J. Dent. Ass. S. Afr. Vol. 28, No. 11, pp. 624 - 629

An In Vivo Evaluation of an Iontophoretic Technique for Increasing the Surface Fluoride Content in Enamel

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SUMMARY

Surface enamel fluoride concentrations were determined in 18 subjects before and after topical fluoride applications. These 18 subjects were divided into 2 groups, one of which received the fluoride applications iontophoretically whereas the control group had the identical fluoride applications without the use of the current. The results indicate that the greatest increase in fluoride concentrations resulted after 3 applications done at weekly intervals when the iontophoretic technique was employed. Two iontophoretic fluoride applications with an interval of a week between them resulted in a less marked increase whereas no increase was observed when fluoride was applied once only. The increase in the fluoride concentration of the surface enamel was not as significant when the fluoride was applied without the use of current.

OPSOMMING

Die fluoried-konsentrasie van glasuuroppervlakke is vóór en ná plaaslike aanwending van fluoried in 18 persone bepaal. Die 18 persone is in 2 groepe verdeel. In één is die fluoried aanwendings iontoforeties gedoen terwyl in die kontrolegroep identiese aanwendings sonder die gebruik van elektriese stroom toegedien is. Die resultate dui aan dat die grootste vermeerdering in fluoriedkonsentrasie plaasgevind het na 3 weeklikse aanwendings van die iontoforetiese tegniek. Twee weeklikse iontoforetiese aanwendings het 'n kleiner vermeerdering teweeggebring, terwyl geen vermeerdering gevind is na die fluoried slegs eenmaal aangewend is nie. Die vermeerdering in die fluoriedkonsentrasie van die glasuuroppervlakke was nie so betekenisvol in die gevalle waar die fluoried sonder die gebruik van elektriese stroom aangewend is nie.

INTRODUCTION

Topical fluoride therapy is widely used as an aid in the prevention of dental caries (Torell, P and Ericsson, Y 1965; Brudevold, F *et al.* 1967; and Horowitz, H S 1969). It is recommended particularly in areas where the fluoride content of the public water supply is below the optimal level of 1ppm. The Witwatersrand area, supplied with drinking water by the Rand Water Board, is one such example, with a yearly average of approximately 0,2 ppm of fluoride (Rand Water Board, 1969). A property common to all these topical agents is their ability to increase the fluoride content of the surface layers of enamel. Iontophoresis is one method available for topical fluoride application. The principle of this technique is that an electric current is passed through a fluoride-containing gel which is in intimate contact with the teeth. The electric current assists the dissociation of the fluoride salts in the gel into the component anions and cations. In addition, during the flow of the current, the teeth are made electropositive and thus the negative fluoride ions are attracted to the electropositive enamel.

The influence of an electrical potential on fluoride ion migration in human normal tooth enamel was studied on isolated teeth *in vitro* (Pouëzat, 1970). Fluoride accumulation and penetration were determined by means of an electron probe X-ray microanalyzer. This author reported that no notable difference was observed between teeth treated with fluoride by topical application and those treated by iontophoresis.

The purpose of this investigation was to assess the ability of enamel to acquire and retain fluoride when applied iontophoretically *in vivo*. As a control, the same form of fluoride was applied without the use of current.

MATERIALS AND METHODS

The source of fluoride was a gel containing sodium fluoride (1,10 per cent) and sodium monofluorophosphate (11,40 per cent), with a total fluoride ion concentration of 2,0 per cent (Fluocaril, Lab Goupil, Paris, France). The variable source of current was a "Galvanoster" (Lab Goupil, Paris, France) which supplied a direct current of up to 5 mA. The duration of the fluoride application varied indirectly with the strength of the current. The current strength was adjusted between 3 mA and 5 mA depending on the tolerance of each individual and the corresponding times varied between 2 min and 3,3 min. The product of current strength and time was always constant at 10 mA min. Within this range, discomfort was never found to be great enough for treatment to be discontinued. The fluoride was applied for 2 min to each subject of the control group.

In this investigation 18 subjects, resident in the Witwatersrand area, 18 – 35 years of age, were chosen at random and divided into 2 series of 9 patients each. The control series received fluoride applications without the use of current (Series A), whereas the fluoride was applied iontophoretically to the other series (Series B). The 9 subjects in each series were further subdivided into 3 equal groups (Fig. 1).

Prior to any fluoride applications, enamel biopsies were taken from all the available teeth, up to and including the first molars in the upper and lower jaws, of each subject. The *in vivo* enamel biopsy method developed by Hotz, Mühlemann and Schait (1970) was used. This was followed immediately by the first fluoride application.

The patients in Group 1 of the experimental and control series (Fig. 1), each received a single fluoride application. Those in groups II and III received 2 and 3 fluoride applications respectively with an interval of one week between each application. In each individual a further enamel biopsy was performed 2 weeks after the final application of fluoride.

The enamel samples obtained during the biopsy procedures were placed in plastic tubes containing 2 ml of a dilutant incorporating 0,08 ppm fluoride as sodium fluoride and 50 per cent Tisab at pH 5,1. These containers were stored sealed for not less than 3 days to allow the solution to reach equilibrium, before determining the fluoride concentrations of the solutions by means of a combined fluoride ion selective electrode (Orion Research, Model 96-09) coupled to a digital pH meter (Orion Research, Model 701). The solutions were then diluted 25 times and their calcium concentrations determined by atomic absorption spectrophotometry (Carl Zeiss Spectrophotometer, Model PMQII with flame attachment F.A. II).

Using the assumption that the calcium content of human enamel is 37,03 per cent (Retief *et al*, 1971), the weight of enamel removed from each tooth during the biopsy procedure was determined by means of the following formula:

$$\text{Weight of enamel removed } (\mu g) = \frac{[Ca^{++}] \times 100}{37}$$

The fluoride concentration of the enamel solution was then calculated as follows:

$$[F^-] \text{ ppm} = \frac{\text{Total fluoride content of the biopsy sample} \times 10^6}{\text{Weight of enamel removed}}$$

The depth to which the enamel had been etched during the biopsy procedure was determined for each tooth assuming the density of human enamel to be 2,95 gm/cc (Manly and Hodge, 1939). In addition, it was assumed that the areas of enamel which were exposed to the acid demineralisation during the biopsy procedures, were constant and that the etching of the tooth surfaces was uniform.

The following formula was used in calculating these depths.

$$\text{Depth of biopsy } (\mu m) = \frac{\text{Weight of enamel removed}}{\text{Density of enamel} \times \text{area etched}}$$

RESULTS

Frequency distribution histograms (Figs. 2 and 3), were constructed to demonstrate the biopsy depths obtained from Series A and Series B respectively. The depths were subdivided into 3 separate ranges. The first range included all depths below 4,0 μm, the second those between 4,0 and 9,0 μm and the third extended from 9,0 to 13 μm.

Examination of the results from the subjects who had the fluoride applied without current (Fig. 2), showed that prior to the fluoride applications the depths of etching of 3 teeth fell within the 0 – 4 μm range. After the fluoride applications, this number increased to 8 teeth. The results of 122 teeth fell within the second range prior to application of fluoride, whereas this number remained fairly constant at 129 teeth after the fluoride applications. Finally, 66 teeth were initially biopsied to depths within the limits of the third range, which again remained constant at 64 teeth after the fluoride applications.

The results obtained from the subjects who received iontophoretic fluoride applications (Fig. 3), showed that before the fluoride treatment, 12 teeth fell within the first range and that this number remained practically constant at 14 after the treatment. Initially 109 teeth fell within the second range, whereas this number increased markedly to 160 after the fluoride applications. Finally, the results of 61 teeth fell within the limits of the third range initially but dropped markedly to only 18 teeth after the iontophoretic applications of fluoride.

Figures 4 and 5 are frequency distribution histograms of fluoride concentrations in surface enamel before and after the fluoride applications. For the sake of convenience the histograms were divided into 2 segments representing fluoride concentrations above and below 1200 ppm. The fluoride concentration in figures 4 and 5 were not related to the depths of etch.

The results obtained from the subjects who received the fluoride applications without the use of current (Fig. 4) showed that before fluoride application, 86 per cent of the teeth had fluoride concentrations below 1200 ppm (48 per cent less than 600 ppm and 38 per cent between 600 ppm and 1200 ppm). After the fluoride applications however, this figure decreased slightly to 79 per cent. Conversely, the number of teeth with fluoride concentrations greater than 1200 ppm increased from 14 per cent before, to 21 per cent after the fluoride treatment.

Results from the subjects in Series B (Fig. 5) showed that prior to the fluoride applications, 66 per cent of the teeth

GROUPING OF SUBJECTS

GROUP I			GROUP II			GROUP III		
B	B	B	B	B	B	B	B	B
A	A	A	A	A	A	A	A	A
I	I	I	I	I	I	I	I	I
I	I	I	A	A	A	A	A	A
B	B	B	I	I	I	I	I	I
			I	I	I	A	A	A
			B	B	B	I	I	I
						I	I	I
						B	B	B

B... BIOPSY

I... PERIOD OF ONE WEEK

A... FLUORIDE APPLICATION

Fig. 1 Subdivision of the subjects in the control and experimental series and the experimental design.

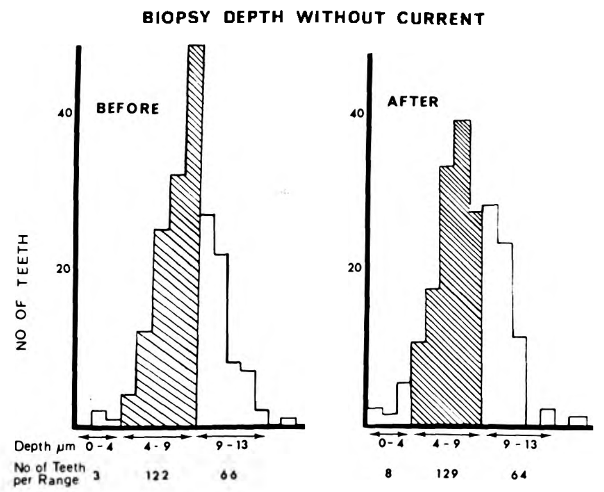

Fig. 2 Frequency distribution histograms of depths of etch before and after the application of fluoride without the use of current.

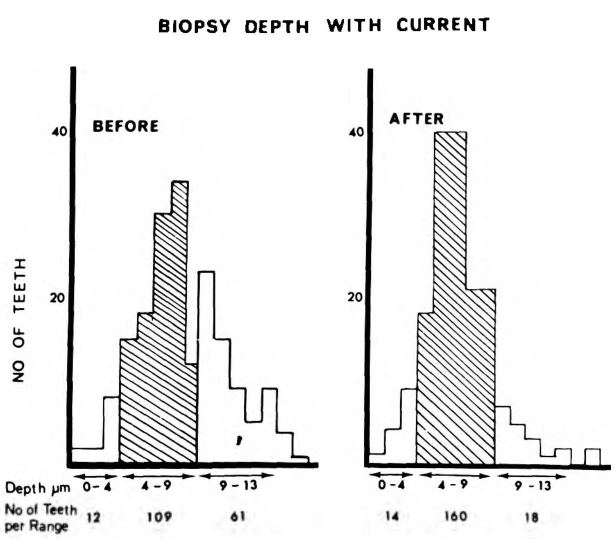

Fig. 3 Frequency distribution histograms of depths of etch before and after the iontophoretic application of fluoride.

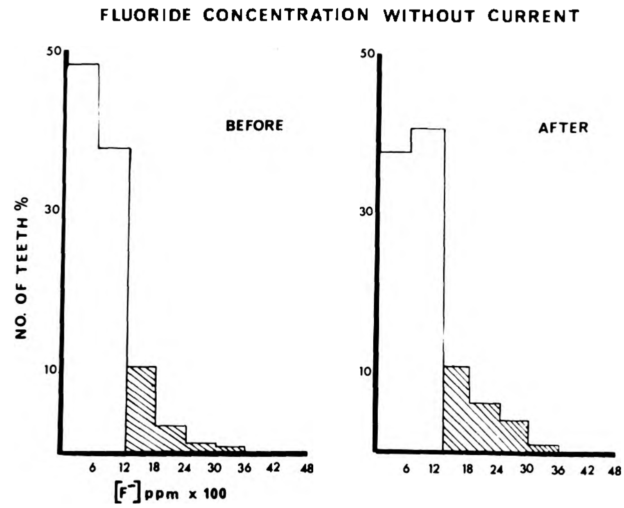

Fig. 4 Frequency distribution histograms of fluoride concentrations of surface enamel before and after the application of fluoride, without the use of current.

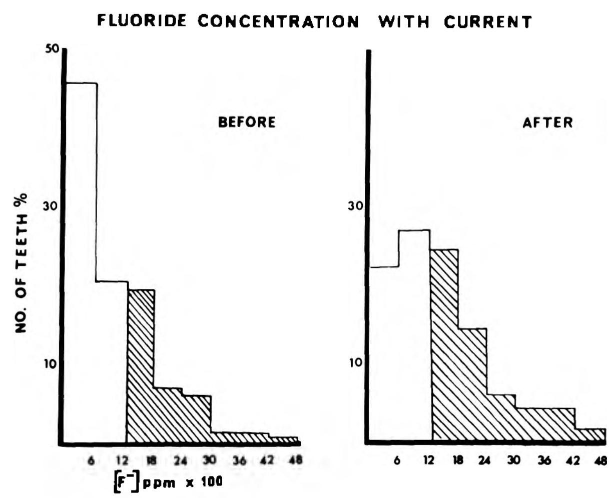

Fig. 5 Frequency distribution histograms of fluoride concentrations of surface enamel before and after the iontophoretic application of fluoride.

had fluoride concentrations below 1200 ppm (20 per cent less than 600 ppm and 46 per cent between 600 ppm and 1200 ppm) whereas in the remaining 34 per cent, the fluoride concentrations were greater than 1200 ppm. After the fluoride had been iontophoretically applied, 48 per cent of the teeth had fluoride concentrations less than 1200 ppm whereas in 52 per cent these were greater than 1200 ppm.

DISCUSSION

Weatherell and Hargreaves (1966) showed that the concentration of fluoride in the surface enamel of teeth was higher than that in the deeper layers of the enamel. Furthermore, the relationship between the fluoride concentration and the depth from the enamel surface for any particular tooth is not linear but follows a negative exponential curve. These findings were confirmed by Barbakow, De Kock and Retief (1971), for persons resident in the Witwatersrand area (Fig. 6). For this reason results of fluoride determinations, expressed as concentrations of fluoride, should be related to the depths of the samples from the enamel surface.

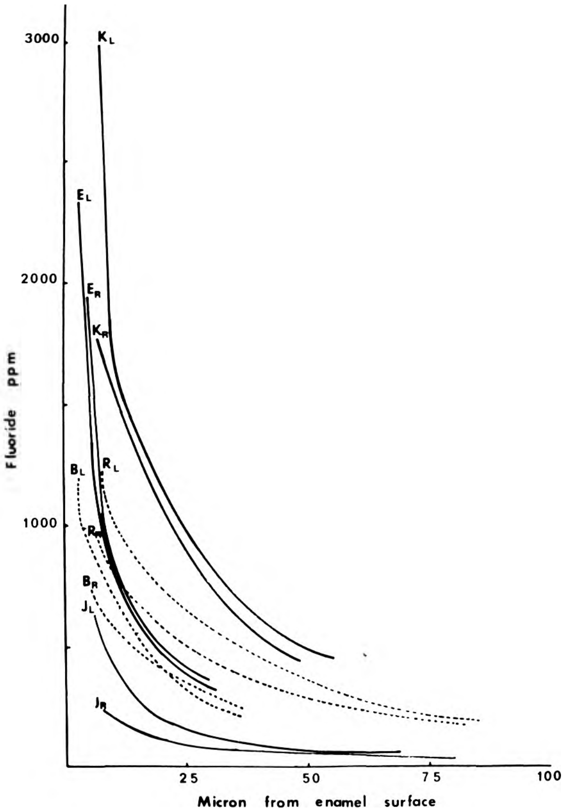

Fig. 6 Fluoride distribution curves of five pairs of central incisors, constructed from the results of an *in vitro* study (Barbakow, De Kock and Retief, 1971).

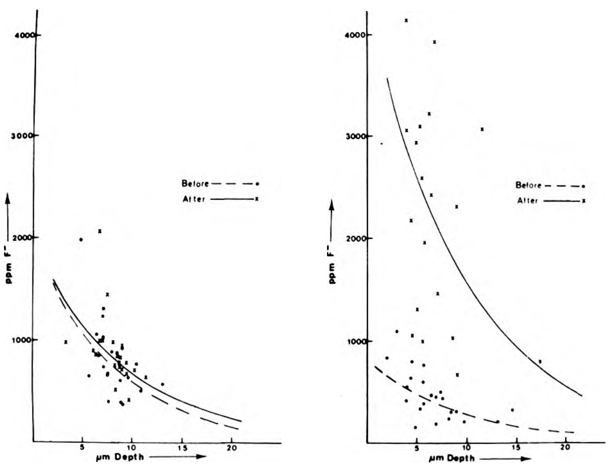

Fig. 7 Examples of the model fitted to the data from 2 patients. The left hand figure shows no significant increase in fluoride concentrations after treatment whereas the right-hand figure shows a significant increase (0,1 per cent significance level).

The *in vivo* biopsy method described by Hotz *et al* (1970) is an acid etching procedure. A limitation of this technique is that although it is possible to determine the depth of the etching, it is not possible to control this depth accurately. On the other hand, besides being a convenient method of clinically obtaining enamel samples, the acid etching technique provides a good measure of the acid resistance of the enamel.

The reaction between fluoride and hydroxyapatite consists either of the formation of calcium fluoride or of the substitution of hydroxyl ions in hydroxyapatite to form fluoroapatite (McCann, 1968). It is the fluoride in this latter compound which is more firmly bound in the enamel and which has been shown to increase the resistance of the enamel to acid demineralisation (Young and Elliott, 1966). Fait *et al* (1970) demonstrated this altered enamel resistance to acid attack in an *in vitro* study and cited a 25 – 37 per cent increase. We therefore used the parameter of depth of etch in analysing our results with respect to the resistance of enamel after fluoride treatment, to acid demineralisation.

The values obtained for the depths of the biopsies before and after the fluoride applications in the control group (Series A), showed minimal changes. From this it may be deduced that the change which occurred in the resistance of the surface enamel to acid demineralisation was insignificant. A significant change was, however, observed in the second and third depth ranges for the results obtained from the subjects in Series B, or that group which had the fluoride iontophoretically applied (Fig. 2).

Initially only 109 teeth were biopsied between 4,0 and 9,0 μm whereas after the iontophoretic fluoride application, this number increased to 160 teeth. This indicates that the enamel of a greater number of teeth was made more resistant to the acid demineralisation during the biopsy procedure. Conversely the number of teeth which fell within the 9,0 – 13 μm range decreased from 61 to 18 teeth after the fluoride treatment indicating a decrease in the number of teeth with low resistance to acid demineralisation.

Mellberg, Laakso and Nicholson (1966) showed that the greatest loss of fluoride, in the form of unreacted fluoride and CaF_2, from the surface enamel occurs within 24 hours after a topical application. For this reason it was decided to do the second enamel biopsies two weeks after the final fluoride application, in an attempt to assess the retention of the fluoride after the fluoride applications.

Although the subjects were selected at random, the initial fluoride concentration in 86 per cent of the teeth in the group which received fluoride application without current was below 1200 ppm. The corresponding figure in the experimental group was 66 per cent. After completion of the fluoride treatment these figures decreased to 79 per cent and 48 per cent respectively, the reduction of 7 per cent in the first case being smaller than that of 18 per cent in the second.

Conversely the number of teeth with fluoride concentrations greater than 1200 ppm increased from 14 to 21 per cent in the control group and from 34 to 52 per cent in the group which received iontophoretic fluoride, indicating that fluoride retention was greater in the latter group.

The results were statistically analysed (see Statistical appendix) in order to establish whether any fluoride increases in the surface enamel observed as a result of topical application of fluoride, either with or without the use of current, were in fact statistically significant.

In the case of the subjects who received fluoride applications without the use of current (Series A) significant increases occured in 2 individuals. In the first the increase was significant at the 1 per cent level after one application and in the second it was significant at the 2,5 per cent level after 3 applications. There was no significant change in the fluoride concentrations of the remaining 7 subjects.

When the fluoride was iontophoretically applied (Series B) one of the 3 subjects who had had 2 fluoride applications showed an increase in enamel fluoride concentration significant at the 5 per cent level. Of the 3 subjects who each had 3 applications, one showed an increase significant at the 5 per cent level and 2 at the 0,1 per cent level. In the remaining 5 subjects of this series there was no statistically significant increase in the fluoride concentrations of their surface enamel.

It should be born in mind that besides the 2 known parameters i.e. the fluoride concentrations and the depths of the biopsies, other factors not considered in this investigation may have an important effect on the final results. These include chemical laws such as the laws of mass action and Fick's laws of diffusion (Stearns, 1970), as well as inconsistencies in the biopsy technique (van der Merwe, Retief and Barbakow). Finally, the individual genetic make-up, pre- and post-natal dental development, as well as the local oral environment may influence the final results.

STATISTICAL APPENDIX

Models of the form

$$y = ab^{-x}$$

$$\text{or } \log y = \alpha - \beta x$$

where y = fluoride concentration at a depth of x below the surface of the tooth

were fitted by the least squares method to the data from each patient. Separate models were fitted to the before treatment and after treatment data, and a one-sided t-test was performed for each patient to test for a significant increase in α, which could be attributed to the treatment (Fig. 7).

The results are given in table 1, in which the patients are divided up into 6 groups of 3 patients each, corresponding to the different types of treatment.

A one-way analysis of variance was then applied to the t-values, to test whether there were any significant differences between the responses in the 6 groups. The result was found to be significant at the 7 per cent level, but not at the 5 per cent level ($F = 2{,}91$ on 5° and 12° of freedom).

A multiple comparison test to see whether the responses in group 6 (3 applications, with current) were stronger than those in the other 5 groups gave a result significant at the 5 per cent level ($t = 2{,}17$ on 12° of freedom – one sided test). This suggests that the maximal treatment of 3 applications with current could have increased the fluoride retention in the patients' teeth, to a greater extent than in the other 5 types of treatment.

CONCLUSIONS

A comparison of the fluoride concentrations of surface enamel of individuals who had the same fluoride gel applied topically, either with or without the use of an electric current, indicated that:

(1) fluoride concentration increases were greater after the iontophoretic applications,
(2) the resistance of enamel to acid demineralisation was increased as a result of the iontophoretic applications,
(3) three iontophoretic applications were most effective as supported by statistical analysis of the results.

Table I Significance levels for the increases in fluoride levels after treatment for each of the 18 patients (One-sided t-tests)

Without Current

Group No.	1	2	3
No. of applications	1	2	3
Patient 1	1%	Not significant	Not significant
Patient 2	Not significant	Not significant	Not significant
Patient 3	Not significiant	Not significiant	2,5%

With Current

Group No.	4	5	6
No. of applications	1	2	3
Patient 1	Not significant	Not significant	5%
Patient 2	Not significant	Not significant	0,1%
Patient 3	Not significant	5%	0,1%

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