

## FLUORIDE UPTAKE IN VIVO BY ENAMEL WITH "FLUOCARIL BIFLUORE 250" AND "COLGATE"

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### INTRODUCTION

It has been demonstrated that one of the major factors against caries attack is the fluoride content of surface enamel.<sup>4)</sup>

Mühlemann suggested that a level of approximately 1000 ppm F (in 30  $\mu\text{m}$ ) be necessary for cariostatic action.<sup>11)</sup> Therefore, the fluoride released from F-containing toothpaste must be fixed to the enamel and incorporated into the enamel apatite in a stable form (Fluoroapatite) rather than in a labile fluoride reservoir ( $\text{CaF}_2$ ). The mechanism of F incorporation into the surface enamel is influenced by F concentration in a toothpaste. In addition, F uptake is probably affected by the F-compound employed and general composition of a toothpaste.

Sodium monofluorophosphate (MFP) is currently thought to be one of the better form of fluoride for use as a cariostatic agent in toothpaste.<sup>5)</sup> MFP is less susceptible to inactivation by dentifrice abrasives than NaF and it does not stain the enamel as does stannous fluoride. The caries reducing effect of MFP is suggested to be due to F released by hydrolysis rather than MFP itself which is probably not the active agent in these toothpastes.<sup>7,13)</sup>

A new bifluoride toothpaste which combines MFP with NaF to obtain a more efficient source of F has been recently developed.

The purpose of present investigation is to quantitatively compare the F-uptake and retention of outermost enamel layer (12-15  $\mu\text{m}$ ) in situ after brushing with Fluocaril 250 and Colgate and to evaluate their comparative effectiveness.

The F content in surface enamel will be determined by the enamel biopsy technique recently developed by Hotz et al.<sup>9)</sup>

### MATERIALS AND METHODS

#### \* Patient Selection

51 dental students were selected for study according to the following criteria:

- 1) Each subject must have 2 upper central incisors and has not had a topical fluoride treatment within the previous 6 months.
- 2) Incisors must be free from caries, restorations and enamel defects on labial surface.
- 3) The subjects have not been served by fluoridated water. This experimentation consisted of two phases.

- 1) 1st phase: All the students used toothpaste without fluoride for a month in order to eliminate fluoride on enamel. After this period, a control of enamel fluoride content will be effected to select the subjects with no more than 500 ppm F on the enamel surface.
- 2) 2nd phase; The subjects were divided into 3 groups; One group continued to use the toothpaste without fluoride, the other group used Fluocaril 250, the third group used Colgate 1450.

Table I. Data relating to toothpastes used.

Group	No. of Subjects	Toothpastes	F-compounds	Fluoride concentration	Remarks
A	17	Control	none	0 ppm	France
B	17	Fluocaril	MFP & NaF	2500 ppm	France
C	17	Colgate	MFP & NaF	1450 ppm	U.S.A.

Each students were instructed to brush for 3 min. twice a day. 3 areas on the labial surface of incisors were biopsied as indicated in Fig. 1. Enamel biopsies were performed immediately before (A), 4 weeks (B), and 8 weeks (C) after brushing with different toothpastes.

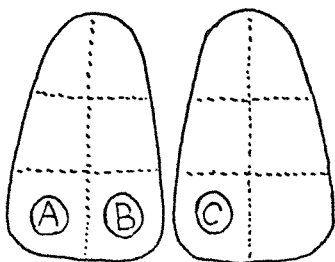


Figure 1. Distribution of sampling areas on the tooth surface.

\* Method of Enamel Biopsy<sup>9)</sup>\*

The enamel to be biopsied was isolated with cotton rolls, cleaned with a slowly-rotating rubber cup without pumice, and dried with compressed air. A round disc (3mm in diameter) stamped from scotch tape was pasted onto the enamel to be biopsied, and after that, the entire tooth surface and scotch tape disc were painted with Copalite.\*

10 ul demineralizing solution (2N perchloric acid) was pipetted onto the filter paper disc (1 mm in thickness and 3mm in diameter), which was held with the vacuum pipette.

The scotch tape disc on the prepared tooth was removed and the drenched filter paper disc was put on the Copalite-free area. A slight, intermittent pressure with the vacuum pipette on the filter paper disc caused an agitation of the demineralizing solution. Exposure time of the tooth

\* Copalite: Bosworth Company, U.S.A.

surface to perchloric acid was 8-9 sec. Afterwards the filter paper disc was removed from the tooth surface, and perchloric acid residue was absorbed immediately with a second filter paper disc. Both discs were transferred into a plastic tube containing 2ml of dilutant (5% TISAB II: pH 5.1), and vigorously shaken.

Fluoride in solution was measured with the fluoride ion specific electrode and calcium was determined with the atomic absorption spectrophotometer.

Assuming that enamel contains 37% calcium and that the density of enamel is 2.95, the amount of enamel removed and the depth of sampling are determined knowing the enamel surface area to be constant. The thickness of the etched layer is estimated from the following formula:

$$D = \frac{W \times 1000}{S \times 2.95}$$

D : Depth (um)

W : Weight of each enamel sample (mg)

S : Sampled surface (um<sup>2</sup>)

The enamel is usually rough and dull after demineralization but with polishing and topical fluoridation, no esthetic problem remains.

## RESULTS

The difference of fluoride uptake in control group was not demonstrated by Student t-test during experimental period (8 weeks) (Table II).

The findings from two different toothpastes were indicated in Table III and IV. Measurement of enamel fluoride after 4 weeks treatment showed marked increase of fluoride uptake rate of 226.9% in Fluocaril group as compared to a mean increase of 146.3% in Colgate group (Fig. II)

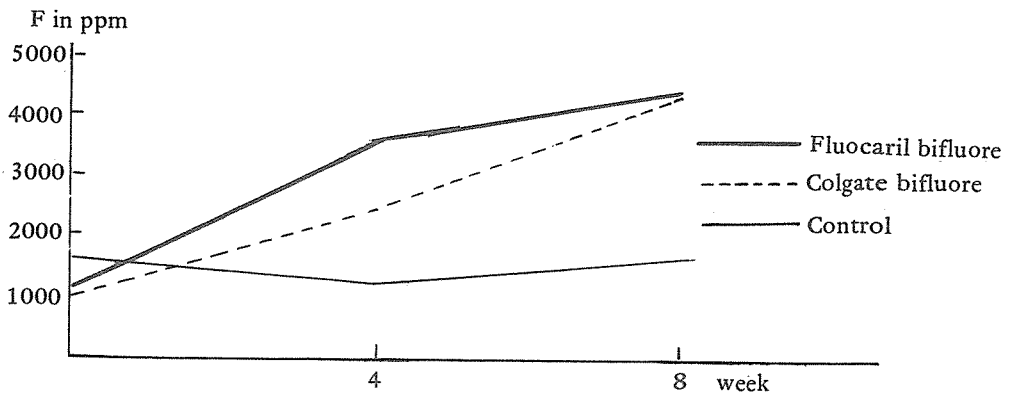


Figure II. Fluoride concentration in outer enamel after toothbrushing with fluoride containing toothpastes.

These findings showed that Fluocaril toothpastes had much higher F uptake rate than Colgate toothpastes especially in the early period. However, the surface enamel fluoride concentration was similar in Fluocaril and Colgate toothpastes in 8 weeks. The difference between Fluocaril and Colgate groups in 4 weeks was statistically significant ( $P < 0.01$ ), but insignificant in 8 weeks.

The mean surface enamel fluoride concentration was similar in Fluocaril and Colgate toothpaste groups after a month wash-out period. However, the mean surface enamel fluoride concentration of the control group was different from those of the other two groups after a month wash-out period.

Table II. The mean surface enamel fluoride concentration after toothbrushing with control toothpaste (N=17)

Weeks	Enamel Weight±S.E. (mg)	Depth±S.E. (um)	ppm F±S.E.	Uptake rat (%)
Before	0.242±0.035	11.606±1.679	*1654.80±217.40	
4	0.260±0.021	12.475±1.008	*1227.20±456.20	(-) 25.2
8	0.252±0.014	12.091±0.672	*1611.60±133.00	(-) 2.6

\* P>0.01

Table III. The mean surface enamel fluoride concentration after toothbrushing with Colgate toothpaste(N=17)

Weeks	Enamel Weight±S.E. (mg)	Depth±S.E. (um)	ppm F±S.E.	Uptake rate (%)
Before	0.242±0.018	11.611±0.864	*1094.20± 71.67	
4	0.209±0.023	10.028±1.104	*2448.80±391.04	+146.3
8	0.201±0.009	9.644±4.318	*4303.60±151.50	+332.9

\* P<0.01

Table IV. The mean surface enamel fluoride concentration after toothbrushing with Fluocaril toothpaste (N=17)

Weeks	Enamel Weight±S.E. (mg)	Depth±S.E. (um)	ppm F±S.E.	Uptake rate (%)
Before	0.207±0.015	9.949±0.720	*1103.00±112.13	
4	0.208±0.029	9.932±1.391	*3606.60±308.60	+266.9
8	0.212±0.011	10.172±0.528	*4401.20±136.70	+299.0

\* P<0.01

Table V. Difference in fluoride uptake between two groups (Fluocaril and Colgate)

Weeks	t-value	significance
Before	0.27	P>0.01
4	3.56	p<0.01
8	0.71	p>0.01

## DISCUSSION

### 1. TECHNIC OF THE STUDY

Comparisons of F levels between groups have usually been made on the basis of the group mean F concentrations of enamel samples. Since there is a F-gradient in enamel,<sup>12,16)</sup> such comparisons would be strictly valid only if all the samples were taken to a uniform depth. Therefore, the tooth surface was exposed to perchloric acid for 8-9 seconds. In 1971, Aasenden and Moreno<sup>1)</sup> concluded that, with the biopsy method used, only central incisors can definitely be used interchangeably as matched pairs in comparative studies. In this study, by standardizing the sampling areas (3mm diameter), amounts of the F-uptake before and after brushing with F-containing dentifrices could be compared.

### 2. MFP EFFECT

The fluoride uptake by enamel from MFP is related to different explanations:

Pearce<sup>13)</sup> found that uptake of F by enamel was related more to fluoride concentration of the dentifrices than the MFP concentrations and that other factors such as the binder were also involved. He also observed that fluoride arose in part from a slow hydrolysis of MFP in the tube after manufacture. Analysis of commercial MFP used has shown that the fluoride is also incorporated with the MFP initially.<sup>7,15)</sup> Grön showed that the uptake of F from MFP dentifrice

- 1) the fluoride amount included as an impurity during manufacture.
- 2) the subsequent rates of addition by hydrolysis of MFP
- 3) removal by reaction with the abrasive,
- 4) pH

The complex monofluorophosphate anions  $(PO_3F)^{2-}$  contain F in a covalently bound form and therefore its reaction with hydroxyapatite might be expected to differ from that of the fluoride anion,  $F^{-2}$

Ericsson (1963)<sup>5)</sup> concluded that the uptake reaction involved exchange of MFP solution with apatite orthophosphate followed by an intracrystalline transposition according to the scheme  $(PO_3F)^{2-} + OH \longrightarrow (PO_4)^{3-} + H^+ + F^-$ : the released F replacing the OH to form fluorapatite.

But Ingram<sup>10)</sup> has proposed that hydroxyapatite takes up the MFP anion from solution and that it remains intact in the apatite lattice, exchanging for the  $HPO_4$  groups which occur in calcium deficient apatites.

Ericsson<sup>6)</sup> later considered the possibility that  $F^-$  may be released from MFP in the mouth by enzymatic hydrolysis (the action of acid phosphatase).

Grön et al.<sup>7)</sup> reported that a catalytic splitting of MFP by the hydroxyapatite crystal as well as an enzymatic action occurs. No mechanism for this catalysis was suggested but it could be possible due to calcium ions.

### 3. NaF-MFP-EFFECT

When the same F compound (MFP)-containing dentifrices were employed, bifluoride (MFP with NaF) dentifrice had superiorly F uptake as compared to other MFP-containing dentifrice in foregoing study.<sup>14)</sup> These results were probably due to the difference of F concentration of the dentifrices rather than MFP concentration, although other various factors seemed to be involved. And, in the same bifluoride dentifrices, the higher the fluoride content in bifluoride dentifrices, the more would be the fluoride uptake on surface enamel as shown in this investigation.

High F uptake in bifluoride dentifrice might be attributed to added sodium fluoride. Pearce and More<sup>13)</sup> reported that when the four MFP-containing dentifrices were equalized with respect to fluoride concentration by addition of NaF, uptake become more uniform.

### 4. OTHER ELEMENTS

The kind of teeth, its natural F level, and its age are likely to influence the F-uptake after brushing with various F-containing toothpastes. But the natural fluoride level is more important than the age of teeth. Its F-uptake rate was shown low if the natural F-content was high.

In this study, enamel fluoride content after a month using toothpaste without fluoride was so variable (400-2000 ppm F) that we couldn't sufficiently select the subjects with no more than 500 ppm F on the enamel surface. These results were probably due to the F uptake from F-containing foods during wash-out period and the effect of subjects with original high F content in enamel before this study.

## CONCLUSION

Sodium monofluorophosphate seems largely to be present as  $\text{PO}_3\text{F}^{-2}$  in solutions. However, in the NaF systems no complexing seems to have taken place. Therefore, the addition of NaF to a dentifrice would be preferable to the addition of  $\text{Na}_2\text{PO}_3\text{F}^{8)}$  according to fluoride uptake by enamel.

In addition, Grön et al.<sup>7)</sup> reported that the uptake of F from MFP was greater at low pH than at neutral pH because acidity increases the ionization ratio. Brudevold et al.<sup>3)</sup> observed that a low pH furthered the uptake. Even at a neutral pH however, NaF has been found to be a more efficient source of F than  $\text{Na}_2\text{PO}_3\text{F}^{10)}$  at neutral pH,  $\text{Na}_2\text{PO}_3\text{F}$  is not hydrolysed but NaF is ionized. This pH dependence of F may therefore not be pronounced in bifluorided dentifrices but may occur in the mouth during brushing.

When we compared the salivary fluoride concentration following 1½ mn brushing and washing, we observed that fluoride remains a longer time (about 1 low) after brushing with Fluocaril (ref; Finidori-Lamendin). It may be due to slow fluoride release from MFP.

In our study we can observe a low and slow fluoride uptake by enamel from Colgate toothpaste (1450 ppm fluoride). on the opposite, fluoride toothpaste Fluocaril (2500 ppm fluoride) induces a very important fluoride uptake in few weeks: enamel becomes quickly protected against acid attack.

These observations suggest that a bifluoride toothpaste containing MFP and NaF at high F content will be more effective to prevent dental caries owing to;

- 1) the rapid fluoride incorporation in enamel (may be due to the high NaF content).
- 2) the slow activity spreaded over time by
- 3) the high fluoride content of the toothpaste (2500 ppm)

Although our results were statistically significant we should require further and long term study to validate the advantage of high bifluoride toothpaste because of our limited number of subjects and short term study.

### SUMMARY

A microsampling technique was used to quantitatively compare enamel fluoride concentrations on the maxillary central incisors of fifty one dental students before, 4 weeks and 8 weeks after toothbrushing with two bifluorided toothpastes (NaF-MFP).

The results were obtained as follows:

- 1) after four week treatment, enamel F uptake rate was greater than that of Colgate group and statistically different.
- 2) after 8 weeks treatment the same occurs but the difference is not statistically evident.

These results suggest that bifluoride Fluocaril (2500 ppm) is superior to Colgate (1450 ppm) as high fluorided toothpaste for its early cariostatic action.

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## Fluocaril bifluore 250과 Colgate에 대한 법랑질의 생체내 불소 흡착

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.....<국문초록>.....

본 연구는 법랑질의 불소 흡착량을 측정하기 위하여 치과대학생 51명을 대상으로 상악중절치를 이용하여 4주간의 세정기(wash-out period)를 거친후 두가지의 bifluore tooth pastes (NaF-MFP) 인 Fluocaril 250 (2500ppm F) 과 Colgate (1450ppm F) 로 치솔질하게 하여 4주, 8주 후에 법랑질 생검을 시행하였으며 법랑질 불소농도를 정량한 결과 다음과 같은 결론을 얻었다.

1. 실험 4주후, 법랑질 불소 흡착율(F-uptake rate)에 있어 Fluocaril 250이 Colgate군에 서 보다 높았으며 통계학적으로 유의성이 있었다.
2. 실험 8주 후에는 Fluocaril 250과 Colgate군이 비슷한 법랑질 불소 흡착율을 보였으며, 통계학적으로 서로 차이점이 없었다.

이러한 결과로 볼 때, 초기 치아우식 저지 작용에 있어 Fluocaril 250이 Colgate보다 고농도불소치약(high fluorided toothpaste)으로서 작용이 우수하다.