In vivo Effects of a SnF_2 Gel on Acid-softened Enamel

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The purpose of this investigation was to study the intra-oral rehardening of acid-softened enamel and fluoride uptake from SnF_2 gel. Bovine enamel slabs were softened with 0.1 mol/L lactate buffer at pH 4.0 for 14 hrs and then mounted in a mandibular removable Hawley appliance. Control slabs were worn for 96 hrs by seven adult males whose teeth were brushed daily with a fluoride-free dentifrice. Test slabs were exposed once/day to 0.4% SnF_2 gel. The gel was swabbed onto the slabs for one minute before being replaced in the mouth unrinsed. The natural dentition was brushed 4x/day with a fluoride-free dentifrice. Microhardness testing was performed after intra-oral exposure (IOE) and after acid-resistance-testing (ART) following immersion in 0.01 mol/L lactate buffer for 24 hrs at pH of 4.0. Fluoride uptake was measured on separate controls, test slabs, and test slabs after ART, with 0.5 mol/L $HClO_4$ etches of from 15 to 60 sec. The F content was measured with a F-ion-specific electrode and the phosphate content by spectrophotometry. Following IOE, microhardness recovery was 35.6% for control and 37.9% for test slabs, and control slabs retained 1.4% resistance to acid, as compared with 18.6% for the test slabs. The F content of control slabs was significantly less than that of SnF_2-treated slabs from 5 to 60 μm in depth, and the F content of SnF_2-treated slabs after ART was significantly less at depths of from 5 to 35 μm than that of SnF_2-treated slabs not exposed to ART. Both control and SnF_2 enamel slabs demonstrated rehardening after IOE, but only SnF_2-treated enamel retained a significant fraction of that rehardening after ART.

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Introduction.

The topical application of fluoride agents over the past four decades has produced significant anticaries benefits to the human dentition. Among the frequently used fluoride topical agents was stannous fluoride (SnF_2), utilized as a professionally applied solution (Jordan *et al.*, 1959; Mercer and Muhler, 1961; Peterson and Williamson, 1962), incorporated into a dentifrice (Muhler *et al.*, 1955; Muhler, 1957), and into a mouthrinse (Radike *et al.*, 1973). The rationale for the use of SnF_2 was that it greatly reduced the solubility of enamel in acid (Muhler and van Huysen, 1947) and exhibited significant antiplaque properties (Svatun, 1978; Bay and Rølla, 1980; Øgaard *et al.*, 1980).

A variety of topical fluoride agents and methods of application has been investigated in both laboratory and intra-oral studies following recognition that demineralized enamel could be remineralized by saliva and that this was enhanced by the presence of low levels of fluoride (Koulourides, 1968). Despite significant anticaries benefits of SnF_2, its capacity to enhance remineralization of demineralized enamel has received little attention. Koulourides *et al.* (1980) demonstrated *in vivo* that 8% SnF_2 solution more effectively remineralized acid-softened enamel than did 2% NaF, and SnF_2 provided greater acid resistance to acid-softened enamel than did APF (Koulourides and Cameron, 1980). Perhaps the lack of interest in SnF_2 was related to a prior need to prepare the topical agent fresh for each application and its unacceptable taste. Likewise, SnF_2-containing dentifrices, once used extensively, are no longer widely marketed (Accepted Dental Therapeutics, 1984), having been replaced by NaF- or monofluorophosphate-containing products, mainly because of their relative ease of formulation and lower cost of production.

Recently, the use of SnF_2 preparations has been revived in the form of a self-applied 0.4% SnF_2 gel due in part to its antibacterial properties (Tinanoff, 1985), its improved taste, and its ease of application. It was the purpose of this study to investigate the *in vivo* capacity of a 0.4% SnF_2 gel, applied daily, to remineralize acid-softened enamel using microhardness and acid-resistance-testing, and analysis of fluoride uptake to evaluate the results.

Materials and methods.

Sections of enamel (3 × 3 mm × 2 mm) were cut from the labial surfaces of bovine permanent mandibular incisors by means of a diamond wheel saw and were mounted on acrylic disks with sticky wax. Approximately 50-100 μm of the surface enamel were removed by polishing with a paralleling device (Koulourides *et al.*, 1976). A smooth flat surface was produced with wet emery paper (240-, 400-, and 600-mesh). The polished slabs were inspected with a dissecting microscope to eliminate samples with cracks or hypoplastic defects.

The mounted slabs were coated along their lateral borders with acid-resistant varnish to seal the cut sides from subsequent acid demineralization. Each enamel slab was demineralized by exposure to 20 mL of 0.1 mol/L lactic acid-sodium hydroxide buffer for 14 hrs at 37°C. The buffer contained 1% sodium carboxymethylcellulose with 3 mmol/L calcium and 1.8 mmol/L phosphate, and the pH was adjusted to 4.0. The slabs were then washed in de-ionized water and sterilized by exposure to 1% ethylene oxide vapor for 8 hrs.

An alginate impression of the mandibular arch was secured for each of the seven healthy male subjects (aged from 23 to 55 years). Yellow stone models were poured, on which removable mandibular acrylic appliances were fabricated. Recesses large enough to accommodate up to 16 slabs each were cut into the right and left sides of the appliance. This appliance is a modification of that described by Zimmermann *et al.* (1985). Pre-softened slabs were removed from the acrylic disks, and 16 were mounted on each side of the appliance, with sticky wax. The wax sealed the lateral borders of the slabs and created a smooth contour for comfort for the appliance-wearer. Thirty-two control slabs were worn for 96 hrs by each subject, 16 of which were used for microhardness testing and 16 for fluoride analysis. All control data were obtained prior to test treatments. The same number of slabs and procedures was used for the experimental phase using SnF_2 gel. An additional set of 16 test slabs was worn subsequently for 96 hrs prior to exposure to acid-resistance-testing (0.01 mol/L lactate buffer for 24 hrs). These test slabs were then analyzed for F content, and the values compared with those of control slabs and treated

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slabs used for F analysis only. The appliance was worn by each test subject continuously except at meal times, when it was wrapped in paper tissue moistened with de-ionized water and stored in an orthodontic appliance box. All subjects continued their usual dietary habits, and all lived in a fluoridated area (1 ppm). The subjects gently cleansed the test slabs with a moist cotton swab following thorough brushing of the natural dentition with a fluoride-free dentifrice (Pepsodent, Lever Brothers Co., New York, NY) 4x/day during both the control period and experimental period.

During the experimental phase of the intra-oral exposure, a stannous fluoride gel (GEL-KAM, 0.4% Stannous Fluoride Gel, Scherer Laboratories, Inc., Dallas, TX) was applied to the slabs once/day (before bedtime). The appliance was removed from the mouth and the gel applied to the slabs with a cotton tip applicator; it was allowed to remain in contact with the surfaces of the slabs for one minute. No effort was made to wash the slabs following the one-minute extra-oral gel application. The appliance was then replaced in the mouth and the gel allowed to mix with the saliva. Following the final application on the third day, all subjects wore their appliances for an additional 24 hrs and continued brushing the dentition with the fluoride-free dentifrice. Control slabs were not exposed to SnF_2 gel, but were exposed to saliva for the same period of intra-oral exposure. Subsequently, the slabs were recovered, rinsed with double-distilled water, and then analyzed.

Microhardness testing was performed at four different points. Initial microhardness readings were recorded prior to acid-softening. The second readings followed acid-softening. The third followed intra-oral exposure (IOE), to measure the rehardening effect of the regimen, and the final microhardness test of each slab was performed following acid-resistance-testing (ART). Each prior set of measurements was re-measured to ensure that no changes in lengths of previous indentations had occurred.

All slabs were re-mounted with sticky wax on their original coded acrylic disks, with the paralleling device used to make the enamel surface parallel to the base of the disk (Koulourides *et al.*, 1976). The Tukon Microhardness Tester (Wilson Instrument Division, Bridgeport, CT) with an elliptical diamond and a 500-g load was used to determine the hardness of the enamel surface. The lengths of three indentations were measured in filar units, averaged, and converted to depth (μm) by multiplying the filar number by the penetration factor of the microscope objective. The method described by Gelhard *et al.* (1979) was used to calculate the percentage changes in microhardness following both IOE and ART.

Acid-resistance-testing was accomplished by immersing each slab in 20 mL of 0.01 mol/L lactic acid-sodium hydroxide buffer (pH 4.0) in 1% sodium carboxymethylcellulose containing 3 mmol/L calcium and 1.8 mmol/L phosphate at 37°C for 24 hrs. The lateral borders of each slab were coated with acid-resistant varnish to prevent decalcification by the acid through exposure of the lateral aspect of the slab.

Baseline values for F content were determined prior to acid-softening. Sixteen randomly selected bovine incisors from different animals were prepared to provide one enamel slab from the middle one-third of the labial surface. These slabs were analyzed by the same method as that used for control and test slabs.

Analyses of separate sets of 16 slabs for each subject for fluoride content followed intra-oral exposure for control, test slabs, and test slabs following ART. Each slab was removed from the appliance and glued on the end of a plastic rod (0.64 cm in diameter). The lateral borders were sealed with blue inlay wax, and the slabs were immersed in 0.5 mL of 1.0 mol/L KOH for 24 hrs at room temperature so that the alkali-soluble fluoride would be removed from the surface (Caslavska *et al.*, 1975). After being rinsed with de-ionized water, the slabs were inspected to ensure that the lateral borders remained sealed with wax.

Five layers were removed from the 3×3 mm enamel surface by immersion in separate vials containing 1.0 mL of 0.5 mol/L perchloric acid for 15, 30, 30, 60, and 60 sec. The specimens were rotated at 100 rpm for a standard agitation. The slabs were rinsed with 1 mL of total ionic strength adjusted buffer (modified TISAB II with CDTA, Orion Research, Inc., Cambridge, MA), and dried with a cotton pellet which was added to the vial after each etch. The modified TISAB II was prepared by combining 100 mL of TISAB II with 15 mL of 1 mol/L NaOH with a final pH of 5.6. The fluoride concentration was determined directly using an Orion Ion Analyzer 901 (Orion Research, Inc., Cambridge, MA) equipped with a fluoride-ion specific electrode (Orion 96-09). Phosphate concentrations were determined by spectrophotometry using the method of Gee *et al.* (1954).

The depth of enamel removed by each etch was calculated from the etched area of enamel (3×3 mm) and the amount of phosphate in the aliquots. The phosphate content of bovine enamel was assumed to be 51.2% and the mineral density 2.88 g/cm^3 (Davidson *et al.*, 1976). This density value, and therefore the calculations, were approximations, since the density after partial intra-oral remineralization was not known. However, this procedure provided a convenient means of presenting the F levels in enamel, assuming that the error in enamel density was similar in both regimens.

In order to describe the overall relationship between the depth (μm) and the fluoride content (ppm) for control, SnF_2-treated, and SnF_2-ART slabs, $\bar{X} \pm SD$ values for F content were calculated for every 5 μm from 0-50 μm and every 10 μm from 50-80 μm. Tests for differences between the mean values of these three groups at each of the depths were performed using the analysis of variance (ANOVA) for each of the separate depths. Bonferroni's correction was applied to these multiple tests to control the overall level of significance of the tests (Glantz, 1981). In the performance of k tests in order to control the probability of making one or more type I errors, Bonferroni's correction amounts to doing each of the individual tests at the level of significance α/k, or $0.05/13 = 0.0038$. After each significant ANOVA, three pair-wise comparisons were made using the Student's *t* test, again in conjunction with Bonferroni's correction.

Results.

Microhardness. — Tables 1 and 2 summarize the microhardness values expressed as depth of indenter penetration (μm). Included are $\bar{X} \pm SD$ values for the seven subjects, each of

TABLE 1
MICROHARDNESS VALUES (CONTROLS); (DEPTH OF INDENTER PENETRATION IN μM); $\bar{X}$ + SD

N = 7	Sound Enamel	Post-softened	Intra-oral Exposure (IOE)	Acid-resistance-testing (ART)
A	5.54 ± 0.12	16.74 ± 1.22	12.43 ± 0.91	17.01 ± 1.19
B	5.50 ± 0.11	18.02 ± 1.59	11.97 ± 1.70	17.98 ± 2.59
C	5.49 ± 0.10	18.68 ± 1.48	13.48 ± 1.44	19.34 ± 2.03
D	5.43 ± 0.12	18.29 ± 1.54	13.61 ± 2.36	18.17 ± 1.37
E	5.52 ± 0.12	17.00 ± 1.39	13.18 ± 1.80	16.24 ± 1.23
F	5.51 ± 0.13	17.36 ± 1.16	13.18 ± 0.95	16.96 ± 1.69
G	5.46 ± 0.10	20.65 ± 1.95	17.52 ± 2.63	19.87 ± 2.64
$\bar{X}$	5.49 ± 0.03	18.11 ± 1.22	13.62 ± 1.68	17.94 ± 1.22

TABLE 2
MICROHARDNESS VALUES (SnF_2); (DEPTH OF INDENTER PENETRATION IN μM); $\bar{\chi} \pm SD$

N = 7	Sound Enamel	Post-softened	Intra-oral Exposure (IOE)	Acid-resistance-testing (ART)
A	5.52 ± 0.09	18.09 ± 0.76	12.38 ± 0.61	14.83 ± 0.63
B	5.50 ± 0.10	17.43 ± 1.48	12.44 ± 1.00	14.67 ± 1.12
C	5.48 ± 0.10	16.32 ± 1.02	11.89 ± 0.44	14.18 ± 0.79
D	5.57 ± 0.14	13.23 ± 2.35	10.47 ± 1.98	11.74 ± 2.11
E	5.49 ± 0.12	16.01 ± 0.96	12.02 ± 1.17	14.02 ± 1.17
F	5.51 ± 0.12	17.18 ± 2.50	13.20 ± 1.63	15.70 ± 1.62
G	5.49 ± 0.13	18.42 ± 1.39	14.67 ± 1.37	17.08 ± 1.41
$\bar{\chi}$	5.51 ± 0.03	16.67 ± 1.62	12.44 ± 1.19	*14.60 ± 1.52

*SnF_2 value significantly different from that of control ($p<0.05$).

TABLE 3
MICROHARDNESS VALUES (% RECOVERY)

N = 7	SnF_2		Control	
Subject	ART	IOE	ART	IOE
Group 1				
A	25.93	45.42	−2.41	38.48
B	23.13	41.83	0.32	48.32
Group 2				
C	19.74	40.87	−5.00	39.42
D	19.45	36.03	0.93	36.39
E	18.92	37.93	6.62	33.28
Group 3				
F	12.68	34.10	3.38	35.27
G	10.36	29.00	5.13	20.61
$\bar{\chi}$	18.55	37.90	1.35	35.58
±SD	± 5.06	± 5.04	±3.81	± 7.70

whom contributed 10-16 samples for controls and approximately the same number for the test regimen. The number of test slabs ranged from 10-16 for each subject because of the occasional loss or surface destruction of slabs during the experimental procedure. Additionally, any slabs which developed cracks or iatrogenic surface defects were discarded.

The remeasurement of prior indentations revealed no changes in length of indentation, indicating that the integrity of the surface layer was maintained throughout the acid-softening, IOE, and ART procedures.

The microhardness values for both sound enamel and post-softened enamel demonstrated no significant difference between control and experimental slabs. The IOE produced almost exactly the same apparent rehardening for both control and test slabs. Conversely, control slabs demonstrated the loss of virtually the entire acquired rehardening following ART, whereas the test slabs retained a significant portion of their rehardening after acid exposure.

Table 3 illustrates the results of microhardness testing for control and experimental groups expressed in percent of recovery of hardness following IOE and ART, and shows three distinct groups within the seven subjects related to ART values. The subjects have been grouped in Table 3 according to the SnF_2-ART% in descending order from highest to lowest values. There were three distinct groups which emerged from this method: Group 1 (Subjects A and B), Group 2 (Subjects C-E), and Group 3 (Subjects F and G). An analysis of variance (ANOVA) of these three groups for SnF_2-ART% (mean values for each group) revealed a statistically significant difference ($p = 0.0015$). Scheffe's method of multiple comparisons was then used to make all the pair-wise comparisons among the three groups. Each group was significantly different from each of the others.

The same analysis for SnF_2-IOE% revealed a significant difference only between Group 1 and Group 3 ($p = 0.031$). There were no significant differences between any of these three groups for either control-IOE% or control-ART%.

When all subjects were compared for SnF_2-IOE% *vs.* SnF_2-ART% as well as control-IOE% *vs.* control-ART%, there were statistically significant differences between the values of each respective comparison ($p<0.0002$). Similarly, a comparison of SnF_2-ART% with control-ART% revealed a statistically significant difference for the entire group ($p<0.002$). There was, however, no significant difference between SnF_2-IOE% and control-IOE% ($p>0.3$). No consistent site-to-site variations in IOE or ART values were observed for individual subjects.

Pearson's product moment correlation coefficient revealed a close direct relationship between percentages for SnF_2-IOE% and SnF_2-ART% for the combined groups ($r = 0.95$). There was also a direct relationship between control-IOE% and SnF_2-IOE% ($r = 0.79$) and between control-IOE% and SnF_2-ART% ($r = 0.76$). Conversely, there was a moderate inverse relationship between percentages for control-ART% and SnF_2-ART% ($r = -0.60$) and between control-ART% and SnF_2-IOE% ($r = -0.68$).

Fluoride uptake. — The fluoride content of the sound bovine enamel slabs was uniformly low (50-75 ppm) for all depths.

The F content for control, SnF_2-treated, and SnF_2-ART slabs is shown in the Fig. There were significant differences between SnF_2-treated and control slabs at the depths of 5-60 μm. The SnF_2-treated slabs contained significantly greater F content than did the SnF_2-ART slabs from 5-35 μm, and the SnF_2-ART slabs in turn contained significantly greater F content than did controls at the same range of depths (5-35 μm).

Discussion.

The 0.4% SnF_2 gel used in this study represents a relatively new and different approach from previous SnF_2-containing agents (*i.e.*, professionally applied 8% SnF_2 solutions, or 0.4% SnF_2-containing dentifrices). The gel contains approximately the same fluoride content as a dentifrice (1000 ppm), but without an abrasive system of a dentifrice and is therefore not formulated for plaque removal. Additionally, the SnF_2 gel is recommended for daily application and does not resemble the more potent professionally applied solutions which are rec-

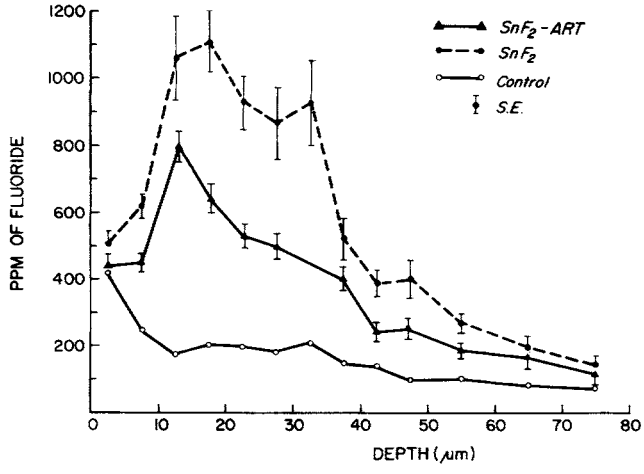

Fig. — Fluoride concentration (ppm) *vs.* depth (μm) from the enamel surface.

ommended for semi-annual application. Nevertheless, the gel fulfills many of the requirements for *in vivo* remineralization of decalcified enamel. It has a relatively low fluoride content in contrast to more potent agents such as the 1.23% APF gels, is recommended for repetitive daily application, and is relatively cheap and easily self-applied using a "brush-on" method.

The results of this study demonstrated the value of several methods of evaluation of the intra-oral effects of fluoride upon enamel remineralization. Surface microhardness values reflect average hardness for a given degree of mineral content of the enamel (Arends *et al.*, 1980b), but do not clarify the crystalline structure of the redeposited mineral (Koulourides and Housch, 1983). Comparison of the microhardness recovery data for control and SnF_2-treated slabs revealed that the 0.4% SnF application did not enhance the rehardening of the test slabs over the controls following IOE (37.90% and 35.58%, respectively). However, when the enamel slabs were exposed to a prolonged acidic challenge (ART), the mineral redeposition exhibited by the controls was almost entirely lost (Table 3), which may be explained by the relatively low uptake of F by the control slabs (Fig.). Conversely, microhardness values following ART for SnF_2-treated slabs were significantly higher than those of the controls (Table 3), as were their respective values for F content, indicating that the increased mineral content included alkali-insoluble mineral (FAP and/or FHAP) (Hercules and Craig, 1978), which contributed to the increased resistance to acid exposure. The stability of the F deposited in the SnF_2-treated enamel became evident when such slabs were exposed to ART, with the retention of acquired F significantly greater than controls from 5-35 μm (Fig.).

The rehardening of controls appeared due to the ingestion of fluoridated water, since low levels of fluoride (1.0 ppm) show a strong affinity for pre-softened enamel (Koulourides *et al.*, 1974), but there was still considerably less F uptake by controls as compared with that of the test slabs following SnF_2 applications (Fig.).

Of special interest was the natural grouping of subjects based on percentage SnF_2-ART values. The ANOVA test for the SnF_2-ART%, and to a lesser extent the SnF_2-IOE%, demonstrated that significant differences do exist among subjects, who could be characterized as high, average, and low remineralizers. Variable remineralizing capacity among subjects was previously recognized by Featherstone *et al.* (1982). The realization that individual subjects may have a widely varying ability to remineralize softened enamel suggests that future studies would benefit by use of larger numbers of test subjects, larger numbers of samples per individual, and concurrent analysis of saliva in order to define better the cause for individual differences. The lack of significant differences among these three groups for control-IOE and ART values makes it difficult to predict the remineralizing capacity of individual subjects on the basis of intra-oral exposure alone. The high correlation between percentages for SnF_2-IOE and SnF_2-ART suggests that subjects with high or low SnF_2-IOE% values will have correspondingly high or low SnF_2-ART% values as well.

Wei and Forbes (1974) studied the reaction of SnF_2 with enamel and suggested that, initially, the surface dissolves and subsequently reacts to form a surface layer containing $Sn_3F_3PO_4$ and CaF_2 following the application of 8% SnF_2 for a minimum of four minutes. However, remeasurement of prior indentations indicated that surface dissolution did not appear to occur in this study, and there exists little evidence to support the contention that the formation of such a layer containing $Sn_3F_3PO_4$ would occur with the *in vivo* application of the much weaker 0.4% SnF_2 applied for only one minute.

The use of the acid-etch method of determining fluoride uptake, although quantitatively less precise than analytical methods such as secondary ion mass spectrometry (SIMS) (Arends *et al.*, 1980a), provides a useful method of comparing the F profiles of the control and SnF_2-treated enamel specimens. The difference in the F content between the control and the treated enamel was sufficient to illustrate the significant treatment effects of the SnF_2 applications.

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