schuthof 1975; Riethe et al. 1977; Arends et al. 1980; Retief et al. 1983; Seppä 1983). For a review see Dijkman (1982) and Chapter 3 of this thesis.
In the literature there is consensus on the following points:

(1) fluoridation of enamel with varnishes delivers considerable amounts of fluoride in and on enamel;
(2) fluoride is deposited on the enamel as a CaF_2-like layer which is denoted as F [on] (Caslavska et al. 1975; Dijkman et al. 1982; Nelson et al. 1983b; Nelson et al. 1984);
(3) fluoridating varnishes deposit fluoride deep into the enamel denoted as F [in] (Dijkman et al. 1982) (see Chapter 2);
(4) a substantial amount of fluoride leaches away in vivo both from the F [on] and F [in] types in the period following application (Mellberg et al. 1966);
(5) an advantage of varnish applications is that, as far as F^- deposition is concerned, the loss of fluoride immediately after application is relatively small due to the long contact period (Retief et al. 1980).

Two varnish systems have been investigated thoroughly:

1. Fluor Protector - polyurethane based with 0.7 wt% F^- as difluorosilane (Vivadent, Schaan, Liechtenstein);
2. Duraphat - colophonium based with 5 wt% sodium fluoride (Woelm Pharma GmbH & Co, Eschwege, FRG.)

Previous studies have always been carried out with these relatively high F^- levels in the varnishes. Nowadays, however, there is, for various reasons, a tendency to decrease the F [content] in fluoride gels (Sluiter and Purdell-Lewis 1984) as well as in toothpastes.
It is therefore interesting to investigate the effect and efficiency of varnishes with lower F [content] on fluoride acquisition ON and IN enamel.

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* The experiments and results described in this chapter have previously been published and are reprinted with permission.

De Bruyn, H., Hummel, M., Arends, J.: In vivo effect of a fluoridating varnish with various fluoride contents on human enamel.
Caries Res. 19: 407-413 (1985).
De Bruyn, H., Arends, J.: Wirksamkeit von Fluoridlacken.
Oralprophylaxe 7: 131-137 (1985).
De Bruyn, H., Arends, J.: Fluoride-opname in menselijk tandglazuur na applicatie van Fluor Protectorlak.
J. Head & Neck Pathol. 5: 93-96 (1986).

AIMS

The aims of this part of the study were to investigate for polyurethane varnishes with differing fluoride concentrations:

1. The amount of fluoride acquired as F [on] and F [in] human enamel after a single 24 hours application in vitro.
2. The amount of F [on] and F [in] acquired in vivo one week after application.
3. The comparison of the fluoride uptake after 24 hours with that after one week, in order to assess fluoride loss and efficiency.
4. The fluoride release in vitro in water as a function of fluoride concentration and time.

MATERIALS AND METHODS

General.

Sound human enamel was used in this study. The varnish was always in place during 24 hours. In the 'in vivo' part of the study, enamel blocks were positioned in the oral cavity for 1 week. In the 'in vitro' part fluoride was measured immediately after varnish removal. The experimental design is depicted schematically in Figure 4.1..

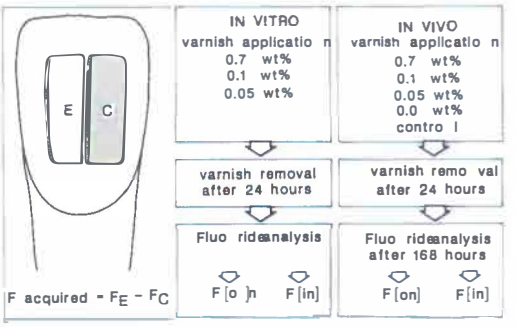

Figure 4.1. Experimental design of the study.

Prosthesis.

A new method for in vivo experimentation was developed using frame prostheses, shown in Figure 4.2.. Only Kennedy classification III dentures in the premolar-molar region were employed. The major connector, the clasps and the experimental frame body (b) were made of Wironit (a chromium-cobalt-molybdenum alloy, Bego, Bremen, FRG). The slots indicated by e in Figure 4.2. were always of the same dimensions, 4 x 6 mm^2 in area and 2 mm in width. In general 5 slots could be made in one frame body. The top of the frame body was covered by a removable occlusal part (S) made of a palladium-silver alloy and fixed to the frame body by a stainless steel screw. The enamel specimens placed in the slots were easily removable without damage. The connector parts of the frame were kept as small as possible to avoid excessive plaque accumulation.

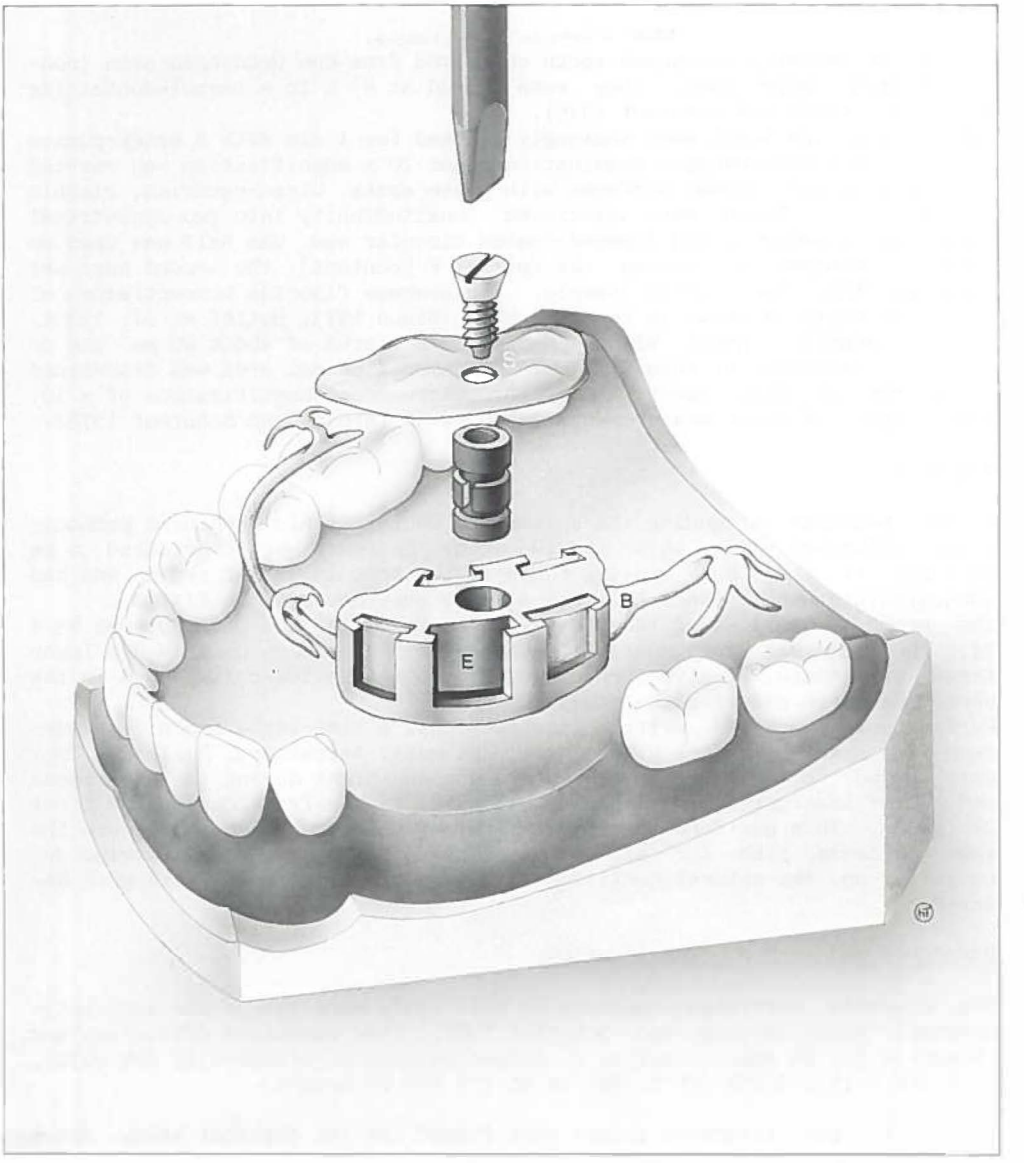

Figure 4.2.
The intra-oral device used in the clinical study (S: occlusal part; E: enamel; B: frame body).

Enamel.

Sixty-nine recently extracted teeth collected from the Groningen area (non-fluoridated) were used; they were stored at 4° C in a thymol-containing solution (Arends and Schuthof 1975).
Before use the teeth were thorougly cleaned for 1 min with a water-pumice slurry. Stereomicroscopic examination under 10 x magnification was carried out to eliminate enamel surfaces with white spots, micro-cavities, visible defects etc.. Teeth were sectioned longitudinally into two symmetrical parts by a water-cooled diamond-coated circular saw. One half was used as internal control to assess the initial F [content]; the second part was used as the experimental sample. The average fluoride concentration of these two parts is known to be comparable (Bruun 1973; Retief et al. 1980). The rectangular enamel blocks had a surface area of about 20 mm^2 and an average thickness of about 1.5 mm. The exposed enamel area was determined by micrometer measurements under stereomicroscopic magnification of x 10. The accuracy of these measurements is about 3% (Arends and Schuthof 1975).

Patients.

Eleven patients attending the University Dental Clinic Groningen participated voluntarily in this in vivo study. Patients were considered to be suitable if they had missing teeth in the premolar-molar region and had adequate interdental space to allow a frame prosthesis to be fitted.
The average age ± SD of the participants, 8 females and 3 males, was 43 ± 12, the range was 26-59 years. 1 participant had both an upper and a lower frame prosthesis. Twelve frame dentures, 8 in the lower jaw and 4 in the upper jaw, were made for the study.
Participants received written instructions, a time-table and a fluoride-free toothpaste (Prodent Ultra Actif, Intradal, Amersfoort, Holland). They were asked to wear the frame denture day and night during the experiment and were instructed not to brush the enamel in the frame during the first 24 hours. This was done to avoid removal of the varnish and to ensure the same exposure time for all enamel samples. Oral hygiene was carried out normally on the natural dentition with the non-fluoridated toothpaste described.

Varnishes and method of application.

The fluoride varnishes employed in this study were all on the same polyurethane base (Arends and Schuthof 1975). They contained difluorosilane (Figure 4.3.) in amounts giving F^- concentrations of either 0.7% (FP 0.7%), 0.1% (FP 0.1%), 0.05% (FP 0.05%) or 0% (FP 0%) by weight.

Five different treatment groups were formed for the **clinical study**. Since the number of slots in the frame appliances was 52 at least 5 enamel specimens could be measured per treatment. In each prosthesis there always was an untreated control and 4 varnished enamel specimens, each treated with a different varnish. The varnishes were painted carefully with a small brush on the exposed enamel surface taking care that cut surfaces were not touched. Application of the agent was carried out under stereomicroscopic magnification of x 10. The layer thickness of the varnish is about 0.2 mm (Dijkman 1982). The enamel blocks were subsequently positioned at random in the buccal or lingual slots of the frame immediately after application. After 24 hours participants were recalled and the varnishes removed. The removal was carried out with a scalpel under stereomicroscopic magnification x 10.

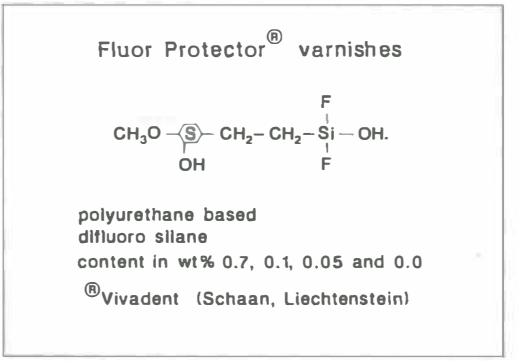

Figure 4.3.
Details of the varnish system used.

Samples were replaced into the frame body and kept in the oral cavity for 6 additional days.

In the **in vitro study** 18 enamel samples were randomly devided into 3 treatment groups: FP 0.7%, FP 0.1% and FP 0.05%. Each varnish was applied on 6 teeth. After being air-dried the enamel samples were placed in a closed polyethylene jar and stored at 37.4° C for 24 hours in a 100% humidity. One day after application varnishes were peeled off carefully with a scalpel. (SEM checks proved that, using this method, varnish particles were removed nearly completely.)

Determination of F [on].

Following the experiment, samples were cleaned with cotton wool and distilled water. The KOH-extraction procedure described by Caslavska et al. (1975) was used to estimate the alkali-soluble F [on]. The enamel blocks were immersed in a polyethylene bottle containing 0.5 ml 1 M KOH stirred (150 rpm) at room temperature for 24 hours. Subsequently, 0.5 ml 1 M HNO_3 was added to neutralize the alkaline solution and 0.5 ml 1 M trisodium citrate was added as buffer. Fluoride concentration in the solution was determined directly with a specific fluoride ion electrode (Orion Research Inc.) coupled to an expanded scale millivolt meter (Orion 401). The apparatus was callibrated twice a day with standard solutions containing 2.0, 1.0, 0.5 and 0.05 ppm fluoride. The thickness of the CaF_2-like layer was calculated using:

$$d[CaF_2]\ (\mu m) = \frac{W[CaF_2]\ (g)}{3.18\ (g.cm^{-3}) \times A\ (cm^2) \times 10} \qquad (1)$$

In which d is the thickness of the layer, $W[CaF_2]$ is the amount of CaF_2, A is the surface area, and 3.18 is the estimated calcium fluoride density (Dijkman et al. 1982). In the calculation it is assumed that the CaF_2-like layer is distinct and uniform (Nelson et al. 1984).

Determination of F [in].

Subsequently the enamel blocks were positioned on a plastic rod with sticky wax. This rod could be fixed on a stirring device for etching procedures. The cut surfaces were covered with nail-varnish leaving only the experimental surface exposed. This procedure was carried out under stereomicroscopic examination (10 x magnification). F [in] determination was carried out

using the method described by Dijkman et al. (1981).
In order to remove five consecutive layers of enamel these rods were rotated at a constant speed of 100 rpm in a polyethylene jar, containing 0.5 ml 0.1 M $HClO_4$ for 30, 30, 60, 120 or 120 s.
After a given etch period 2.0 ml 0.1 M trisodium citrate was added to stop the etching process. Samples were then thoroughly washed with distilled water before the next layer was etched away. Fluoride concentration of the etching solution was determined and expressed in ppm as described above with the F^- electrode system. To estimate layer thickness the calcium content of each layer was determined by atomic absorption spectroscopy (Perkin Elmer 370). 0.5 ml of the solution was diluted 20 times with 1 ml 0.1% lanthanum oxide and 8.5 ml distilled water.
Thickness of the etched enamel layer was determined using the equation

$$d[Ca]\ (\mu m) = \frac{WE\ (\mu g)}{2.95\ (g.cm^{-2}) \times A\ (mm^2)} \qquad (2)$$

In this equation A is the area of exposed enamel, 2.95 is the average enamel density and WE is the weight of enamel in the solution. WE is calculated assuming an average human enamel content of 37.4% calcium (Dijkman et al. 1982).
The weight of fluoride from the 5 biopsies was calculated per square centimeter of exposed enamel surface and expressed in $\mu g.cm^{-2}$.
F [in] + F [on] = F [total], expressed in $\mu g.cm^{-2}$.

F [release].

The apparatus employed for F [release] measurements consisted of a 100 ml polyethylene container, a magnetic stirring device (100 rpm) and a combination fluoride-electrode (Orion Res. Inc. type 96-09) connected to an expanded-scale millivoltmeter (Orion Res. Inc. type 401).
The F^- electrode was callibrated with standard solutions containing 1.0, 0.4, 0.2 and 0.1 ppm fluoride, respectively. The fluoride varnishes were applied to one side of a flat glass plate with a surface area of 3.24 cm^2. After air-drying for one minute the samples were placed in a 50 ml solution made up of 10 ml Tisab II (total ionic strength activity buffer) and 40 ml distilled water.
F [release] from the varnish in the liquid was measured as a function of time. F [release] was determined up to 48 hours after application. The varnish weight on the glass surfaces was determined using a microbalance (Sartorius-Werke GmbH, Göttingen, FRD type 2474). The maximum F [content] in a varnish per cm^2 area was estimated using the equation:

$$F\ [max]\ (\mu g.cm^{-2}) = \frac{W\ (\mu g)}{A\ (cm^2)} \cdot f\ (wt\%) \qquad (3)$$

W is the weight of the varnish applied; f is the fluoride concentration in the varnish and A is the surface area; F [max] is the maximum F [content] in a varnish.

RESULTS

The amount of fluoride acquired by the enamel after varnish application was obtained by subtracting the value of the internal control from the value of the experimental sample. This procedure was used both for F [on] (obtained after KOH-extraction) and for F [in] (measured from etching).

F [on] acquired.

The amounts of F [on] acquired after varnish applications are compiled in Figure 4.4.. The results show that the acquired F [on] value strongly increases with increasing F [content] in the varnish. Differences are highly significant ($p < 0.001$) between FP 0.7% and FP 0.1%.

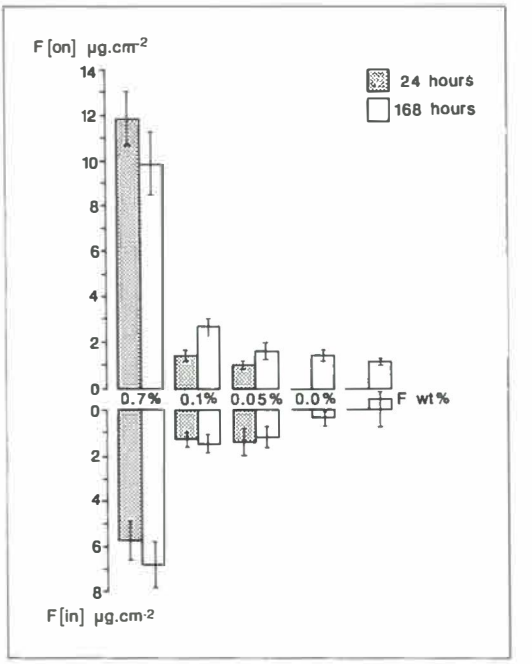

Figure 4.4.
F [on] and F [in] totally acquired in 5 layers for the various applications expressed in $\mu g.cm^{-2}$ (mean ± SE).

There is, however, no statistically significant difference between FP 0.1%, FP 0.05% and FP 0%.
One week after application in vivo there was no difference between the FP 0% values and those of the untreated control.
The layer thickness d [CaF_2] as calculated from formula (1) is presented in Table 4.I..

Table 4.I *Calculated layer thickness of "CaF_2-like material" precipitated on the enamel after 168 hours and 24 hours. Values are given in µm ± SE.*

Varnish	$^{d}CaF_2$ 168 hours		$^{d}CaF_2$ 24 hours	
FP 0.7%	0.06±0.01	(11)	0.08±0.008	(6)
FP 0.1%	0.02±0.003	(10)	0.01±0.001	(6)
FP 0.05%	0.01±0.003	(11)	0.01±0.001	(6)
FP 0%	0.01±0.002	(9)		
control	0.01±0.006	(10)		

Figures in parentheses are number of specimens.

F [in] acquired.

After determination of alkali-soluble fluoride, fluoride in the enamel was determined by etching five consecutive layers. The etching depth was found

to be nearly independent of the fluoride concentration in the enamel and was not influenced by the varnish applications as seen in Table 4.II.. These findings are in agreement with previous studies by Caslavska et al. (1975), Arends et al. (1980), Dijkman et al. (1983).

Table 4.II *Average etch depth in μm (mean ±SE) after application of the various varnishes for the 168 hours and 24 hours experiments.*

Varnish	Etching periods	Etch depth 168 hours	24 hours
FP 0.7%	30	2.2±0.1 (11)	2.5±0.1 (6)
	30	2.4±0.1 (11)	2.6±0.1 (6)
	60	4.3±0.2 (11)	4.9±0.2 (6)
	120	7.5±0.3 (11)	8.6±0.3 (6)
	120	7.5±0.4 (11)	8.3±0.3 (6)
FP 0.1%	30	2.4±0.1 (10)	2.5±0.1 (6)
	30	2.3±0.2 (10)	2.7±0.1 (6)
	60	4.6±0.3 (10)	5.0±0.2 (6)
	120	8.3±0.2 (10)	9.2±0.3 (6)
	120	8.2±0.4 (10)	9.5±0.3 (6)
FP 0.05%	30	2.4±0.1 (11)	2.4±0.2 (6)
	30	2.8±0.2 (11)	2.8±0.2 (6)
	60	4.4±0.2 (11)	5.2±0.3 (6)
	120	8.4±0.3 (11)	9.1±0.5 (6)
	120	8.3±0.2 (11)	9.2±0.5 (6)
FP 0%	30	2.7±0.2 (9)	
	30	2.9±0.1 (9)	
	60	4.8±0.3 (9)	
	120	8.6±0.3 (9)	
	120	8.6±0.3 (9)	
Control	30	2.7±0.1 (10)	
	30	2.7±0.1 (10)	
	60	4.8±0.2 (10)	
	120	8.7±0.3 (10)	
	120	8.4±0.2 (10)	

Figures in parentheses are number of specimens.

The acquired fluoride per etched layer (expressed in ppm) is given for each application in Table 4.III..

Table 4.III *Acquired fluoride F[in] following treatment with varnishes after 168 and 24 hours; given for each etched layer. Values are given in ppm (mean ±SE).*

Varnish	Treatment	Layer 1	Layer 2	Layer 3	Layer 4	Layer 5
FP 0.7%	168 h (11)	11150 ± 2026	9166 ± 1971	8022 ± 1466	7223 ± 1211	3972 ± 928
	24 h (6)	5858 ± 1522	4370 ± 1085	3210 ± 836	2577 ± 824	2087 ± 694
FP 0.1%	168 h (10)	4911 ± 1507	3260 ± 1232	2047 ± 1285	1453 ± 645	617 ± 426
	24 h (6)	3259 ± 1243	1927 ± 875	916 ± 518	702 ± 341	-209 ± 227
FP 0.05%	168 h (11)	5462 ± 1713	3080 ± 1345	2013 ± 912	1347 ± 597	437 ± 410
	24 h (6)	2568 ± 664	795 ± 516	382 ± 296	317 ± 211	-199 ± 206
FP 0%	168 h (9)	2369 ± 1709	1706 ± 1437	988 ± 1133	1464 ± 883	670 ± 528
Control	168 h (10)	25 ± 1740	166 ± 1436	395 ± 1035	525 ± 763	154 ± 497

Figures in parentheses are number of specimens.

The 168 h results show that there was a significantly higher uptake from FP 0.7% then from FP 0.1% (t-test, $p < 0.05$). The higher uptake was found in all five etched layers. This is also evident from Figure 4.5..
There was no significant difference between FP 0.1%, FP 0.05% either after 24 hours or 168 hours.
It should be noted that the difference between FP 0% and the untreated control is not statistically significant.
The ranking order in fluoride uptake is:

FP 0.7% » FP 0.1% ≈ FP 0.05% ≈ FP 0% ≈ control.

and this is valid for F [on] and F [in] after 24 hours and after 168 hours, as visualized in Figure 4.4..

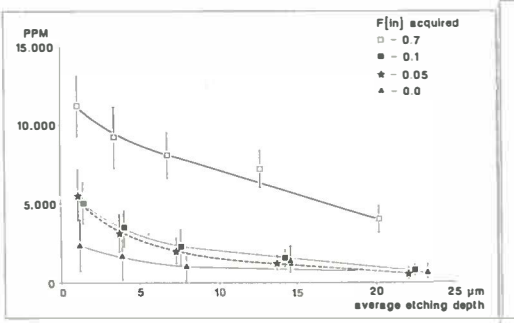

Figure 4.5.
Fluoride uptake in vivo per etched layer for the various applications, expressed in ppm (mean ± SE); depth is in µm.

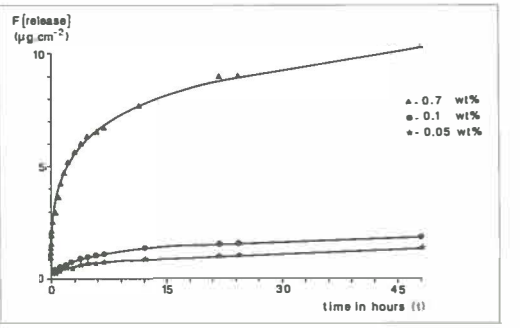

Figure 4.6.
F [release] in $\mu g.cm^{-2}$, for the fluoride varnishes as a function of time.

F [release].

Figure 4.6. shows the fluoride release in water from the different fluoride containing varnishes as a function of time.
The amount of fluoride released in the liquid in 24 hours is expressed in $\mu g.cm^{-2}$ (Table 4.IV.). F [max] was determined using equation (3) described above with an average weight of 2.13 ± 0.08 mg (mean ± SD) per cm^2 covered with varnish material.

Table 4.IV *F[release] during application of fluoride varnishes with various F[content]. Values are given in $\mu g.cm^{-2}$ (mean ± SE).*

Varnish	F[max] in varnish	F[release] 24 hours
FP 0.7%	14.9 ± 0.6 (4)	9.0
FP 0.1%	2.1 ± 0.1 (4)	1.5
FP 0.05%	1.1 ± 0.05 (4)	1.2

Figures in parentheses are number of specimens.

F [total] acquired.

The total amount of fluoride acquired after application of varnishes with varying fluoride concentrations is given in Table 4.V..

Table 4.V *The total amount of fluoride acquired after application and after one week. Values are given in $\mu g.cm^{-2}$ (mean ± SE). The total enamel biopsy depth is given in µm (mean ± SE).*

Varnish	Treatment	Biopsy depth	F[total]
FP 0.7%	168 h (11)	23.9 ± 1.1	16.7 ± 1.7
	24 h (6)	26.9 ± 1.0	17.7 ± 1.4
FP 0.1%	168 h (10)	25.8 ± 1.2	4.1 ± 0.6
	24 h (6)	28.9 ± 1.0	2.5 ± 0.4
FP 0.05%	168 h (11)	26.3 ± 1.0	2.9 ± 0.5
	24 h (6)	28.7 ± 1.7	2.3 ± 0.5
FP 0%	168 h (9)	27.6 ± 1.2	1.6 ± 0.3
Control	168 h (10)	27.3 ± 0.9	0.5 ± 0.9

Figures in parentheses are number of specimens.

The efficiency of the varnishes in the sense of fluoride uptake/loss has been calculated from these results.
The total biopsy depth was not influenced by the varnish treatment, a result obtained by summing the 5 etching depths.
The ranking of F [total] ($p < 0.05$) is after application:

FP 0.7% » FP 0.1% ≈ FP 0.05%.

and after one week:

FP 0.7% » FP 0.1% ≈ FP 0.05% > FP 0% ≈ control.

DISCUSSION

A new intra-oral device was introduced in this study (Figure 4.2.). Koulourides (1966) was the first author to demineralize human enamel in vivo by means of intra-oral devices. He enhanced demineralization by covering tooth enamel slabs placed in an acrylic appliance with teflon gauze, thus increasing plaque retention. In 1974 he developed a technique in which enamel slabs were mounted in the buccal flanges of lower dentures and subsequently exposed to the oral environment (Koulourides et al. 1974). Similar but more advanced methods have been used by several authors (Gelhard 1982; Dijkman 1983; Sluiter et al. 1984; Van Herpen and Arends 1986; Dijkman et al. 1986). In recent studies the tooth specimens have been embedded about 1.5 mm below the surrounding acrylic of the denture to enhance plaque retention and thus demineralization. In remineralization studies the experimental samples have been positioned flush with the surrounding acrylic to avoid plaque accumulation (Dijkman et al. 1986). The microbiology of patients wearing a full denture might be different by dentate or partially dentate patients. Hayes et al. (1983) showed that the acidogenicity of dental plaque is related to the DMF number: as patients with a full denture could have had a considerable DMF value, they may have a different plaque flora. One can therefore argue whether it is useful to investigate caries under these circumstances. Samaranayake and MacFarlane (1980) have also

shown that considerable amounts of yeasts are present in the oral environment when acrylic dentures are in place.
Recently, Creanor et al. (1986a) developed an in situ appliance for the investigation of caries processes using a single section technique. In their study the enamel was placed in a chamber on the lingual side of the dentate lower arches. They described that the microbiology and acid/anion profiles of the appliance samples are **similar** to interproximal plaque (Creanor et al. 1986b).
In the Koulourides model the position of the enamel specimens in the buccal flanges of dentures is quite unnatural because salivary flow, physiological movements and plaque composition are different from the situation on teeth. Sluiter et al. (1985) showed that the plaque composition in the intra-oral cariogenicity test changed strongly in a three week period. Lactobacilli, not present at the start of the experiment in the plaque, had increased to 66% while streptococci decreased to half the original concentration.

In the light of these findings an appliance was developed whereby the experimental specimens are placed in the dental arch substituting missing teeth. The selected patients only had a few teeth missing and had no partial dentures. The objectives were also that the positioning and removal of the experimental specimens could be done easily and quickly. The dimensions of the slots should be uniform, in order to allow for standardized preparation of experimental samples in the laboratory.
This method is flexible and allows experimentation as close as possible to the real life model.

The results of this work clearly show that the amount of fluoride present after application and in vivo after 6 days is strongly dependent on the F [content] in the varnish.
Considering first F [on], Figure 4.4., shows that a decrease in F [content] from 0.7% to 0.1% reduces F [uptake] with a factor 3-4.
There is also a lineair relation between F [on] and F [content] in the varnish (Figure 4.7.). This conclusion can also been drawn for the calculated thickness of CaF_2 in Table 4.I..

It is interesting to compare the values of the FP 0% with the control values. A small fluoride uptake of about 1 $\mu g.cm^{-2}$ is measurable in both groups. This is most likely due to F [uptake] from the diet. The F^- level in saliva may be slightly enhanced due to F^- loss from the other fluoridated specimens in the same mouth during the same experimental run.

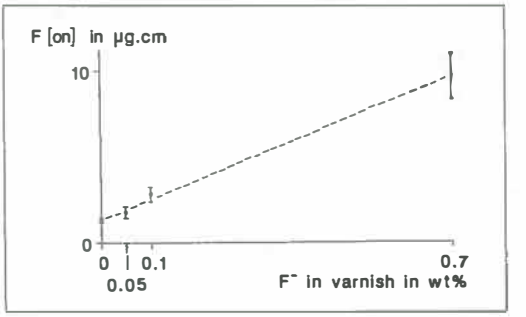

Figure 4.7.
F [on] in $\mu g.cm^{-2}$ deposited on enamel as a function of the F [content] in the varnish in wt% one week after application (mean ± SE).

The same ranking of the fluoride varnishes is observed immediately after varnish removal. No statistically significant difference between FP 0.1% and FP 0.05% was measurable (Figure 4.4.).

The fluoride uptake in enamel was measured by etching 5 successive enamel layers. In Figure 4.4. the total amount of fluoride acquired in 5 layers at a **total** depth of 26 ± 3 µm (mean ± SD) is given for 24 hours and 168 hours. The ranking is the same as observed for F [on]:

FP 0.7% > FP 0.1% ≈ FP 0.05% ≈ FP 0% ≈ control.

If one considers the fluoride uptake per enamel biopsy, as expressed in Table 4.III., FP 0.7% has a significantly greater uptake for each layer compared with FP 0.1% after 168 hours.

An interesting aspect is seen in the F [in] values of FP 0% and the untreated control. Although not statistically significant ($p < 0.05$), a trend can be seen that the F [uptake] is slightly greater for FP 0% than for the control, the latter being nearly zero. This measurable F [uptake] in the FP 0% treated samples is most likely due to a local superficial etching caused by the varnish. This etching is shown in Figure 4.8.. It is known that application of polyurethane-based varnishes leads to minute etch pits at the prism borders (Nelson et al. 1983b). The removal of the varnish leaves the

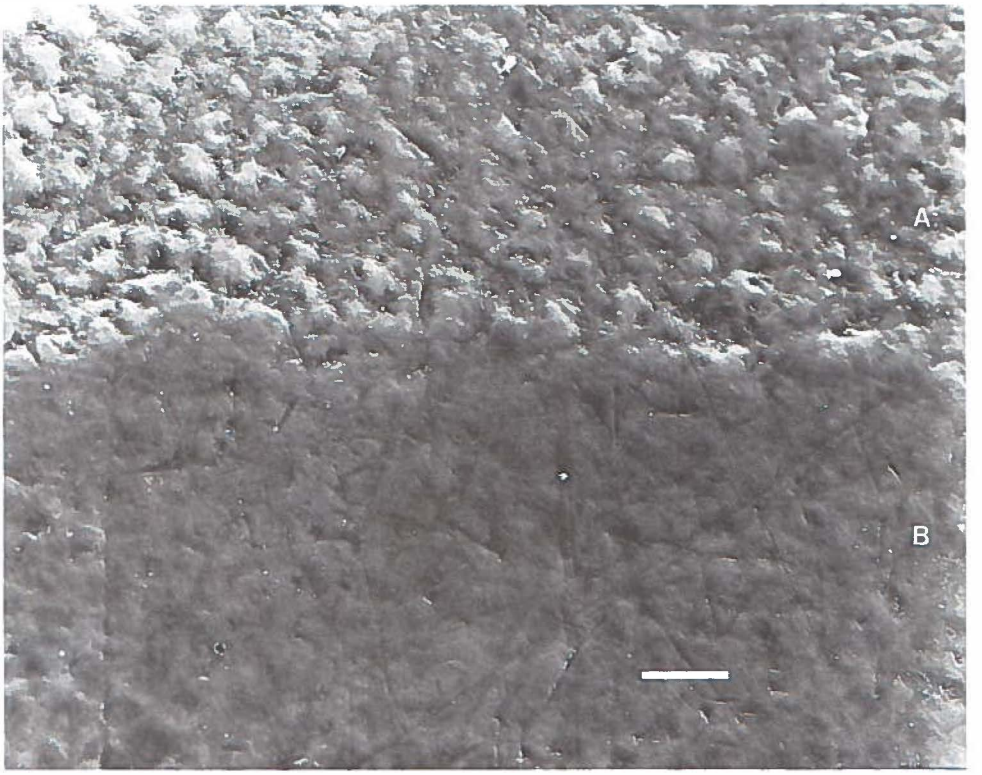

Figure 4.8.
SEM of an enamel surface partially pretreated (24 hours) with FP 0% after varnish removal. The etch pattern of the pretreated surface (A) compared with an untreated part (B) is clearly visible. The bar denotes 10 µm.

enamel in such a state that F [in] is obviously enhanced.
It is clear from the control data (Table 4.III.) that the cross over effect in F [in] is negligible.

The etch depth values found in this study are in good agreement with results in the literature (Dijkman et al. 1983) and are not substantially influenced by the varnish treatments. This can be seen clearly in Table 4.II.. The total biopsy depth, obtained by adding the 5 layers, is statistically comparable (α = 0.05) except for the FP 0.7% at 168 hours; it is slightly less than in the other groups. This does not influence the conclusions.

The mean total biopsy depth in the enamel for the 24 hours experiment is 28 ± 3 µm. This is not different from the 1 week data, mean 26 ± 3 µm and allows one to compare the one week results directly with the results obtained immediately after application. It is clear from Figure 4.4. that in a one week period a slight increase of F [in] and a decrease of F [on] is indicated for FP 0.7% (although not statistically significant).
Considering Table 4.III. it seems that fluoride on the outer enamel surface is "converted" into fluoride in deeper enamel layers. This is only significant for the FP 0.7% application. This tendency is also observed for the FP 0.1% and FP 0.05% although no statistical evidence is found. This is probably due to the short experimental period. In other publications, however, fluoride uptake in enamel has been described as increasing up to 3 months after application (Dijkman et al. 1983) and measurable amounts of fluoride are available even after 6 months (Bruun 1973).

A comparison of the total fluoride uptake after 24 and 168 hours clearly indicates that no measurable fluoride loss occurs in one week. Statistically there is no difference in F [total] between the 2 experiments for FP 0.7% and FP 0.05%. A slight increase is even observed for FP 0.1%.
From Table 4.V. it is clear that FP 0.7% > FP 0.1% ≈ FP 0.05% > FP 0% = untreated control. The 0% application is not different from the untreated control, in both cases fluoride gain is probably due to fluoride from saliva and food.
We conclude therefore, that the varnishes are efficient as far as F [uptake] is concerned and that the loss of fluoride from the various varnish treatments is rather slow.

Some authors have stated that fluoride uptake is influenced **inversely** by the fluoride concentration in the tooth **prior** to application (Retief et al. 1980; 1983; Nicholson and Mellberg 1969). In this study a correlation analysis on the individual data was carried out in order to assess these possible correlations. After one week inverse correlations were found for the total amount of fluoride acquired in the enamel, with a very small correlation coefficient compared to the initial concentration. The coefficients for FP 0.7%, FP 0.1% and FP 0.05% are r = -0.46, r = -0.47 and r = -0.36, respectively. No significant correlation was found using t-test ($p < 0.05$). This is in agreement with data presented by Heifetz et al. (1970). No difference in the fluoride content was found for the various experimental groups. We conclude therefore that the initial F [content] in the enamel does not influence F [uptake] from the varnishes.

In Figure 4.6. one can see, as far as F [release] from the varnishes in water is concerned, that nearly 50% of the available fluoride has been released after 6 hours. After 24 hours 60%, 70% and 100% of the available

fluoride was in solution for FP 0.7%, FP 0.1% and FP 0.05%, respectively. Although the F [release] set-up is simple and different from the situation in the oral cavity (and there is no interaction with enamel) the amount of fluoride released in 24 hours (Table 4.IV.) is linearly correlated to the F [on] values after 24 hours (shown in Figure 4.9., correlation coefficient being 0.97.).

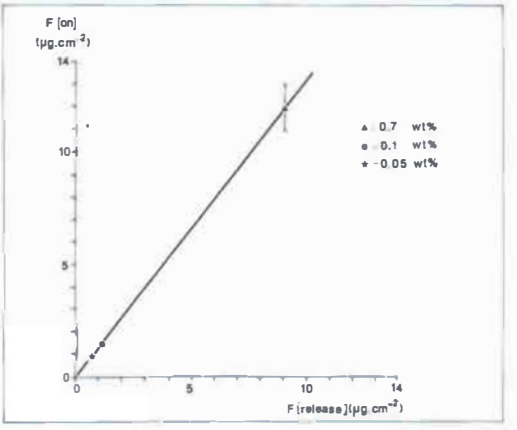

Figure 4.9.
F [on] in $\mu g.cm^{-2}$ (mean ± SE) deposited on enamel after application as a function of the F [release] of the varnishes expressed in $\mu g.cm^{-2}$.

Concluding one can say that as far as fluoride uptake is concerned the fluoridating varnishes investigated are:
i) effective in delivering fluoride ON and IN enamel and
ii) the fluoride loss in vivo in one week is small.

For the caries preventive effects of the various varnishes one should refer to Chapters 5 to 7.

inhibition of in vitro demineralized human enamel after varnish applications

a constant composition study at pH = 5

5

INTRODUCTION

Fluoride varnishes are useful topical fluoridating agents with caries preventive properties. This has been shown in laboratory investigations as well as in clinical trials; for reviews see Clark (1982), Yanover (1982) and Chapter 3 of this thesis.
Two types of varnishes are commonly used in dentistry in Europe, namely, Duraphat (containing 5 wt% NaF - Woelm Pharma, Eschwege, FRG) and Fluor Protector (containing 0.7 wt% silanefluoride - Vivadent, Schaan, Liechtenstein). Several studies have shown that as far as fluoride uptake is concerned Fluor Protector enhances fluoride uptake more than any other topical application (Edenholm et al. 1977; Kolehmainen et al. 1978; Arends et al. 1980; Retief et al. 1980; Tveit 1980; Dijkman et al. 1982).
Higher fluoride uptake is, however, not proportional to the caries inhibition This has been shown in animal studies (Gibbs et al. 1981; Seppä et al. 1982c; Seppä and Luoma 1983) and in clinical trials (Seppä et al. 1982b; Kolehmainen and Kerosuo 1979).

The aim of this study was to evaluate the inhibiting effect of fluoride varnish applications on enamel demineralization as a function of the fluoride content under severe demineralizing (constant composition) conditions in vitro.
There were two main reasons for chosing the constant composition technique described by Buskes et al. (1985) to assess enamel demineralization.
The first reason was that in this system the liquid flowing over the enamel specimens reflects the salivary flow in the oral situation. This is a feature which is not found in most laboratory techniques available at the present time. Secondly, the constant composition of the solution provides a reproducible demineralization method.

MATERIALS AND METHODS

Enamel.

Thirty human incisors collected in the Groningen area were cut into rectangles using a water-cooled diamond saw. The buccal surfaces were flattened with grinding paper (Siawat grid 600). Stereomicroscopical examination (x 10) was carried out to eliminate samples with visible defects or lesions. The enamel slabs were embedded in polymethylmetacrylate and polished with polishing paper (Siawat grid 800). Prior to varnish application the speci-

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H. De Bruyn, J.A.K.M. Buskes and J. Arends: The inhibition of demineralization of human enamel after fluoride varnish application as a function of the fluoride content.
Jour. Biol. Buccale 14: 133-138 (1986).

mens were cleaned ultrasonically for 3 minutes in order to remove polishing slurry.
The embedded slabs were than divided at random into 5 experimental groups each with 6 samples. The average surface area of each enamel specimen was approximately 16 mm^2.

Varnishes.

The varnishes were polyurethane based and contained 0.7%; 0.1%; 0.05% or 0% difluorosilane in weight percent, respectively. They were applied to the enamel surface in a thin layer with a small brush. The samples were then stored for 24 hours at 37.4° C under 100% humidity. After one day the varnish was peeled off with a scalpel. The enamel was cleaned with acetone in order to avoid fluoride contamination due to varnish remnants.
After the varnish had been removed the enamel slabs were demineralized in the constant composition apparatus. The FP 0.7% and FP 0.1% groups were demineralized for a period of one week whereas the FP 0.05% group was studied for two weeks. Both the unfluoridated groups were kept under the experimental conditions for as long as possible. There was, however, little point in making further measurements after 50 hours because of severe softening of the enamel surfaces.

Demineralization.

The constant composition apparatus used in this study (Figure 5.1.) has recently (Buskes et al. 1985) been described. Ten litres of the demineralizing solution is pumped continuously over the enamel slabs. The solution enters the apparatus from a polyethylene container and is divided over the enamel embedded in the PMMA by means of a manifold. The demineralizing liquid flows over the specimens at a controlled rate resulting in a thin liquid film on the enamel. The composition of the solution is kept constant within the biological variations. Calcium and phosphate variation is less than 3%, pH variation within 0.03 pH unit. A liquid sensor controls the fluid level and keeps it constant.
The demineralizing solution used consisted of 3 mM $CaCl_2.2H_2O$; 3 mM KH_2PO_4; 50 mM CH_3COOH and was brought to pH 5 by adding 48 ml 10 M KOH. A trace of thymol was added as fungistat. All the chemicals which were used were from Merck (pA) except for the thymol (Reinst).

Microhardness and microradiography.

Hardness measurements were carried out with a Leitz hardness tester with a 100 g load perpendicular to the enamel surface. Ten indentations were made on each enamel block **before** varnish application, after varnish removal and during given periods of the experimental run. Microradiograms were made at the end of each experiment in order to determine lesion depth and mineral distribution.
The enamel specimens were sectioned for microradiography into slices of approximately 600 µm thickness with a water-cooled diamond saw. These slices were then polished on grinding paper (Siawat 1200 grid) to a thickness of 80 ± 10 µm, measured with a micrometer gauge.
Microradiograms were made on photographic film (Kodak SO-253) with a (CuKα) X-ray source (Philips X-ray diffractometer PW 1730 at 20 KV and 15 mA). After having been developed the film was scanned on a microdensitometer (Leitz MPV) connected to a microcomputer (De Josselin de Jong and Ten Bosch 1985). Students' t-test ($p < 0.05$) was used for statistical analysis.

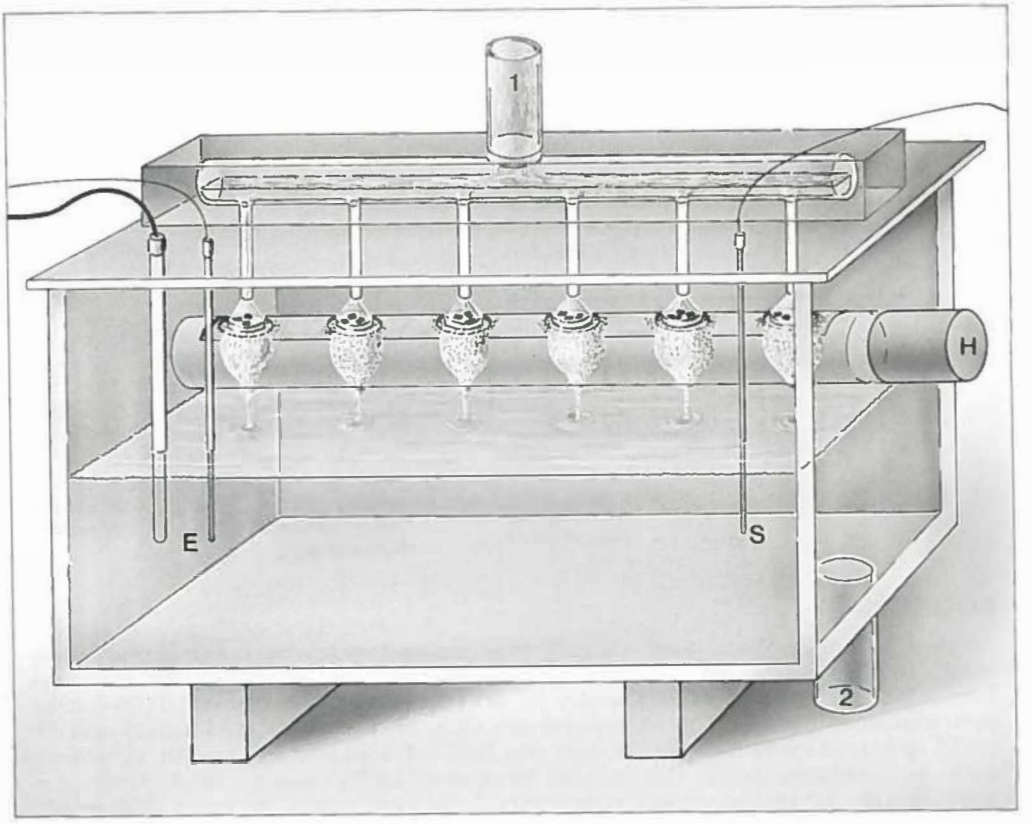


Figure 5.1.
The constant composition apparatus used in this study: H: specimen holder; E: electrodes; S: sensor; P: perspex apparatus; 1: inlet; 2: outlet. (Buskes et al. 1985).

RESULTS

Figure 5.2. shows the microhardness indentation length of the enamel after a fluoride application with the varnishes, given as a function of the demineralization period. Each value in this figure is an average of six enamel slabs each with ten indentations.
Statistical analysis revealed no differences in microhardness between the fluoridated enamel slabs. After two weeks the average indentation length of the FP 0.5% group was 75 ± 5 μm. This was comparable with the average value of 77 ± 10 μm after one week of demineralization. After 4 hours of demineralization the indentation length of the unfluoridated enamel was already statistically significantly higher than the fluoridated enamel. Unfluoridated enamel slabs could be demineralized up to the dentin in approximately 50 hours.

Microradiograms made after a varnish treatment with FP 0.7%, FP 0.1% and FP 0.05% showed no mineral loss at the end of each experimental run. This is in agreement with the hardness data. Typical microradiograms for the fluoridated enamel are shown in Figure 5.3.. They are not distinguishable from sound enamel curves.

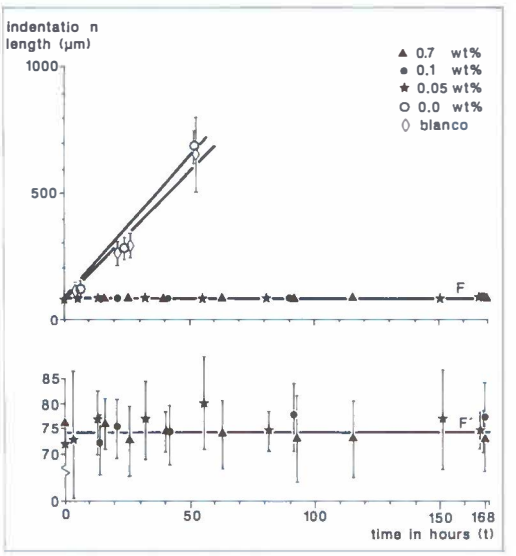


Figure 5.2.
The microhardness indentation length as a function of the demineralization period for the different experiments. F' shows the values of the fluoridated enamel on an expended scale. Each value is an average of six enamel slabs, each containing ten indentations; ± standard deviation.

DISCUSSION

It has clearly been demonstrated that under the in vitro test conditions used in this study, no demineralization of fluoridated enamel was observable after a period of one week. No statistically significant differences were measured between the microhardness data of the FP 0.7%, FP 0.1% and FP 0.05% applications. The results of the FP 0.7% application are in agreement with a previous study (Dérand and Petersson 1981), where lesion formation and lesion progression was completely inhibited after 10 weeks demineralization in a acidified gel-system at pH 4.5..
Application with the (same) polyurethane **fluoride-free** varnish had no preventive effect. No difference with respect to untreated control enamel specimens has been found.

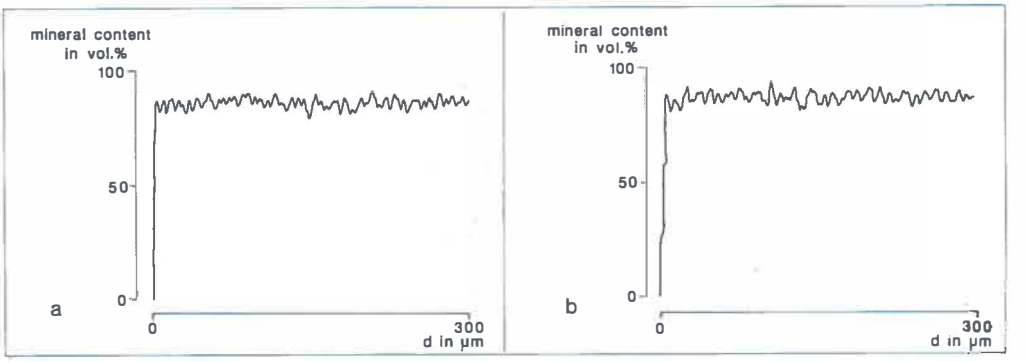


Figure 5.3.
Typical microradiogram of enamel fluoridated with a: FP 0.7%, FP 0.1% and FP 0.05% after 1 week and b: FP 0.05% after 2 weeks of demineralization.

It has been suggested that filaments of resin (tags) might interfere with diffusion phenomena and could be one of the mechanisms for the demineralization inhibition by varnish systems (Arends et al. 1980; Dérand and Petersson 1981). The data of the 0% varnish experiment in this study, however, show that this is of minor importance in the demineralization process which was investigated. It was checked whether the inhibiting effect on demineralization was **not** due to fluoride **in** the solution but to the pretreatment with fluoride varnishes. This was done in the following method.
The varnish weight applied to the experimental enamel slabs was determined using a microbalance (Sartorius-Werke GmbH, Göttingen, FRG, type 2474). The maximum fluoride concentration that could be introduced from the varnish in the solution was calculated using $F^- = W.f$ (W: varnish weight in µg; f: fluoride content in the varnish in wt%).

Table 5.I. shows the maximum possible fluoride leakage into the demineralization solution (10 litres), assuming a complete loss of the fluoride from the enamel blocks. The assumed F^- concentration in the solution ranges between 0.06 and 0.004 ppm.

Table 5.I *The amount of varnish applied on the experimental samples and the maximal amount of fluoride that could have been in the demineralization solution assuming a complete loss of fluoride.*

Varnish	Amount mg	F solution PPM
FP 0.7%	83.3	583.10^{-4}
FP 0.1%	82.5	82.10^{-4}
FP 0.05%	83.4	42.10^{-4}
FP 0%	82.7	0

A 0.2 ppm F^- concentration in the demineralization solution tested under the same conditions leads to deep subsurface lesion formation with high mineral loss (Figure 5.4.). It is, therefore, clear that the demineralization inhibition measured in this study, is due to fluoride in and on the enamel.
It is surprising that the low fluoride content in the varnish of 0.05% is so effective in the inhibition of demineralization. Arends and Christoffersen (1986) suggested that fluoride in the liquid phase is effective even in extremely low concentrations (between the crystallites) and dominates the demineralization inhibition mechanism.

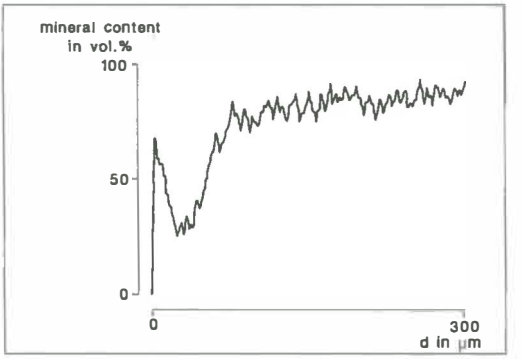


Figure 5.4.
Typical microradiogram of an enamel lesion after 168 hours demineralization with 0.2 ppm F^- in solution.

This experiment suggests that the amount of fluoride in varnishes can be decreased without a substantial loss of effect. A long-time *in vivo* effect can, however, not be inferred from this work and further clinical research is needed to confirm the conclusions which have been drawn. A clinical study is presented in Chapter 7.

in vitro lesion formation of bovine enamel pretreated with fluoride varnishes

a constant composition study at pH = 4.5

6

INTRODUCTION

The inhibiting effect of a 24 hours application of a fluoridating varnish with differing fluoride concentrations on human enamel demineralization has been described earlier (De Bruyn et al. 1986; Chapter 5). A complete inhibition of demineralization was observed in the fluoridated enamel at pH 5 after an acid attack of 336 hours. Although the varnishes investigated in vitro had differing fluoride concentrations, no differences in lesion depth or mineral loss were observed. This suggests that the fluoride concentration in varnishes can be reduced.
In the oral cavity demineralization and remineralization alternate several times a day and the effect is, therefore, always a combination of both processes. Therefore, both processes have to be taken into account when choosing the fluoride concentration in varnishes. As high fluoride concentrations inhibit demineralization and low concentrations enhance remineralization, there is presumably an optimum fluoride concentration for maximum caries prevention.

It may well be that the amount of fluoride in the fluoridating varnishes influences de- and remineralization by different mechanisms. Varnishes with a high fluoride concentration may, for example, have better caries preventive properties than varnishes with a lower concentration but the opposite could well be the case with remineralization.

The aim of this study was to check the effect of varnish applications (with differing fluoride concentrations) on bulk bovine enamel demineralization at low pH by means of microhardness measurements, microradiography and scanning electron microscopy.

A recently developed microradiography technique to follow demineralization in enamel single-sections was used to check lesion formation as well as with conventional microradiography (Buskes et al. 1987). With this technique it is possible to measure the enamel specimens repeatedly at the same area after different periods of demineralization.

MATERIALS AND METHODS

In this study 30 bovine incisors were demineralized under constant composition conditions (Buskes et al. 1985, Chapter 5). The demineralization solution which was used had a pH of 4.5.

The **polyurethane varnishes**, Fluor Protector with differing fluoride concentrations (abbreviated FP 0.7%; FP 0.1% and FP 0.05%) were applied on 8 polished enamel blocks, each for 24 hours. A control area of each enamel sample was covered with nail-varnish prior to demineralization in order to have an intra-tooth control. Four untreated specimens were used as a control.
Microhardness measurements were carried out on enamel blocks (fluoridated samples and control) after given periods of demineralization, as has previ-

ously been described in Chapter 5. After demineralization, part of the enamel surfaces were covered with nail-varnish in order to obtain microradiographic tracings of several demineralization periods, namely 4, 6, 14, 18 and 48 hours.

Two "types" of microradiography were carried out in these experiments:
i) **microradiography** was done at the end of the experiment on bulk enamel cut into thin sections as has been described in detail in Chapter 5.
ii) **microradiography** using the single-section technique (Buskes et al. 1987).

In the case of the second technique two **single enamel sections** of approximately 225 µm thickness were cut from the buccal surface of a polished bovine incisor. These sections were mounted in a sample holder and covered by nail-varnish, leaving only the original enamel surface exposed to the demineralizing solution. The sample holder fits into the constant composition apparatus (Figure 6.1.). The enamel section can be exposed to X-ray radiation (CuKα-radiation at 20 kV and 40 mA for 30 seconds) together with the aluminium stepwedge. The images of tooth section and stepwedge are fixed on Kodak SO-253 photographic film. Mineral assessment is made from densitometric tracings, the tracing position being defined by means of two reference points. The computer always directs the densitometer to the same defined spot and repositioning errors are smaller than 3 µm (Buskes et al. 1987). Lesion formation can be followed non-destructively during de- or remineralization using this technique.

Some enamel specimens were studied using **scanning electron microscopy**. The bulk enamel blocks were fractured to allow both scanning of the lesion and the outer enamel surface. The enamel was fractured and mounted onto aluminium stubs with a fast curing epoxy-resin in such a way that the fractured cross-section was more or less parallel to the specimen stub and the treated top-surface was perpendicular to the stub. The surface was cleaned of most of the loose material using compressed air. Finally, the specimens were sputter-coated (Balzers Union) with Au, approximately 10 nm, and examined in a JEOL SEM, type 35C operated at 25 kV.

RESULTS

Microhardness.

The indentation length, measured on bulk enamel for each varnish treated group, is given as a function of the demineralization period in Table 6.I..

Table 6.I *Average microhardness indentation length in µm (mean ± SD) after demineralization at pH = 4.5. Each value is an average of 4x10 indentations; for statistical analysis the number of teeth (4) was used. Statistically significant differences are connected by bars.*

Demineralization in hours	Varnish FP 0.7%	Varnish FP 0.1%	Varnish FP 0.05%	Control
0	78 ± 9	70 ± 8	69 ± 5	63 ± 9
4	80 ± 6	133 ± 54	132 ± 55	198 ± 60
6	72 ± 6	162 ± 62	135 ± 72	493 ± 44
14	164 ± 27	149 ± 68	568 ± 192	
18	110 ± 44	530 ± 116	917 ± 103	
48	167 ± 32			
66	354 ± 154			
102	654 ± 279			
180	936 ± 489			

Statistical analysis using student t-test ($p < 0.05$) revealed differences between the fluoridated enamel and the control as well as between the various varnishes.
The mean indentation length values were after 14 hours demineralization:

FP 0.7% < FP 0.1% < FP 0.05% < control.

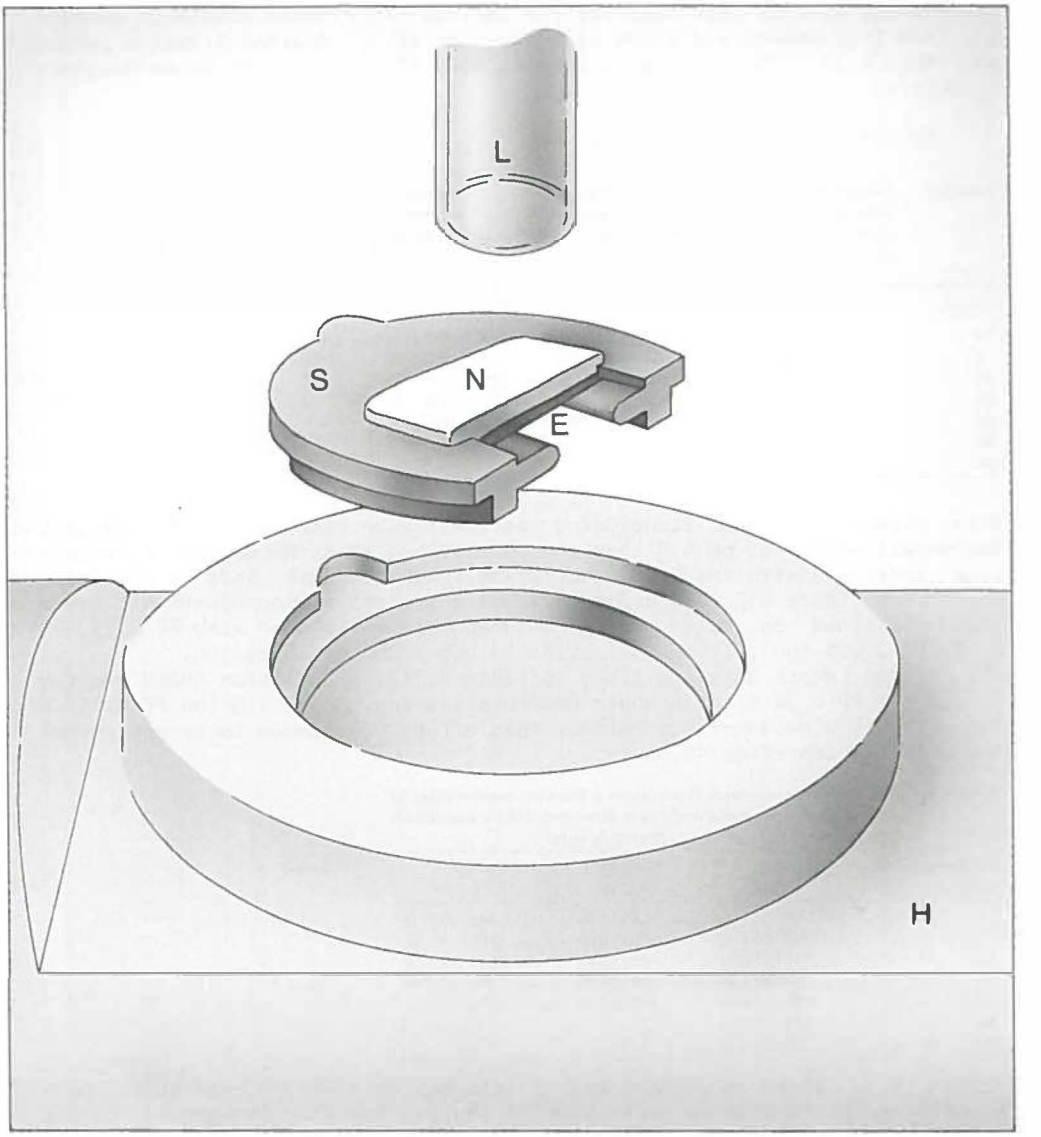


Figure 6.1.
Detail of the single-section sample holder (S) that fits into the specimen holder (H) of the constant composition apparatus. N: nail-varnish; E: enamel surface exposed to the demineralizing solution entering from the liquid tube (L).

The control experiment had to be stopped after 14 hours due to etching of the enamel surface. This phenomenon was also observed in the case of the FP 0.1% and FP 0.05% treated enamel after 48 hours. Demineralization of FP 0.7% treated enamel could be carried out for 180 hours.

Microradiography.

The average mineral loss data for the various fluoridated varnishes obtained from bulk enamel are given as a function of the demineralization period in Table 6.II.. The ranking in mineral loss ΔZ was after 14 hours demineralization:

FP 0.7 < FP 0.1% < FP 0.05%.

Table 6.II *Average mineral loss in vol %. μm (mean ± SD) for the various experimental groups after demineralization at pH = 4.5 The number of tracings is between brackets; statistically significant differences are connected by bars.*

Demineralization in hours	Varnish FP 0.7%	Varnish FP 0.1%	Varnish FP 0.05%
4	581±128 (4)	1677± 690 (6)	1835± 546 (11)
6	1035±727 (4)	1924± 671 (3)	4200±1061 (3)
14	953±505 (4)	2631± 989 (6)	5480±2337 (23)
18	1674±394 (12)	3233± 394(4)	6643±2934 (4)
48	3786±602 (5)	4606±1160 (9)	14575±2883 (4)
66	3791±469 (6)		
180	5041±924 (5)		

This shows that the fluoridated specimens were better protected against demineralization at pH 4.5 than the control and that the degree of protection increased with the F concentration in the varnish. This is also evident from Figure 6.2.. This figure shows 4 typical microradiographic tracings (obtained on **bulk** enamel) of 4 specimens (treated with FP 0.7%, FP 0.1%, FP 0.05% and the control) after 14 hours demineralization.
The lesion depth data are given in Table 6.III.. The lesion depth was for FP 0.05% > FP 0.1% after 6 hours demineralization. At pH 4.5 the FP 0.7% is the only fluoride varnish treatment that offers resistance to severe enamel demineralization after 48 hours.

Table 6.III *The average lesion depth in μm (mean ± SD) after demineralization at pH = 4.5 The number of tracings is between brackets; statistically significant differences are connected by bars.*

Demineralization hours	Varnish FP 0.7%	Varnish FP 0.1%	Varnish FP 0.05%
4	25±17 (4)	54±17 (6)	54± 23 (11)
6	50±27 (4)	70±15 (3)	169± 14 (3)
14	56±23 (4)	93±28 (6)	265±132 (23)
18	59±42 (12)	86±22 (4)	189± 61 (4)
48	131±11 (5)	154±61 (9)	227± 13 (4)
66	113±18 (6)		
180	129±22 (5)		

Figure 6.3. shows a combination of tracings obtained on bulk enamel pretreated with FP 0.7% as a function of the demineralization period. Lesion formation is clearly visible and lesion depths at various time intervals are indicated by arrows.
In Figure 6.4. lesion progression as observed in a single-section pretreated with FP 0.7% is shown. Lesion depths are indicated by arrows. Note that the different tracings were measured in the **same area** of the enamel.

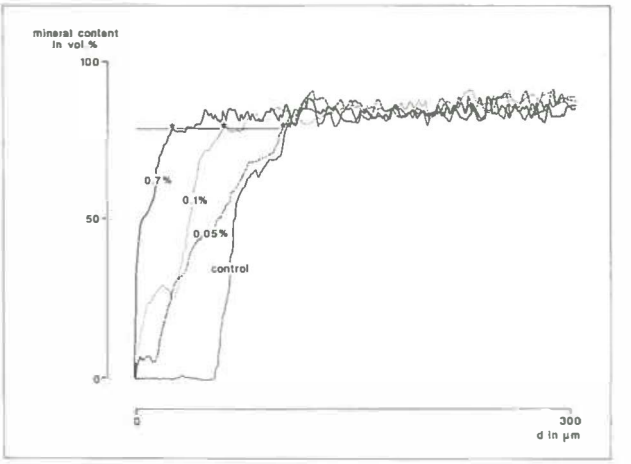


Figure 6.2.
Typical mineral distributions 14 hours after demineralization of the various experimental groups; lesion depths are indicated by asterisks.

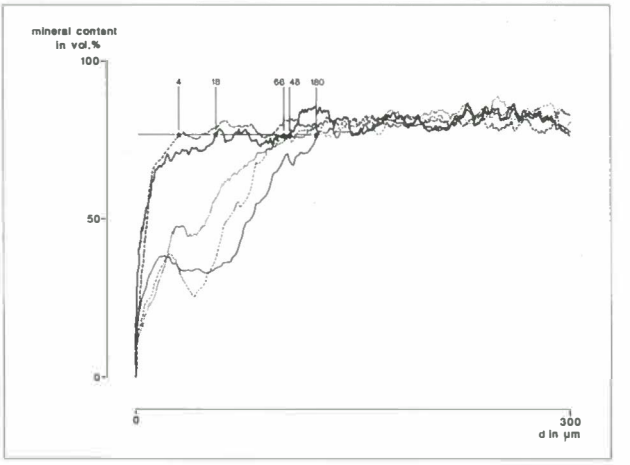


Figure 6.3.
Mineral distribution changes of enamel pretreated with FP 0.7%. This figure is a combination of several tracings obtained on bulk enamel for various demineralization periods; lesion depths are indicated by asterisks.

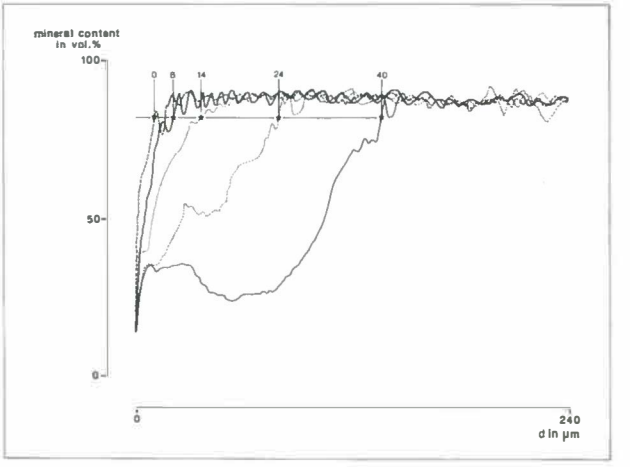


Figure 6.4.
Mineral distribution changes during demineralization of an enamel single-section pretreated with FP 0.7% and followed on the same area for various demineralization periods; lesion depths are indicated by asterisks.

DISCUSSION

In the present in vitro investigation bovine incisors were used instead of human teeth for practical reasons. They are easier to collect and also provide larger experimental enamel surfaces after polishing. The enamel layer is, furthermore, thicker and possesses only a few ppm F^-. It is possible to do more measurements on one tooth because it is possible to cover several enamel areas with nail-varnish during the experimental run. Also a sufficient number of enamel slices can be cut for microradiographic analysis and enough tooth material is left for SEM investigation.
The results of the present experiment indicate that the fluoride concentration in the varnishes is an important parameter in assessing demineralization inhibition under **severe** acid attack. This is evident from both mineral loss and lesion depth data (see Tables 6.II. and 6.III.).
Microhardness changes of the enamel surface are also good indicators of surface demineralization. It is not, however, suitable for studying etched surfaces, as in the untreated control, because the reference position of the enamel surface is unknown and more mineral is dissolved than is predictable from microhardness measurements.

In general, the protection against enamel demineralization of the varnishes is at pH 4.5:

FP 0.7% > FP 0.1% > FP 0.05% > untreated control.

This ranking is clearly illustrated in Figure 6.2.. A combination of typical tracings of the 4 treatment groups varnishes, is given 14 hours after demineralization. Lesion depths (indicated by arrows) decrease with increasing fluoride concentration in the varnishes and are 112; 82 and 42 µm for FP 0.05%, FP 0.1% and FP 0.7%, respectively. If one considers the mineral content of the surface layer formed in the FP 0.1% and FP 0.05% pretreated enamel, one finds 29 and 7 vol%, respectively. The FP 0.7% pretreated enamel shows a mineral distribution without an obvious surface layer. In accordance with microhardness data one can conclude that the enamel is still slightly softened in this stage of demineralization. When SEM images 6.5.a. and 6.5.d. are considered and compared with 6.5.b. and 6.5.c the difference in protection can clearly be seen.

One can see the demineralization process from Figure 6.2.. Fluoride gives a protection against etching and demineralization. Lesion formation will be retarded depending on the fluoride acquired on/in enamel which is in turn related to the fluoride concentration in the varnish (De Bruyn et al. 1985, Chapter 4 of this thesis). Demineralization in the FP 0.05% pretreated enamel is at a more advanced stage than in the FP 0.1% treated enamel.
According to literature (Arends and Ten Cate 1981; Arends et al. 1983; Ten Cate and Duijsters 1983a, 1983b; Borsboom et al. 1985; Buskes et al. 1987) surface softening is the first stage in enamel demineralization. Fourteen hours after demineralization the lesion has a subsurface mineral distribution in the FP 0.1% pretreated enamel while in the FP 0.7% treated enamel it is still in the surface softening stage.

In Figure 6.5. the SEM data of enamel surfaces and of fractured samples show that:

i) **The outer enamel surface** is visibly better protected with increasing fluoride concentration and
ii) the ultrastructure of the enamel observed on fractured samples (at the magnification of Figure 6.5.) is comparable in the 3 cases shown. The prism structure is intact and the demineralization is not "visible".

Subsurface lesion formation in vitro is, in general, due to the presence of an inhibitor e.g. fluoride in the demineralization solution. This was not the case in this study (no inhibitor was added to the demineralization solution), neither do the fluoride varnish treatments of the enamel introduce an appreciable fluoride content into the demineralization solution.
It is, however, very likely that the fluoride acquired after fluoride varnish application is present **locally** in the "liquid state" (between the enamel crystallites) and that this fluoride acts as a **local** inhibitor during dissolution of the enamel.

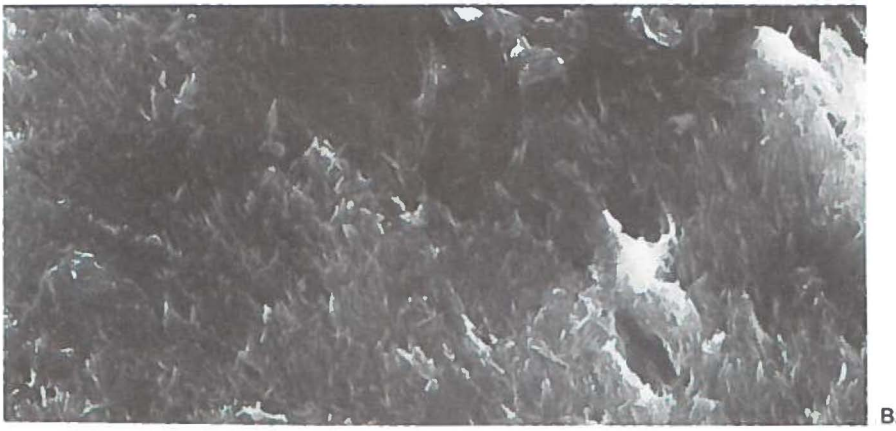
Etching of untreated enamel was measured on the microradiograms by comparing the acid treated area with the sound enamel control (covered with nail-varnish prior to demineralization). An etch depth of approximately 70 μm was calculated from the microradiographical tracing after 14 hours demineralization. The etch pattern is also seen on the SEM micrographs. In Figure 6.5.c. it can be seen that the interprismatic area is removed and the prism structure is observable. (In Figure 6.5.f. etching in the interprismatic regions is visualized. This may, however, be due to artifacts.)

As far as the single-section microradiography technique is concerned, the lesion formation in the FP 0.7% group was studied during demineralization; see Figure 6.4.. No fluoride was present in the demineralizing solution and the lesion has a surface softening mineral distribution at the first stage of demineralization. A surface layer is formed at a later stage. The lesion body beneath the surface layer decreases in mineral content while lesion depth increases with an increase in the period of demineralization.
Ten Cate and Exterkate (1986) recently suggested that acid susceptibility of enamel is strongly increased in single-sections, compared with bulk samples. This was also observed in this work. For example, in the case of the single-section lesion, the lesion depth was 152 μm after 40 hours, whereas the lesion depth observed in bulk enamel was 126 μm after 180 hours.
The single-section technique is very valuable, however, because lesion formation can be monitored in time.

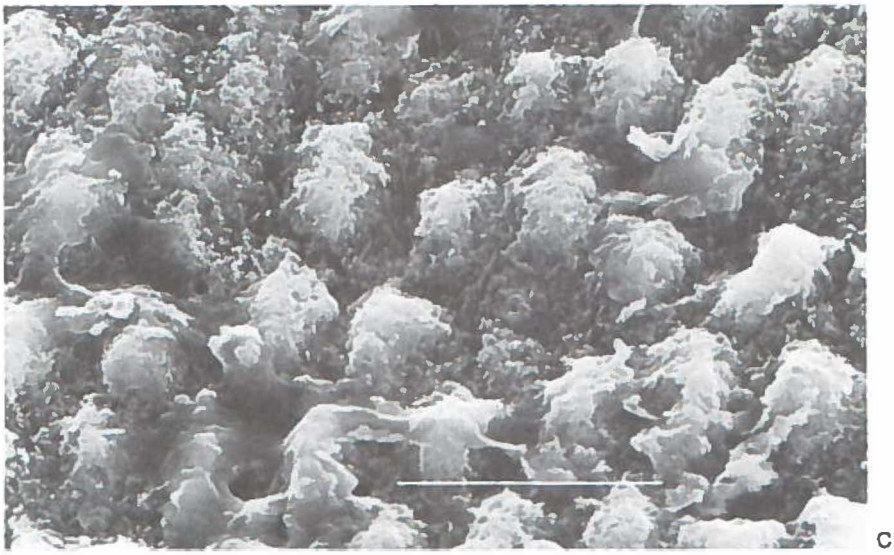
From this in vitro study one can conclude that the demineralization inhibition under a severe acid attack in vitro is dependent on the fluoride concentration in the varnish.
The effect of fluoride varnish applications on **remineralization** will be discussed briefly in Chapter 8.

Figure 6.5. (on the next pages)
Typical electron microscopic micrographs of enamel surfaces (A-C) and in cross-section fractured (D-F) through the demineralized area after 14 hours of demineralization.
Magnification A-C x 3500; D-F x 1000; the bar is 10 μm.

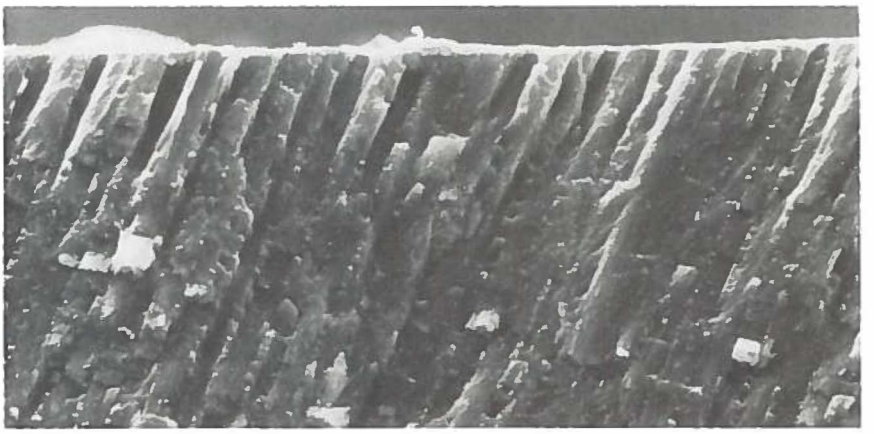
A: FP 0.7%



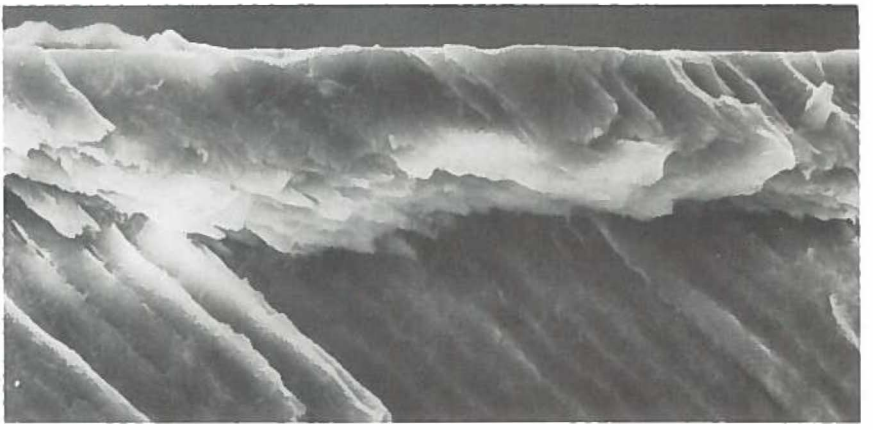
B: FP 0.1%



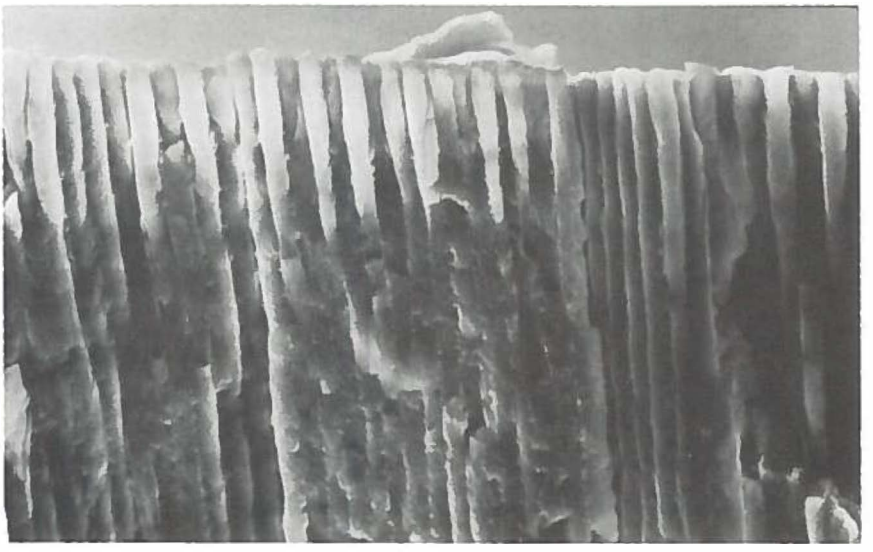
C: untreated



D: FP 0.7%



E: FP 0.1%



F: untreated

caries protection and mineral loss after varnish applications in vivo

7

INTRODUCTION

For many years fluoride varnishes have been employed in preventive dentistry for topical fluoride applications. The most commonly used are Duraphat and Fluor Protector, both of which have been tested in laboratory and clinical trials. Duraphat was first introduced as a natural resin varnish containing 5 wt% sodium fluoride (Heuser and Schmidt 1966). Fluor Protector is a polyurethane based varnish with 0.7 wt% silane fluoride; it was introduced in 1975 in laboratory studies (Arends and Schuthof 1975). Recently, fluoride varnishes on the same base with lower fluoride concentrations (0.1 and 0.05 wt%) have been tested (De Bruyn et al. 1985, 1986).

Fluoride varnishes have two special properties if applied as preventive agents: a relatively high fluoride concentration and a long contact time with the enamel surface. They generate a high fluoride uptake in the enamel as well as on the enamel. Nelson et al. (1983, 1984) studied the acquired fluoride layer on the outer enamel surface in detail using X-ray diffraction, TEM and SEM After various fluoride applications. They described a thick and distinct CaF_2-like layer on the enamel surface after varnish application. Dijkman et al. (1983) and De Bruyn and Arends (1985) found that the CaF_2-like material on the enamel surface is slowly lost in vivo. These findings tend to support the idea that the CaF_2 coating may act as a fluoride depot and consequently play an important role in caries prevention.

Several clinical trials on fluoride varnishes have been published (for a review see Clark 1982). Caries reductions of between 18% and 75% have been reported for Duraphat. It is known that Duraphat is most active if application is repeated after 3 months (Modéer et al. 1984).
The results obtained in studies using Fluor Protector are somewhat confusing due to problems of study design. On the one hand Kolehmainen and Kerosuo (1979); Kolehmainen (1981); Van Eck et al. (1984) found no caries reduction after a single Fluor Protector treatment. There were, however, extremely low caries increments, due to an additional effect of fluoride mouthrinses and normal oral hygiene procedures with fluoride containing toothpastes. The Seppä studies (1981, 1982a) on the other hand, conducted in water fluoridated areas showed an extra caries reduction of 35% after Fluor Protector application. Salem and co-workers (1979) reported a caries reduction of 45% after bi-annual applications of Fluor Protector in a three-year clinical trial.
Fluoride containing varnishes are generally accepted for caries prevention but the clinical effectiveness of Fluor Protector is still uncertain. The

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De Bruyn, H., Van Rijn, L.J., Purdell-Lewis, D.J., Arends, J.: The influence of various fluoride varnishes on mineral loss under plaque.
Caries Res. (accepted 1987).

effect of Duraphat and Fluor Protector on lesion formation and lesion progress in vivo has not yet been quantified using microradiography as a measuring tool.

This study was carried out because, to the authors' knowledge, no clinical study has been carried out in which the effect of additional fluoride administration from fluoridated water, toothpastes or other topical applications during the study has been eliminated. Furthermore, the effect of fluoride varnishes under constantly accumulating dental plaque has never been studied. Also data on the preventive effect of varnishes with a relatively low fluoride concentration are not available.

The aim of this study was therefore threefold:

i) to compare the in vivo effect of Fluor Protector and Duraphat 4 and 6 months after application;
ii) to study the effect of these varnishes on enamel demineralization under accumulated plaque in vivo;
iii) to investigate the caries inhibiting effect of Fluor Protector on demineralization as a function of its fluoride concentration (abbreviated FP 0.7%; FP 0.1%; FP 0.05% and FP 0%).

MATERIALS AND METHODS

General.

In this in vivo study intact sound human enamel was treated with various fluoride varnishes for 24 hours. After removal of the varnish the specimens were positioned in dentures and left under accumulated plaque for periods of either 2, 4 and 6 months. At the end of each experimental period demineralization was studied using quantitative microradiography.

Enamel selection and preparation.

Recently extracted human incisors were collected in the Groningen (non-fluoridated) area. Only incisors were used because the average fluoride concentration of symmetrical areas of the buccal surface is comparable (Bruun 1973; Retief et al. 1980). The teeth were thoroughly cleaned with a water-pumice slurry and stored in a thymol-containing solution before use. Enamel slabs ≈ 4 x 4 mm^2 and 1.5 mm thickness were prepared from the buccal surfaces of the teeth. These unpolished enamel specimens were screened for defects using a stereomicroscope (x 10). Seventy-six of these enamel slabs were selected for the experiments, randomly assigned to five groups and pretreated with either Duraphat or FP 0.7%, FP 0.1%, FP 0.05% and FP 0%, the latter serving as untreated control. The varnishes were applied on the enamel in vitro under a stereomicroscope. All varnishes were removed with a scalpel after 24 hours just before the in vivo part of the experiment.

Experimental design.

Fifteen patients attending the dental clinic in Groningen participated voluntarily in this investigation. Twelve were partially edentulous in the premolar-molar region and received a frame denture suitable for clinical experimentation (De Bruyn et al. 1985); three participants had an acrylic full denture. The second premolars of the lower denture were replaced by metal boxes, each containing two enamel slabs. The participants were in-

structed to carry out normal oral hygiene procedures on the natural dentition with a fluoride-free toothpaste (Prodent Ultra Actif) and to avoid additional fluoride administration. They wore the appliances day and night and were instructed not to clean the enamel samples (leaving plaque accumulation intact). Figure 7.1. shows a detail of a frame denture after 4 months experimentation in vivo.

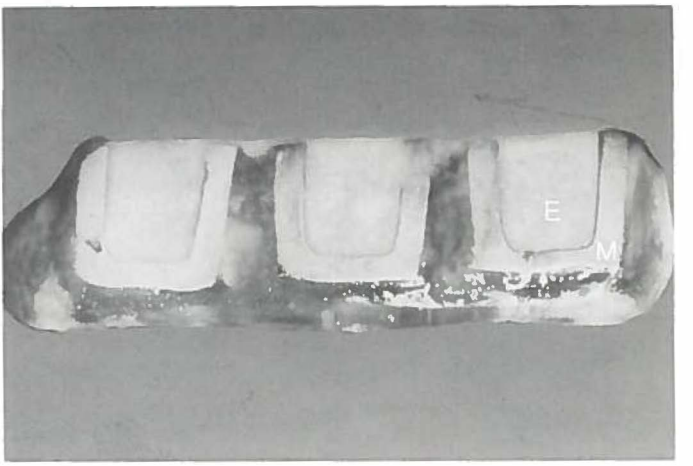

Figure 7.1.
Detail of the experimental frame appliance. M: metal box; E: plaque covered enamel.

A combination of the 5 treatments, FP 0.7%; FP 0.1%; FP 0.05%; FP 0% and Duraphat, was mounted in the appliances of 8 participants for 4 months. 7 participants carried a combination of FP 0.7%; FP 0.1%; FP 0% and Duraphat for 6 months. An extra unfluoridated enamel slab was carried by 9 patients and removed after two months.

Microradiographic preparation and analysis.

At the end of each experimental period the enamel slabs were removed and sectioned into 3 parts of 1 mm thickness. Each slice was subsequently ground and polished to a thickness of 80 ± 10 μm. The thickness was measured using a Sony digital gauging probe (type DG 310) and magnescale (LY-101). Images of the enamel slices and an aluminium stepwedge for calibration were made on Kodak SO-253 photographic film with a Copper Kα X-ray source (Philips X-ray diffractometer PW 1730). Microradiograms were scanned on a microdensitometer (Leitz MPV) connected to a microcomputer and processed using the improved microradiography technique described by De Josselin de Jong and Ten Bosch (1985a).
A representative zone of the upper, middle and lower third of each microradiogram was scanned thus providing in total 9 tracings for each enamel slabs. On each tracing 1) mineral loss, 2) lesion depth and 3) mineral distribution type was determined.

1) Mineral loss
The mineral loss is defined as ΔZ, the difference between the mineral concentration originally present and the mineral concentration actually present integrated over the position (depth) inside the tooth section

(Arends and Gelhard 1983; Gelhard and Arends 1984; Arends and Ten Bosch 1986). ΔZ is expressed in Vol% x μm and depicted in Figure 7.2. by the dotted area. Its value is calculated as follows (De Josselin de Jong 1986; De Josselin de Jong et al. 1987):

i) the computer plots the actual mineral volume percentage V(x) as a function of position (x) in the section.

ii) the value of V(x) measured deep to the lesion is taken as the V of sound enamel: V(sound).

iii) the integral $\int_0^{300\ \mu m} [V(sound) - V(x)]\ dx$

is calculated as an approximation of ΔZ. The unit of ΔZ is then Vol% x μm. In all cases the lesion depth was substantially less than 300 μm thus permitting V(sound) to be determined.

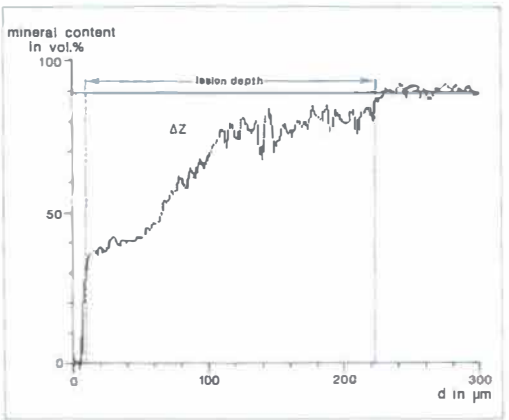

Figure 7.2.
Microdensitometric tracing of an enamel lesion showing the parameters measured. The dotted area indicates mineral loss ΔZ; the arrow (from the approximate outer enamel surface to the point where the mineral volume content is 5% less than the sound enamel value) indicates the lesion depth Ld.

2) Lesion depth

Lesion depth (Ld) is defined as the distance in μm from the enamel surface to the point where the mineral volume content differs by more than 5% from the sound value (Figure 7.2.) (Mellberg and Mallon 1984).

3) Mineral distribution

3 different types of mineral distribution were found on the microradiographic tracings and defined as depicted in Figure 7.3.: 1) sound enamel; 2) mineral distribution without a surface layer; 3) mineral distribution with a surface layer and subsurface lesion. The ranking of these mineral distributions was determined in percent for each different experimental group using all the microradiographic tracings.

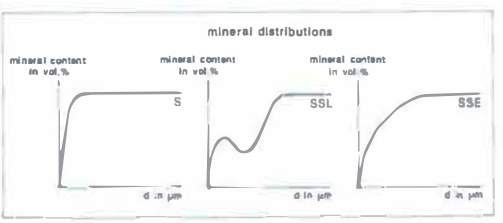

Figure 7.3.
Classification of mineral distribution.
S: sound enamel; SSE: mineral distribution without a surface layer; SSL: subsurface lesion with surface layer.

4) Statistical analysis

The average ΔZ and Ld values were calculated from the 9 tracings obtained from each enamel block.

As within-block standard deviations appeared to be approximately proportional to block means, logarithmic transformation was used to achieve an approximate normality of the distribution of the observations. The block means of the transformed normalized observations were subsequently used for comparison of effects of different applications by means of the analysis of variance. The mean values and respective standard deviations were used to calculate the 95% confidence limits on the original scale. A similar analysis without transformation was carried out for the lesion depths.

RESULTS

Mineral loss and lesion depths.

The mean mineral loss ΔZ, mean demineralization depth Ld and the average percentage protection against mineral loss for the different varnish applications are given in Tables 7.I. and 7.II., for the 6 and 4 months experiments.

Four months after application enamel fluoridated with FP 0.7%; FP 0.1% and FP 0.05% differed statistically ($p < 0.05$) from the unfluoridated and the Duraphat pretreated enamel. The Duraphat group was comparable with the control. No statistically significant difference was found between the various varnishes investigated 6 months after application.

Table 7.I *The average mineral loss ΔZ in vol%.μm, the average percentage protection and the average lesion depth Ld in μm for the various experimental groups 6 months after application. The 95% confidence limits are between brackets.*

Varnish	mineral loss ΔZ (vol%.μm)	protection %	lesion depth Ld (μm)	number of specimens
FP 0.7%	1227 (555; 2714)	42	79 (31; 127)	6
FP 0.1%	1203 (541; 2674)	43	110 (40; 180)	6
Duraphat	1514 (690; 3320)	28	97 (56; 138)	6
Control	2106 (1237; 3587)	0	110 (68; 152)	6

There is no statistical difference at the 5% level between the mineral loss or lesion depth data.

Table 7.II *The average mineral loss ΔZ in vol%.μm, the average percentage protection and the average lesion depth Ld in μm for the various experimental groups 4 months after application. The 95% confidence limits are between brackets.*

Varnish	mineral loss ΔZ (vol%.μm)	protection %	lesion depth Ld (μm)	number of specimens
FP 0.7%	965 (723; 1289)	65	76 (50; 102)	8
FP 0.1%	1303 (1042; 1630)	53	93 (71; 115)	8
FP 0.05%	687 (215; 2192)	75	83 (58; 108)	7
Duraphat	2704 (2102; 3480)	3	138 (94; 182)	8
Control	2787 (1611; 4819)	0	147 (82; 212)	8

The data above and below the dotted line are significantly different at the 5% level. There is no significant difference between Duraphat and control and between the three fluoridated urethane based varnishes.

Table 7.III. shows the data of the unfluoridated control group at 2, 4 and 6 months interval. Demineralization increases between 2 and 4 months ($p < 0.05$); however, no statistically significant difference after 4 and 6 months was found in any of the samples.
(In the 6 months experiment the data from one patient were rejected because calculus formation on the enamel slabs made further measurements impossible.)

Table 7.III *The average mineral loss ΔZ in vol%.μm and the average lesion depth Ld in μm for the untreated control groups, 2, 4 and 6 months after application. The 95% confidence limits are between brackets.*

Experimental period in	mineral loss ΔZ(vol%. μm)	lesion depth Ld(μm)	number of specimens
2	615 (290; 1308)	68 (42; 94)	9
4	2787 (1611; 4819)	147 (82; 212)	8
6	2106 (1237; 3587)	110 (68; 152)	6

The data above and below the dotted line are significantly different at the 5% level. There is no significant difference between 4 and 6 months.

Mineral distribution.

The ranking value in percent of the 3 different mineral distribution types, calculated from all microradiographic tracings, is depicted in Figure 7.4. for the various varnishes at 2, 4 and 6 months.
For Fluor Protector varnished enamel an inverse trend was found between the percentage of distributions without a surface layer and the fluoride concentration in the varnishes, after both 4 and 6 months. It is obvious from Figure 7.4. that the percentage of sound tracings is substantially larger for the FP varnishes than for Duraphat or the untreated controls.
In the control specimens (0 in Figure 7.4.), the percentage of mineral distributions without a surface layer seems to increase between 2 and 4 months and then remains constant; the reverse effect was observed for sound enamel tracings.

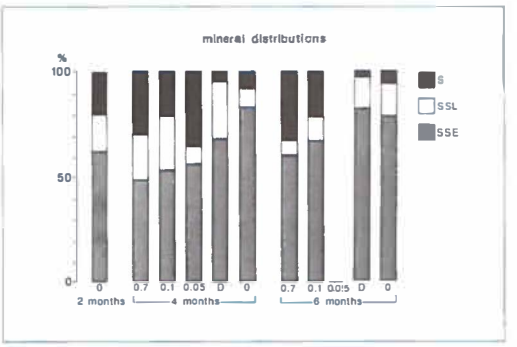

Figure 7.4.
Proportion of the 3 types of mineral distribution in percentage (S; SSE and SSL) for each experimental group.

DISCUSSION

The use of intra-oral devices was first introduced by Koulourides (1966) and has been generally accepted in caries research since then. In this long-term model investigation additional uncontrolled fluoride administration was avoided. By using a caries-model it was possible to create a pla-

que covered environment which promoted demineralization. The enamel was constantly covered by plaque and although this is not a complete mimic of the oral situation, it is unavoidable if a substantial cariogenic challenge is to be created. Under these experimental conditions a high caries risk situation was simulated without causing inconvenience to the participants and ethical problems in the experimental design. In reality dental plaque is likely to be brushed or worn off and subsequently to regrow.

From the literature (Heuser and Schmidt 1966; Seppä et al. 1982b; Seppä et al. 1984a) it is also known that the efficacy of fluoride varnishes in fissures is less than on free or approximal smooth surfaces. There may be considerable differences between the intra-oral device used in our study and the high-risk sites e.g. fissures and interproximal regions.

The protective effect of the various varnishes was measured by contact microradiography since extensive, quantitative information can be gathered from one enamel section (Groeneveld 1974). This technique made it possible to describe the demineralization process not only in terms of mineral loss (ΔZ) (Gelhard and Arends 1984) but also lesion depth (Ld) and mineral distribution. The technique has recently been improved by De Josselin de Jong and Ten Bosch (1985b). Microradiographic tracings made on different slices of one enamel slab showed that there was a large biological variation in each sample.
An example of the variation between 3 tracings on a longitudinally cut enamel slice is shown in Figure 7.5..

From Table 7.II. it can be seen that after 4 months in vivo demineralization, there was no difference between the effects of the different F^- containing FP varnishes with respect to lesion depth and mineral loss. These findings are remarkable because the **in vivo fluoride uptake** in enamel after fluoride varnish application is considerable and strongly related to the fluoride concentration in the varnish. The fluoride uptake has been measured under the same experimental conditions and in the same patients after one week in vivo (De Bruyn et al. 1985). In that investigation fluoride uptake both **ON** the enamel and **IN** the enamel was measured. F [on] was determined as alkali-soluble CaF_2-like fluoride. F [in] was found by etching off 5 enamel layers up to a thickness of 26 µm. It was found that there was a linear relationship between the fluoride uptake and the fluoride concentration in the varnish. The total fluoride uptake after FP 0.7%; FP 0.1% and FP 0.05% was 16.7 µg, 4.1 µg and 2.9 µg, respectively (De Bruyn and Arends 1985). Although the FP 0.05% varnish yields 14 times less fluoride and induces a much lower fluoride uptake than FP 0.7%, there was no **difference** in protective effect. These in vivo results are in agreement with data obtained **in vitro** (De Bruyn et al. 1986) and with the suggestion made by Arends and Christoffersen (1986) that small amounts of fluoride in the liquid phase in enamel play a very important role in demineralization inhibition. These findings indicate that fluoride concentrations in varnishes can be reduced without substantial loss of effect.

In this respect it should be noted that only sound human enamel was used. The fluoride effect is likely to be more pronounced when fluoride varnishes are administred to incipient lesions since fluoride penetration into the lesion plays an important role in the final caries preventive effect.

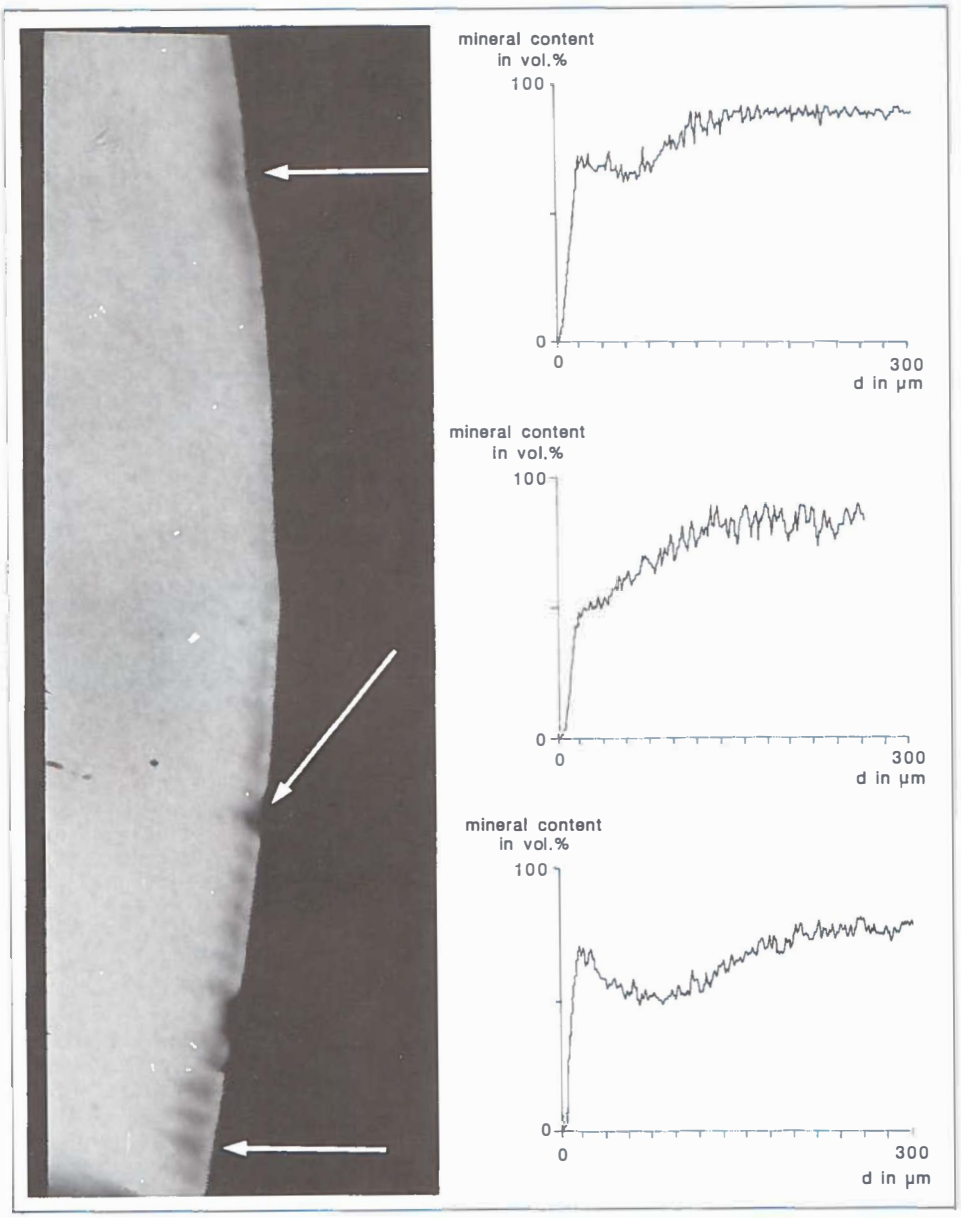

Figure 7.5.
Typical microradiogram of demineralized enamel 4 months after application of FP 0.1%. 3 microdensitometric scans from corresponding areas (indicated by arrows) illustrate the variation in mineral distribution.

A comparison of the average mineral loss of FP 0.7%, FP 0.1% and FP 0.05% with the unfluoridated control reveals a protection* of 65, 53 and 75% respectively 4 months after application. After 6 months, however, there was no statistical significant difference when compared to the control. As far as Duraphat is concerned, no preventive effect on mineral loss or lesion depth was observed at either the 4 or 6 months interval. It is, however, important to note that the experimental conditions of our caries model introduce **a high demineralization challenge** on the enamel slabs. In most papers where Duraphat demonstrated good caries preventive properties, this was not the case. In the literature it has been indicated that in cases with high caries susceptibility Duraphat gives moderate protection (Koch and Petersson 1975) or no protection at all (Murray et al. 1977; Modéer et al. 1984). Murray et al. (1977) therefore suggested to re-apply the varnish every three months.

It is notable that there are no statistical significant differences between any of the groups when changes in mineral loss and lesion depths between 4 and 6 months are compared. Lesion progress obviously stopped or was strongly retarded in the final 2 month period. This phenomenon may be explained by changes in the plaque layer covering the enamel slabs. In this model accumulated plaque remained undisturbed for 4 or 6 months and in one participant of the 6 months group calculus formation occurred. This finding suggests that an increase in calcium in the plaque is likely to occur during the last two months period. It has been reported that calcium in the plaque will depress enamel solubility through a common ion effect (Pearce 1982). It is quite reasonable to assume that in the model system used, the calcium concentration in dental plaque also increased in time.

The difference in mineral distributions (see Figure 7.4.) for the control FP 0% and Fluor Protector varnished enamel with time can also be due to changes in plaque composition. Figure 7.4. indicates a proportional increase in mineral distributions without a surface layer for Fluor Protector varnished enamel in the last two months. This indicates that no remineralization and no inhibition of demineralization occured; there is also a trend towards increased lesion depth. The control enamel however reveals a slight decrease in the percentage of surface softened specimens and lesion depth also tends to decrease. This might suggest that natural remineralization occured more easily in the control.
Due to the high fluoride concentrations deposited in and on the enamel by all the fluoride varnishes, it is most probable that the effects of fluoridation are mainly controlled by CaF_2 deposition on the demineralized enamel. Because, in vivo, CaF_2-like material slowly leaches away in time, it is not unreasonable to pressume a time dependent effect. From the literature it is known that indeed the preventive effect of fluoride varnish treatments is lost when only one application per year is given (Clark 1982) or when the treatment is discontinued (Seppä et al. 1984b).
Seppä et al. (1981; 1982a) and Clark (1982) have suggested that 'fluoride

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* The protection against mineral loss resulting from the fluoride varnishes is defined by:

$$\frac{\Delta Z \text{ control} - \Delta Z \text{ fluoridated}}{\Delta Z \text{ control}} \times 100\%$$

leakage' may play an important role in the results obtained when split mouth techniques are used. In the present study this was not the case since only 4 small enamel samples were varnished and fluoride varnish remnants were peeled off **before** the in vivo experiment. Furthermore the small degree of fluoride leakage has been quantified and described after one week and was found to be 1 $\mu g.cm^{-2}$ (De Bruyn et al. 1985).

It can therefore be concluded from this in vivo model study that under strongly cariogenic conditions Fluor Protector with a concentration of 0.7%, 0.1% and 0.05% is effective in reducing the degree of demineralization. Our results taken in relation with those of other workers also support the premise that Duraphat and probably all fluoride varnishes should be applied more frequently under these conditions.

Topical fluoride agents, such as fluoride varnishes, induce CaF_2 formation on the outer enamel surface. It has been suggested that the precipitated layer is dissolved quickly in vivo, because saliva is undersaturated with respect to calcium fluoride (Brudevold et al. 1967). Recently, however, Øgaard et al. (1983) described that alkali-soluble CaF_2 was retained for at least two weeks. Dijkman et al. (1983) investigated fluoride retention after varnish applications and found a substantial amount of fluoride on the surface three months after application in vivo. The results of the study on fluoride uptake, presented in Chapter 4, confirm these findings. The fluoride loss from the enamel surfaces in one week was negligible. CaF_2-like material is, even under nearly continuous plaque attack, retained for several weeks. One possible explanation for this long fluoride retention was suggested by Rølla and Øgaard (1986). This was, namely that the dissolution rate of CaF_2 could be substantially reduced by the adsorption of proteins and/or phosphates, available in saliva and plaque fluid. An alternative explanation could be that the "CaF_2-like" material, which is precipitated on the enamel surface after fluoride application and contains significant amounts of phosphates, is extremely insoluble.

Although the real solubility product of CaF_2-like material in the enamel is not known, it has a low acid solubility. It can, therefore, **directly** protect the underlying enamel and/or influence the diffusion by blocking natural enamel pores or etch pits as suggested by Nelson et al. (1984b). CaF_2-like material on the outer enamel surface can also act as a fluoride releasing depot. When the pH drops during demineralization, some fluoride is released. This can penetrate into enamel or plaque and can be effective during a subsequent remineralization cycle. This concept could explain why fluoride concentration increases **in** enamel, while the amount of fluoride **on** the surface slowly decreases after topical fluoride application.

In the past the fluoride uptake in the enamel crystallite was regarded as the most important goal in achieving caries prevention. At present the fluoride adsorbed on the outer enamel surface of the crystallites seems to be more important in achieving the inhibition of demineralization. This fluoride (Fl) at the solid-liquid interface directly determines the dissolution reduction and acid solubility reduction of enamel. Furthermore, this Fl can act as a local fluoride reservoir during de- and remineralization cycles. In this thesis the fluoride uptake **in** enamel was measured up to a thickness of 30 µm. Although the varnishes with differing fluoride concentrations (abbreviated FP 0.7; 0.1; 0.05%) realized a variable fluoride uptake, there was no difference in demineralization inhibition at pH 5 in vitro or in caries prevention, as measured in vivo. The very pronounced F [uptake] in enamel after FP 0.7% application does not lead to a more pronounced protection of mineral loss. This suggests, as mentioned by Arends and Christoffersen (1986), that small amounts of fluoride in the liquid state (Fl) are indeed effective in demineralization inhibition.

In the in vitro **demineralization** studies, the CaF_2-layer is probably the main parameter in the inhibition of demineralization. No demineralization of the enamel was observable at pH 5. This is most likely due to the the

coating and reservoir effect of afore mentioned CaF_2-layer. At this pH there was no difference between the varnishes with various fluoride contents. Only the fluoride at the CaF_2-enamel surface interface seems to be effective and the extra fluoride deposited by the varnish with FP 0.7% has no additional effect.
At a low pH value (pH 4.5) the demineralization rate of enamel was influenced by the fluoride content in the varnish. It can be conclude from the fluoride uptake experiments that the alkali-soluble fluoride on the enamel surface was directly related to the fluoride content in the varnish. As shown in Table 8.I. the amount of mineral loss from enamel in vitro at pH 4.5 is correlated with the amount of fluoride acquired 24 hours after application. The linear correlation coefficients between F [on] and ΔZ are high at the beginning of the demineralization and decrease in time. This is most likely caused by the fact that CaF_2 dissolves and consequently its effect becomes less and less noticable.

Table 8.I *Correlation coefficients (assuming linear correlation) between F[on] after application (in μg.cm^{-2}) and ΔZ (in vol%.μm) during demineralization (in hours).*

F[on]		ΔZ			
		4 h	14 h	18 h	48 h
FP 0.7%	11.9	581	953	1674	3786
FP 0.1%	1.3	1677	2631	3233	4606
FP 0.05%	0.9	1835	5480	6643	14575
correlation coefficient		-0.996	-0.802	-0.763	-0.584

Fluoride plays an important role in the protection of enamel against decay as well as in the stimulation of remineralization of caries lesions. In the course of this study the emphasis was on the demineralization inhibiting properties of fluoride varnishes with differing fluoride concentrations. Additional information on remineralization was obtained from a preliminary in vitro study, which will be discussed briefly.

Demineralization of eight bovine enamel blocks was performed under constant composition conditions at pH 5 in a solution consisting of 3 mM $CaCl_2.2H_2O$; 3 mM KH_2PO_4; 50 mM CH_3COOH; 0.22 ppm fluoride. Prior to demineralization the enamel surfaces were partially covered with nail-varnish in order to have sound enamel values as controls. After 144 hours a typical subsurface lesion was obtained with ΔZ 4686 ± 246 vol%.μm, a lesion depth of 95 ± 3 μm (mean ± SE) and a mineral distribution as depicted in Figure 8.1..
After demineralization two enamel specimens were each treated with FP 0.7%; FP 0.1% and FP 0.05%, respectively. Two blocks served as controls. The varnish application period was 24 hours during which the enamel blocks were kept at 100% humidity at 37.4° C. The varnishes were dissolved (one day after application) from the surfaces with acetone, to avoid damage to the surface layer. Part of each enamel surface was covered with nail-varnish again to retain the demineralized situation.
Remineralization was subsequently carried out in the constant composition apparatus with a solution containing 1.5 mM $CaCl_2.2H_2O$; 0.9 mM KH_2PO_4 and 20 mM 4-(2-hydroxylethyl)-1-piperazine ethane sulfonic acid (Hepes). The pH was adjusted to 7 and no fluoride was added.
The amount of mineral gain and the reduction in lesion depth after 18 days of remineralization was measured with microradiography and calculated by subtracting the ΔZ and lesion depth values of the remineralized from the demineralized lesion.

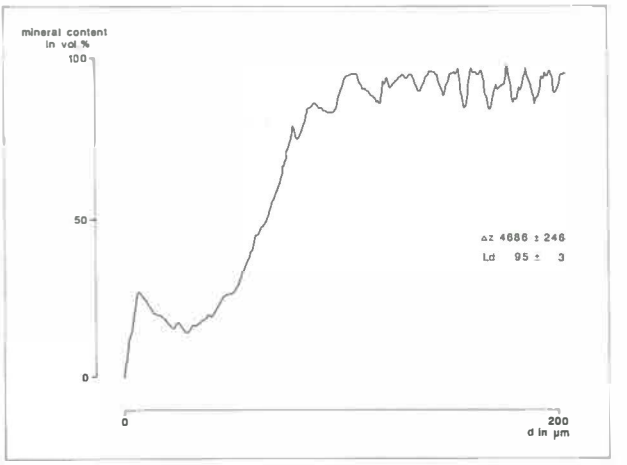


Figure 8.1.
Typical microradiogram of a lesion prior to demineralization.
The average mineral loss ΔZ and lesion depth Ld are calculated from 32 tracings (mean ± SE).

The results (given in Table 8.II.) indicate that the artificial lesions remineralized significantly in the varnished as well as in the control specimens. It is remarkable that no significant difference was observed either between fluoridated and untreated enamel lesions or between the enamel pretreated with the various varnishes. It is reasonable to assume that the lesion is highly reactive to fluoride and that at the beginning of the remineralization large amounts of calcium can react with the fluoride from the varnishes. CaF_2-like material is most likely formed both in the lesion as well as on the surface. This CaF_2 formation obviously has no influence on in vitro remineralization at pH 7.

Table 8.II *Average mineral gain in vol%.µm (mean ± SE), average percentage of remineralization, average lesion depth reduction in µm (mean ± SE) and the average percentage of reduction after remineralization.*

Varnish	Mineral gain		Lesion depth reduction	
	vol%.µm	%	µm	%
FP 0.7%	3009±341	64	45±7	47
FP 0.1%	2124±288	45	40±9	42
FP 0.05%	2207±328	47	33±5	35
control	2579±303	55	33±6	35

Boddé et al. (1985) suggested that high dose fluoride-gel treatments had no positive effect on remineralization because the coating of CaF_2 on the outer surface retarded the inwards diffusion of calcium and phosphate ions. This concept does not explain the present results, because there was no significant difference between varnished or non-varnished specimens.
The fact that the fluoride application had no effect on remineralization may be due to the formation of CaF_2 in the lesion body at pH 7, which does **not effectively** influence the remineralization process. Depletion of fluoride in the lesion, by leakage into the remineralizing solution, is not very likely because fluoride leakage could not be measured in the solution (accuracy) about 0.01 ppm.

Extrapolation of results from in vitro investigations on de- and remineralization to a clinical situation should be interpreted with care. The 'in vivo' environment is more complex, dynamic and the caries process is always a combination of de- and remineralization, alternating several times a day. From the available information one must, however, deduce that CaF_2 formation after varnish application does not stimulate remineralization.

The following section speculates on the long-term effect of fluoride varnish applications on de- and remineralization in vivo.

Fluoride varnishes deliver fluoride on and in enamel in large amounts. The fluoride acquired in enamel is **retained permanently** but **no long-term caries preventive effect** is seen if application is not repeated (Seppä 1984). This has also been observed in some studies where only one Fluor Protector application per year was given and no caries reduction was found (Van Eck et al. 1984; Clark et al. 1985a; Theuns et al. 1985). This can only be explained by the assumption that most of the fluoride is **inactive**. According to Arends and Christoffersen (1986) fluoride is most active when available between the enamel crystallites as unbound fluoride in the liquid phase (Fl). It can be assumed that if CaF_2-like material is formed in lesions (in very insoluble form) it might not be available as Fl.

To obtain "visible" information on the long-term effect of fluoride varnishes enamel samples were also studied by SEM. They were previously treated and demineralized under plaque. The enamel sections which had previously been used for microradiography were fractured and the exposed surfaces were freed of most of the loose material by blowing carefully with air. No chemical treatment was given.

"Globular" mineral deposits around the prisms in the lesion body were observable in Fluor Protector pretreated enamel (Figure 8.2.). Although SEM-micrographs do not provide information about the phase present, it can tentatively be suggest that it is CaF_2 or CaF_2-like material. The Duraphat pretreated and untreated control lesions did not show these globular precipitates (Figures 8.3. and 8.4.). The difference in ultrastructural image could be due to the acidic properties of the polyurethane varnishes, whereas Duraphat is a neutral lacquer.

From the experimental data and the SEM images is is suggested that CaF_2 in the lesion body has no remineralizing effect and does not prevent demineralizaton because the fluoride is inactive. This is a reasonable assumption because the pH ≈ 7 in the lesion body, even under plaque. This would also explain why caries increases further in clinical trials despite the elevated fluoride concentration in the solid unless application is repeated. CaF_2 is, therefore, useful in inhibiting enamel demineralization **before** a lesion is formed (on sound enamel), but seems less important if formed in **existing** lesions.

This suggestion is also confirmed by an additional in vitro investigation using single enamel sections. After varnish application with FP 0.7% lesion formation was followed during demineralization (Figure 8.5.a.). A lesion with a lesion depth of 157 µm was obtained after 40 hours demineralization. The same single-section was **remineralized** (Figure 8.5.b.) in the fluoride-free calcium phosphate solution mentioned above. After 2 weeks of **remineralization** the lesion depth was reduced to 112 µm. The section was **again demineralized**, to see whether the demineralization process was slowed down by the previous remineralization stage (Figure 8.5.c.).
During the remineralization stage a surface layer with a high mineral content (≈ 65%) was formed. This layer did not, however, protect the enamel against renewed acid attack and the lesion depth increased to 196 µm after renewed demineralization for 40 hours. It is striking that no softening was observed during this second demineralization and that the mineral content of the surface layer reached the same level after both D1 and D2 (≈ 40

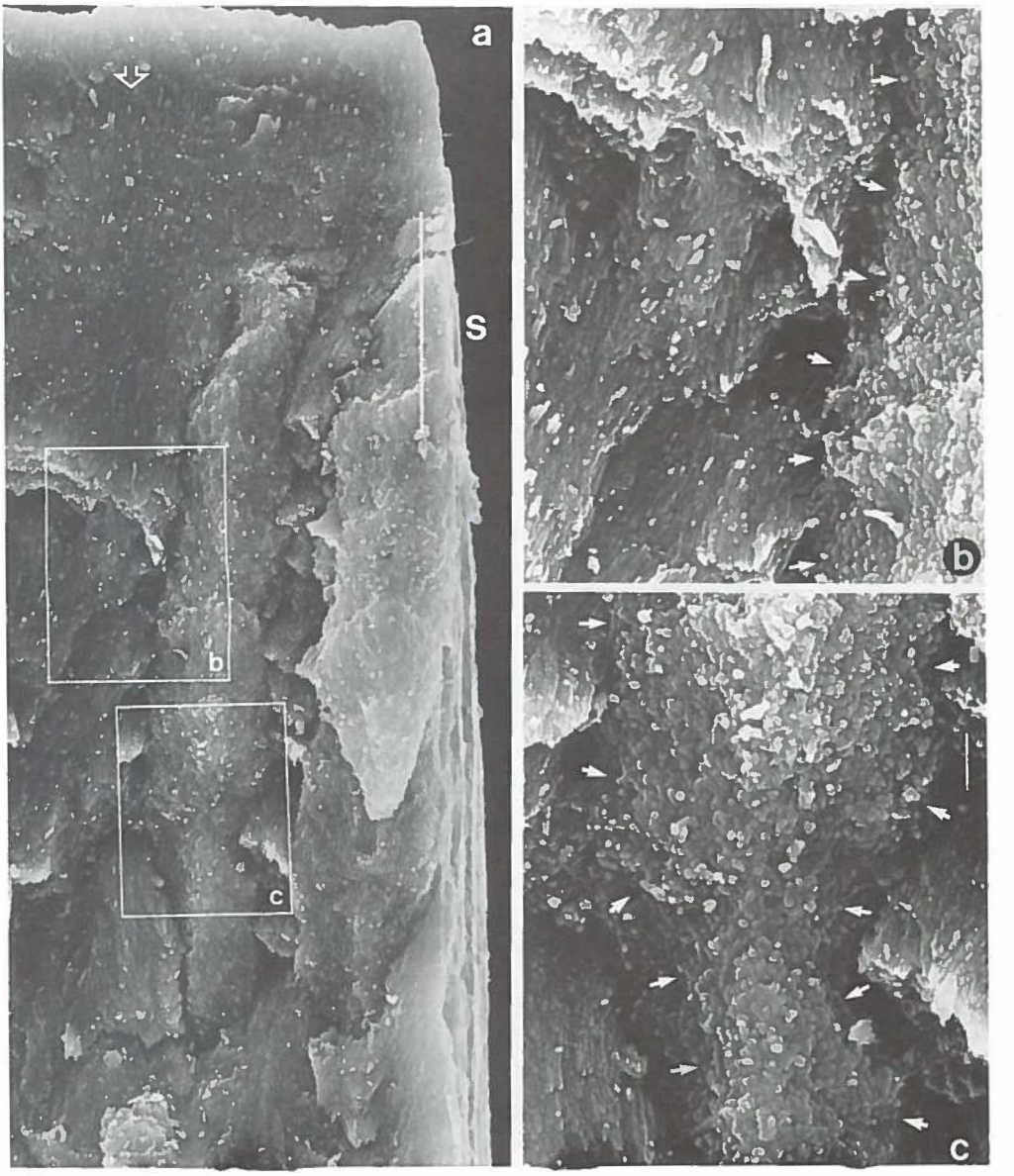


Figure 8.2.
SEM-micrographs of FP 0.1% pretreated enamel after 4 months demineralization.

a. Detail of a broken enamel section (the bar is 10 μm); the cut section surface is indicated by S; the acid attack direction is indicated by the arrow; b and c are enlargements from the white rectangles.
b. Detail of the demineralized area 15-20 μm below the surface (the bar is 1 μm).
c. Detail of the lesion body 20-40 μm below the surface (the bar is 1 μm). The prism zone is covered with mineral globules; some CaF_2-like globules are indicated by arrows.

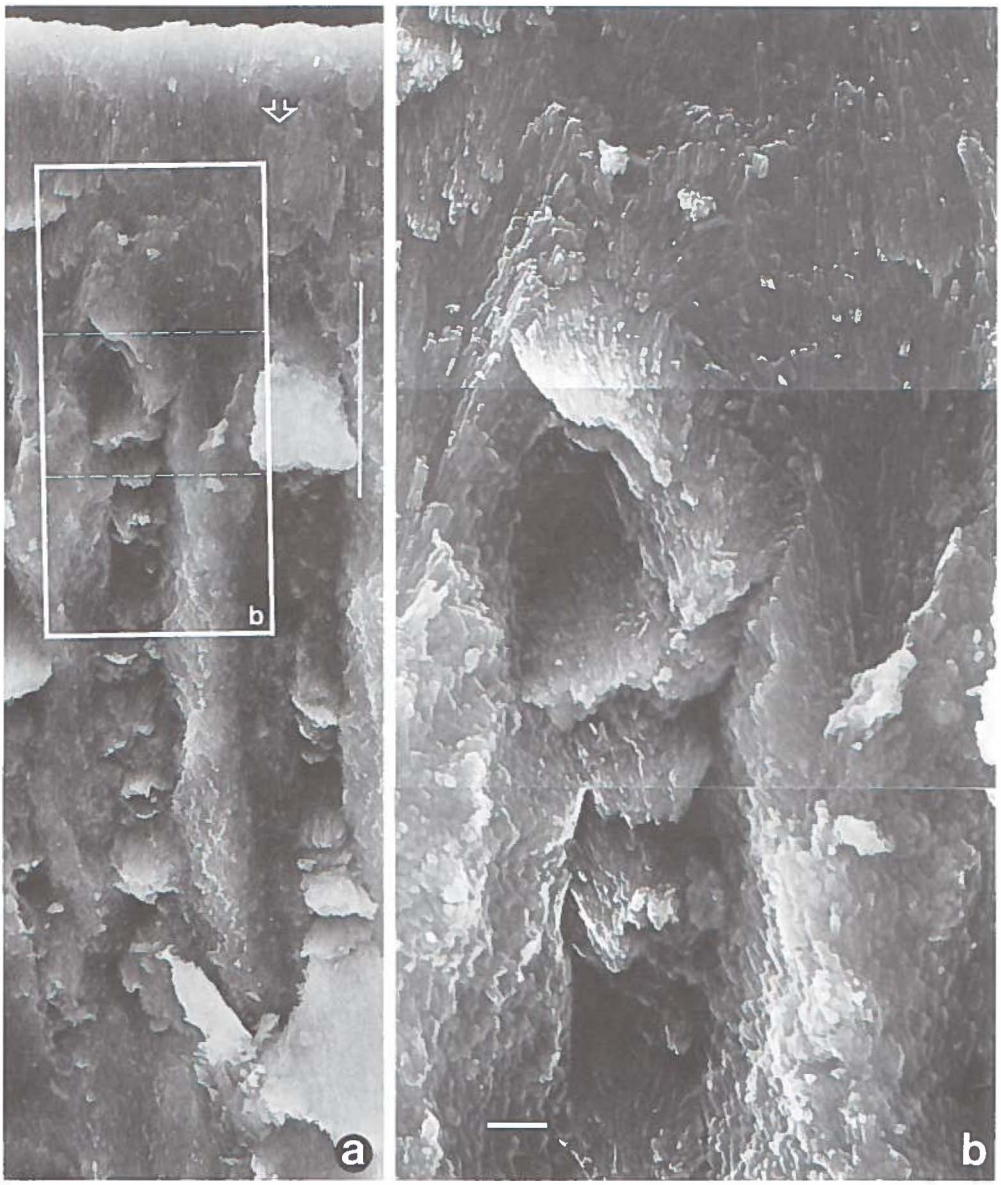
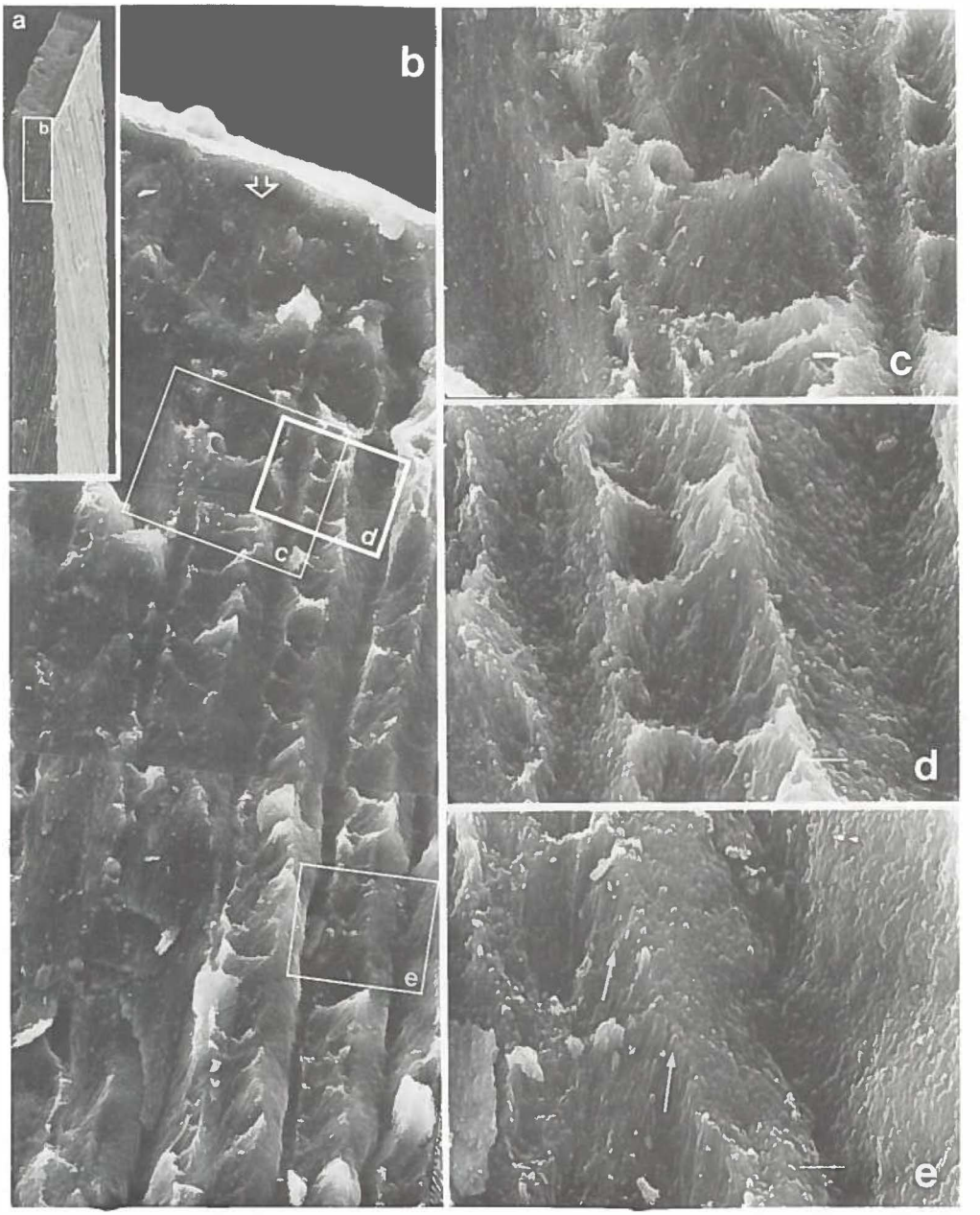
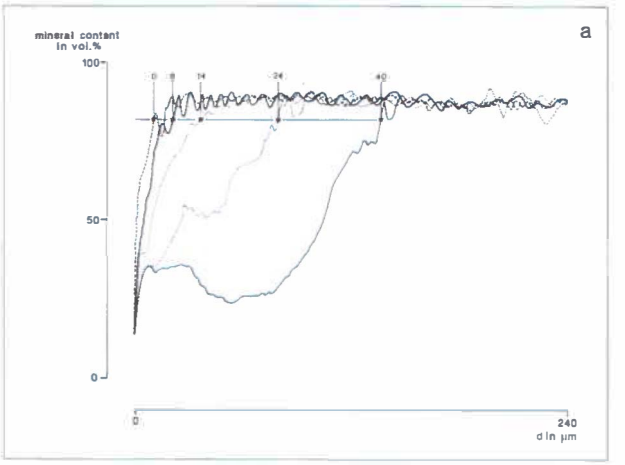


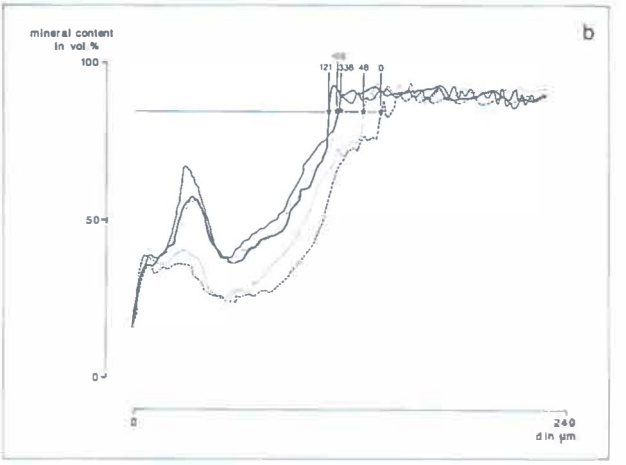
Figure 8.3.
SEM-micrographs of control enamel after 4 months demineralization.
a. Detail of the demineralized area (the bar is 10 μm); the acid attack direction is indicated by the arrow.
b. Detail of the demineralized area 5-30 μm below the surface (the bar is 1 μm); note the absence of the globular structures.

Figure 8.4. →
SEM-micrographs of Duraphat pretreated enamel after 4 months demineralization.
a. Fractured enamel section of ± 65 μm.



b. Detail of the demineralized area (the bar is 10 μm); the acid attack direction is indicated by the arrow.
c. Detail of the demineralized area 20-40 μm below the surface and covering about one prism, indicating by a dotted line (the bar is 1 μm).
d. Detail of the prism (the bar is 1 μm).
e. Detail of the lesion body 60 μm below the surface. The densely packed crystallites are indicated by arrows (the bar is 1 μm).





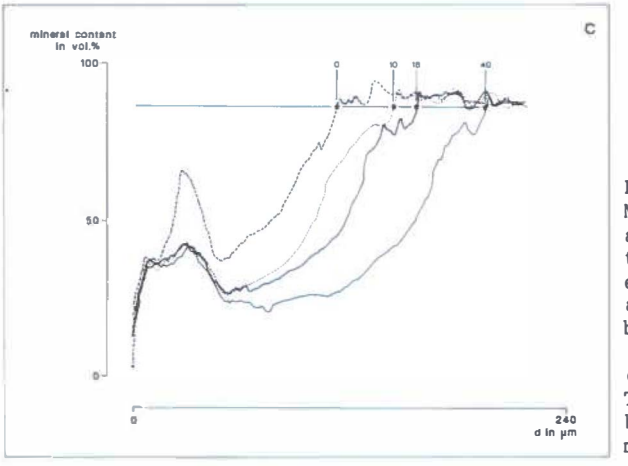


Figure 8.5.
Mineral distribution changes of a single-section of enamel pretreated with FP 0.7% and followed on the same area during:
a. in vitro demineralization;
b. consecutive remineralization and
c. renewed demineralization.
The lesion depths are indicated by an asterisk and the experimental period is given in hours.

vol%). This indicates that the surface is more or less protected by the fluoride treatment.
It can be concluded from these single-section experiments that the enamel is **not** better protected against further demineralization after remineralization of enamel pretreated with Fluor Protector. This means that the fluoride available in the enamel lesion was neither **incorporated in the enamel** mineral as Fs nor effective as Fl.

In some clinical trials, the leakage effect of fluoride from fluoridated surfaces into the oral cavity has been suggested as an important feature in the long-time prevention of enamel decay. The experiments described in this thesis do not support this hypothesis.

Summarizing:

Fluoride varnishes induce a substantial fluoride uptake.
The caries preventive action of this fluoride is most likely related to the CaF_2 formation **on** the outer enamel surface. The varnishes are effective in slowing down enamel demineralization but no long-term effect should be expected unless they are re-applied. This can be explained by the fact that the protective layer on the outer enamel surface is leaching away in time. CaF_2 can be formed in the lesion during demineralization and the acquired fluoride is under these conditions no longer available for the inhibition of demineralization or the enhancement of remineralization.
The use of varnishes with relatively low fluoride contents (0.1-0.05 wt%) seems to have the same effect on demineralization inhibition as well as remineralization.
Finally, it can be concluded that the polyurethane varnishes are effective fluoridating agents in the prevention of enamel decay.

summary 9

Topical fluoride applications have the aim of increasing the fluoride uptake in enamel and consequently reducing caries. In the early 1960s fluoride varnishes were introduced because they had a long contact period with the enamel which resulted in a higher fluoride uptake than from other topical applications. Recently the importance of small amounts of fluoride in caries prevention and remineralization has been stressed and the importance of large amounts of fluoride acquired in the teeth is being seriously questioned.

The aim of this thesis (Chapter 1), was to investigate the effect of fluoride varnishes with a relatively low fluoride content on fluoride uptake, on protection against mineral loss and on remineralization of enamel. The fluoride varnishes were polyurethane based (Fluor Protector, Vivadent, Schaan Liechtenstein; abbreviated FP) and contained 0.7%, 0.1%, 0.05% and 0% fluoride by weight.

Chapter 2 gives a survey of the literature on the cariostatic action of fluoride. The effect of fluoride on enamel solubility, de- en remineralization and plaque metabolism is discussed.
For many years it was believed that fluoride incorporated in the enamel was the main reason for caries reduction. Incorporation of fluoride in the hydroxyapatite mineral leads to less soluble enamel in laboratory investigations. There is, however, in vivo no clear relationship between the fluoride content in enamel and the prevalence of caries. Small amounts of fluoride in the "liquid phase" (between the crystallites) are more important in decreasing the kinetic enamel dissolution than fluoride incorporated in the solid crystallites and they enhance remineralization. This liquid fluoride is supplied continuously by fluoride in saliva, plaque and topically applied agents. Topical fluoride applications, furthermore, provide a "CaF_2-like" coating on the outer enamel surface, which provides a fluoride releasing depot.
In laboratory investigations the effect of fluoride on plaque metabolism has been shown. No indisputable evidence has been presented showing that fluoride directly influences plaque at normal in vivo levels.

An extensive review on fluoride varnishes is presented in Chapter 3. It is clear from the literature that varnishes supply fluoride more effectively than other topical agents. In laboratory investigations and in animal studies, fluoride varnishes have proved to have good demineralization inhibiting properties. Many clinical trials have been carried out especially on Duraphat (Woelm Pharma, Eschewege, FRG) and Fluor Protector. Caries reductions of 18-56% have been reported for Duraphat when applied to permanent teeth. Inconclusive results have been reported concerning the primary dentition. Fluor Protector, on the other hand, has been investigated to a lesser extend and of the papers which have been published it is difficult to draw overall conclusions due to problems of study design.
As well as possessing caries preventive properties the fluoride varnishes are known to be toxicologically safe, convenient and easy to use.

In **Chapter** 4 the fluoride acquisition on and in enamel after a single application of polyurethane varnishes is discussed. The fluoride uptake was measured immediately after the removal of the varnishes in vitro and after one week in vivo. Eleven participants wore a newly developed intra-oral device based on a frame prosthesis in which 5 enamel specimens were held. This method is flexible and allows for experimentation as close as possible to the in vivo situation.
Alkali-soluble fluoride on the enamel surface (F [on]) was measured after KOH extraction; fluoride in enamel (F [in]) was determined by acid etching 5 thin enamel layers.
The results show that the amounts on fluoride present **after application** and **after one week** are strongly dependent on the fluoride content in the varnish.
The fluoride uptake ranks after 24 hours for F [on] and F [in]:

FP 0.7% > FP 0.1% ≈ FP 0.05%.

In vivo the fluoride uptake ranking was:

FP 0.7% > FP 0.1% ≈ FP 0.05% ≈ FP 0% ≈ control.

The large biological variations in the enamel and complexity of the oral environment are the reasons that no differences were measurable between the unfluoridated conditions and the low fluoride varnish concentrations.
A comparison of the 24 hours and one week data revealed that fluoride loss in one week was negligible.
No significant correlation between fluoride concentration in the tooth **prior** to application and fluoride uptake in enamel could be found.

The inhibiting effect of a 24 hours application of a polyurethane varnish with differing fluoride concentrations on demineralization of human sound enamel was evaluated in vitro and is described in **Chapter 5 and 6**. A constant composition technique was used to demineralize fluoride varnished and non-varnished specimens. Microhardness measurements were carried out at different time intervals in order to follow changes in demineralization. Microradiography was carried out at the end of each experimental run to evaluate lesion type, lesion depth and mineral loss.

In **Chapter** 5 the results of demineralization at pH 5 are presented. It is shown that the fluoride varnishes applied for 24 hours on the enamel can inhibit demineralization completely. No demineralization inhibition with the 0% varnish was observed. It was calculated that the inhibiting effect was not due to fluoride leakage into the demineralization solution.

In **Chapter 6** the effect of the fluoride varnishes on demineralization at pH 4.5 was investigated under the same experimental conditions. Additionally, a single-section microradiography technique was used to follow lesion formation at the same area during demineralization. It was concluded that with increasing fluoride content in the varnishes, the enamel surface was better protected against severe acid attack. The ranking in demineralization protection was:

FP 0.7% > FP 0.1% > FP 0.05% > FP 0%.

It could be seen from the single-section microradiographic tracings that subsurface lesion formation was preceded by a surface softening mineral

distribution. Additional information from the SEM indicated that demineralization was slowed down by an increase in fluoride content in the varnishes. Unfluoridated enamel was etched substantially in interprismatic regions.

The caries preventive effects of the polyurethane based varnishes and Duraphat were studied in a clinical caries model (Chapter 7). The plaque accumulation on the enamel promoted a substantial cariogenic challenge.
An unfluoridated varnish was chosen as a control. The varnish application period was 24 hours, after which the varnishes were removed. Fifteen participants, devided into two experimental groups, wore varnished human enamel slabs in a frame appliance for 2, 4 and 6 months. At the end of that period microradiography was carried out on all samples and mineral loss, lesion depth and mineral distribution of the demineralized enamel was measured.
The results of this study showed that in vivo there were no difference in protective effect between FP 0.7%, FP 0.1% or FP 0.05% after 4 or 6 months. After 4 months all 3 polyurethane varnishes showed better protective properties than Duraphat. No difference was found between Duraphat and the unfluoridated control. The effect of all the fluoride varnishes was comparable after 6 months and although trends could be indicated a preventive effect was no longer statistically observable.

In Chapter 8 the mechanism of the fluoride varnish interaction with enamel is discussed.
The formation of CaF_2-like material on the outer enamel surface after fluoride varnish application is most likely the main parameter in the inhibition of demineralization. CaF_2-like material has a low acid solubility and can resist a moderate acid attack. At lower pH (e.g. 4.5) demineralization is substantially retarded and the enamel dissolution rate is influenced directly by the amount of CaF_2 formed on the surface. CaF_2 has also has a "depot" function and the slow fluoride release affects further fluoride uptake in enamel and as a consequence influences de- and remineralization processes.

The effect of fluoride varnishes with ranging fluoride contents on remineralization of initial caries lesions was studied in a pilot study.
The fluoride varnishes do not enhance or retard remineralization, as there was no difference between fluoridated and control enamel.
An important conclusion is that CaF_2 formation in the lesion body does not influence de- or remineralization because the fluoride is lost. This is confirmed by the SEM-micrographs of in vivo demineralized enamel.
Once CaF_2 is formed in the lesion, fluoride is obviously inactivated and is not dissolved easily. This conclusion is in agreement with the results of clinical trials. Fluoride is retained permanently in enamel after fluoride varnish application but no long lasting caries preventive effect is seen unless application is repeated.

The major effect of fluoride varnishes is on sound enamel in preventing a caries attack.

Het direct aanbrengen van fluoriden op het glazuur van gebitselementen heeft tot doel het fluoride-gehalte in het glazuur te verhogen en op die manier het glazuur minder "vatbaar" voor cariës te maken.
In dit kader zijn in de zestiger jaren zogenaamde fluoride bevattende lakken ontwikkeld. Deze lakken bevatten in tegenstelling tot andere fluoride bevattende middelen zoals tandpasta's, gels, spoelmiddelen een hoog gehalte aan fluoriden. Bovendien blijven zij aan de gebitselementen kleven en zijn zij zodoende veel langer dan de andere fluoride bevattende middelen in contact met het glazuur. De tijd waarin fluoriden in het glazuur kunnen diffunderen is derhalve aanzienlijk verlengd.
De vraag op welke manier de fluoride aanwezig in het glazuur van het gebitselement dit element minder gevoelig voor cariës maakt en welke concentratie fluoride daar nu precies voor nodig is, klinkt de laatste tijd steeds luider. Er zijn aanwijzingen dat met name een minimale hoeveelheid fluoride die zich tussen de glazuurkristallieten bevindt van meer belang is voor het voorkómen van demineralisatie en het bevorderen van remineralisatie dan fluoride aanwezig in het glazuur zelf.

In **Hoofdstuk** 1 worden de doelstellingen van het onderzoek beschreven. Onderzocht werd het effect van lakken met een lage fluoride-concentratie op de fluoride-opname door glazuur en op de de- en remineralisatie van het tandglazuur. Deze fluoride bevattende lakken waren allen afgeleid van Fluor Protector (Vivadent, Schaan Liechtenstein), een lak op basis van polyurethaan en bevatten respectievelijk 0.7, 0.1, 0.05 en 0 gewichtsprocent fluoride.

Hoofdstuk 2 geeft een kort overzicht van de literatuur betreffende de preventieve werking van fluoride op het cariësproces.
De inbouw van fluoride **in** het glazuur werd lange tijd beschouwd als het belangrijkste werkingsmechanisme. Laboratoriumstudies toonden immers aan dat inbouw van fluoride-ionen in hydroxyapatietkristallen de oplosbaarheid van het mineraal vermindert. In het mondmilieu is echter geen duidelijke relatie aangetoond tussen de fluoride-concentratie in het glazuur en bescherming ervan tegen cariës. Fluoride aanwezig **in de vloeistof tussen de glazuurkristallieten** is belangrijker dan fluoride ingebouwd in het kristal. Dit fluoride vermindert de demineralisatie en bevordert de remineralisatie van het glazuur. Het fluoride wordt aangeleverd vanuit de plaque, het speeksel en door **lokale** fluoride-applicaties. Deze laatsten zorgen voor een neerslag van CaF_2-achtig materiaal op de gebitselementen. Deze neerslag kan als fluoride-reservoir dienst doen.
In laboratoriumstudies is het effect van fluoride op plaquemetabolisme aangetoond. Klinisch is evenwel nog nooit aangetoond dat fluoride onder normale orale omstandigheden het plaquemetabolisme zou beïnvloeden.

In **Hoofdstuk** 3 wordt een overzicht gegeven van onderzoek over het effect van de fluoride bevattende lakken: Fluor Protector en Duraphat (Woelm Pharma, Eschewege, FRG). Uit de literatuurgegevens blijkt dat bij het applice-ren van deze lakken meer fluoride in het glazuur wordt opgenomen dan bij andere lokale fluoridemiddelen. Dit fenomeen wordt toegeschreven aan de lange contacttijd van de lakken met het glazuur.

Zowel uit dierproeven als uit laboratoriumstudies blijkt dat fluoride bevattende lakken de demineralisatie remmen. Op grond van deze gegevens werd klinisch onderzoek naar het cariëspreventieve effect van de lakken uitgevoerd. Voor Duraphat worden cariësreducties van 18 tot 56% gerapporteerd bij applicatie op het blijvend gebit. De beschermende werking is minder duidelijk op het melkgebit. Het effect van Fluor Protector als cariësremmer is minder vaak onderzocht.
In de meeste studies wordt een lagere effectiviteit gevonden in vergelijking met Duraphat. Deze studies bevatten een reeks ongecontroleerde factoren die de meetresultaten mogelijk hebben beïnvloed. Het is daarom moeilijk uit de tegenstrijdige gegevens eenduidige conclusies te trekken.
Algemeen is aanvaard dat fluoride bevattende lakken cariësremmend werken. Bovendien zijn ze toxicologisch veilig en gemakkelijk toe te passen. Er zijn ook aanwijzingen dat deze lakken bij de behandeling van dentinegevoeligheid kunnen worden gebruikt.

In **Hoofdstuk 4** is de fluoride-afgifte **op** en **in** het glazuur (F [on], F [in]) beschreven. Fluor Protectorlakken werden daartoe gedurende 24 uur (in vitro) op het glazuur aangebracht. De fluoride-opname werd gemeten onmiddellijk na verwijdering van de lak en na één week (in vivo). Elf patienten droegen voor dit doel een frameprothese waarin 5 glazuurblokjes waren aangebracht. Vier blokjes waren met de polyurethaanlakken behandeld, één blokje diende als controle. Voor het reinigen van de natuurlijke dentitie gebruikten de proefpersonen tijdens het experiment een ongefluorideerde tandpasta.
Het op het glazuuroppervlak na applicatie neergeslagen alkali-oplosbaar fluoride (F [on]) werd gemeten via KOH extractie. Het fluoride-gehalte in het glazuur (F [in]) werd bepaald door 5 dunne lagen glazuur af te etsen.
De resultaten van dit experiment tonen aan dat de fluoride-opname onmiddellijk na applicatie en na één week sterk afhangen van de fluoride-concentratie in de lak.
Na 24 uur is voor F [on] en F [in]:

FP 0.7% > FP 0.1% ≈ FP 0.05%.

Na één week in het mondmilieu is de opname:

FP 0.7% > FP 0.1% ≈ FP 0.05% ≈ FP 0% ≈ Controle.

De klinische resultaten worden beïnvloed door de spreiding in samenstelling van het glazuur en de complexiteit van het mondmilieu.
Een vergelijking tussen de gegevens na 24 uur en een week toont aan dat het verlies van fluoride te verwaarlozen is. De fluoride-opname werd in deze studie niet beïnvloed door de oorspronkelijke fluoride-concentratie in het glazuur.

Het effect van polyurethaanlakken met verschillende fluoride-concentraties op in vitro demineralisatie van gezond glazuur wordt beschreven in **Hoofdstuk 5 en 6**. Gefluorideerd en ongefluorideerd glazuur werd gedemineraliseerd onder voor demineralisatie constante omstandigheden. Tijdens het experiment werden hardheidsmetingen uitgevoerd om het demineralisatieproces te kunnen volgen. Met behulp van microradiografie werd aan het einde van het experiment informatie verkregen over lesietype, lesiediepte en mineraalverlies.

Een voorbehandeling van glazuur gedurende 24 uur met fluoride bevattende lakken van verschillende concentraties (FP 0.7%, FP 0.1%, FP 0.05%) geeft een volledige bescherming tegen demineralisatie bij pH 5. Er is geen verschil tussen de verschillende concentraties. Een 0% fluoride bevattende lak geeft geen bescherming en is in deze niet verschillend van de controle.

In **Hoofdstuk 6** werd het beschermend effect van de fluoride bevattende lakken op glazuur gemeten bij pH 4.5. De demineralisatie werd behalve met transversale microradiografie ook bepaald op enkelvoudige glazuurcoupes ("single-section"). Hierdoor was het mogelijk het demineralisatieproces op een zelfde plaats in de tijd te blijven volgen. Bij een extreme zuuraanval blijkt de bescherming afhankelijk te zijn van de fluoride-concentratie in de lak. Het onbehandelde glazuur werd geëtst. Scanning electronen microscopisch onderzoek bevestigde dit. Microradiografie van de enkelvoudige coupes liet zien dat het demineralisatieproces als een verweking van het oppervlak ("surface softening") begint en dat er pas later een lesie met een oppervlaktelaag wordt gevormd.

De cariëspreventieve eigenschappen van de polyurethaanlakken met verschillende fluoride-concentraties zijn klinisch onderzocht en vergeleken met de resultaten verkregen met Duraphat (**Hoofdstuk 7**). Vijftien patienten droegen daartoe een frameprothese waarin 4 gefluorideerde en 1 onbehandeld schijfje glazuur was aangebracht. De schijfjes glazuur waren tijdens het 2, 4 of 6 maanden durende experiment bedekt met plaque, dit om een cariogeen milieu na te bootsten. Tijdens de duur van het experiment gebruikten de proefpersonen een fluoridevrije tandpasta.
Aan het einde van elke periode werden mineraalverlies, lesiediepte en mineraal verdeling van de lesie gemeten met behulp van microradiografie.
Er bleek op geen enkel tijdstip een verschil in bescherming tussen de verschillende fluoride bevattende polyurethaanlakken tegen een cariësaanval. Echter na 4 maanden bieden de lakken op polyurethaanbasis een betere bescherming dan Duraphat. Na 6 maanden is er geen verschil meer tussen met Fluor Protector/Duraphat behandeld en onbehandeld glazuur. Dit geeft duidelijk aan dat de fluoride bevattende lakken regelmatig opnieuw aangebracht moeten worden om een effectieve bescherming te geven.
Duraphat is bij sterk cariogene omstandigheden minder werkzaam dan Fluor Protector. De concentratie fluoride in deze laatste kan worden verminderd.

In **Hoofdstuk 8** wordt het werkingsmechanisme van de fluoride bevattende lakken op glazuur besproken. De neerslag van CaF_2-achtig materiaal op het glazuur oppervlak na applicatie van fluoride bevattende lakken is de belangrijkste beschermende factor. Deze neerslag heeft een afdichtend effect waardoor de diffusie wordt gehinderd. CaF_2 heeft een laag oplosbaarheidsproduct en maakt het glazuur minder gevoelig voor een zuuraanval. Tenslotte kan fluoride uit de CaF_2 laag diffunderen in de vloeistof tussen de glazuurkristallieten en op die manier de- en remineralisatie beïnvloeden.

Het effect van fluoride bevattende lakken op glazuurremineralisatie is in vitro onderzocht. Er werd geen verschil gemeten tussen gefluorideerd en onbehandeld glazuur. De conclusies zijn dat het appliceren van fluoride bevattende lakken geen invloed heeft op het remineralisatie-proces. Dit kan worden verklaard door de vorming van CaF_2 in de lesie. Dit fluoride is dan niet meer beschikbaar in de vloeistof tussen de glazuurkristallieten en kan derhalve de remineralisatie niet bevorderen.

De voor de algemeen practicus van belang zijnde conclusies van dit onderzoek zijn:

- fluoride bevattende lakken aangebracht op gezond glazuur hebben een cariëspreventieve werking;
- applicatie van fluoride bevattende lakken moet regelmatig worden herhaald om een optimale werking te verkrijgen;
- de natuurlijke remineralisatie wordt niet beïnvloed na applicatie van de fluoride bevattende lakken;
- onder sterk cariogene omstandigheden biedt Duraphat minder bescherming dan Fluor Protector.

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curriculum vitae

Hugo De Bruyn werd op 16 april 1960 geboren te Ninove, België. Hij volgde de klassieke humaniora, afdeling Latijn-Wiskunde aan het Heilig Kruiscollege te Denderleeuw. Van 1978 tot 1983 studeerde hij aan de Katholieke Universiteit te Leuven, waar hij in juni 1983 het Licentiaatsdiploma in de Tandheelkunde behaalde met grote onderscheiding.
Vanaf augustus 1983 is hij in dienst als wetenschappelijk assistent bij de vakgroep Parodontologie, Prothetodontie en Sosiodontie van de Rijksuniversiteit te Groningen. In deze periode werd onder leiding van Prof. Dr. J. Arends onderzoek verricht naar de effecten van fluoride houdende lakken op glazuurcariës.
Na zijn promotie zal hij een verdere opleiding tot parodontoloog volgen aan het Postgraduate Center for Dentistry te Malmö, Zweden.