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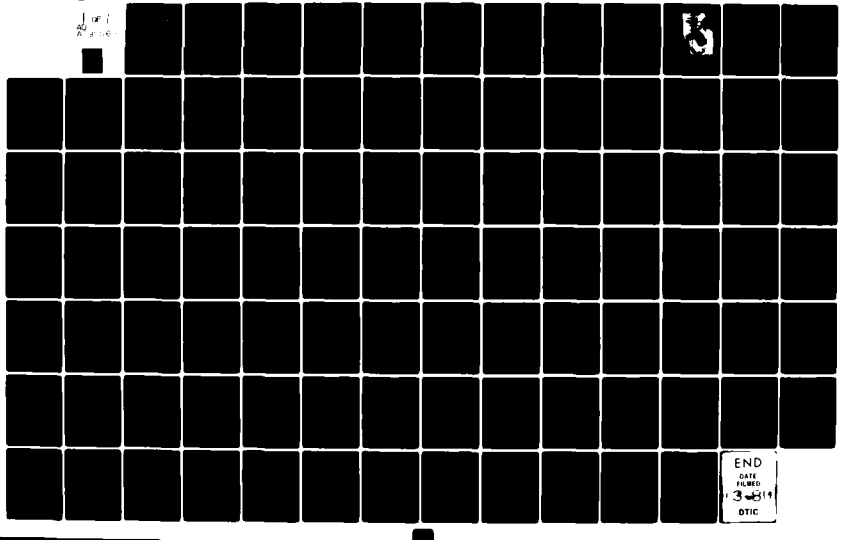

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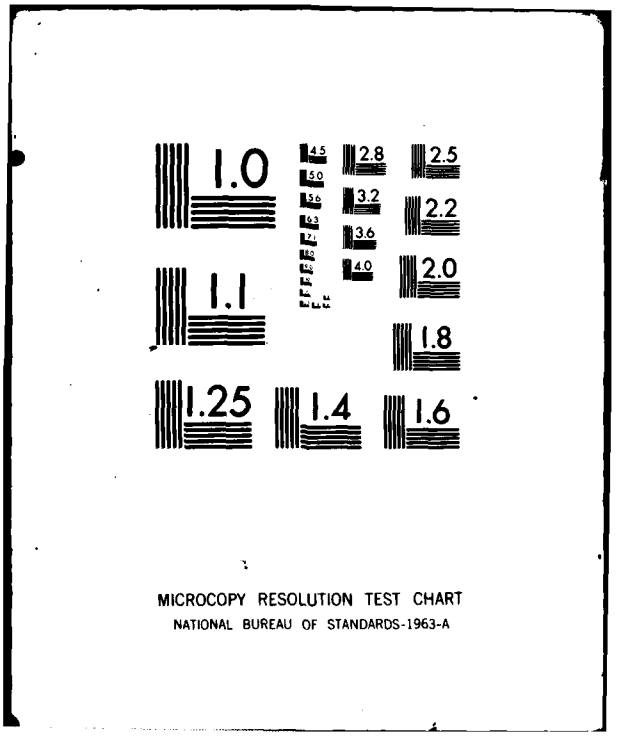

THE EFFECT OF A LOW FLUORIDE DELIVERY SYSTEM ON BACTERIAL METABOLISM

Annual Report

By

Norman Tinanoff, D.D.S., M.S.

David Camosci, B.S.

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compounds at this concentration produced decreased acid production of plaque forming organisms. As well, Na_2SnF_6, NaF and SnF_2 appeared to affect the attachment mechanisms of S. mutans to enamel, with SnF_2 being the most effective. An increase in both alkali and water soluble glucans of the bacteria suggest that fluoride at low levels produces unbalanced growth. The large accumulation of tin found in those organisms treated with SnF_2 may account for the increased effectiveness of SnF_2. From this series of experiments, it appears that continuous exposure of SnF_2 (10 ppm F^-) has the most effect on S. mutans growth, metabolism and attachment.

In the second phase of the experiment we investigated the differences between SnF_2 and $SnCl_2$ effect on plaque formation so that we could further examine the antiplaque determinants of SnF_2. SnF_2 had impressive antiplaque properties with initial plaque formation but with lesser effects noted on preformed plaque. The decreased effectiveness of SnF_2 with preformed plaque may be associated with the adhesion/cohesion altering properties of SnF_2. $SnCl_2$ was not as effective as SnF_2 probably due to the fact that fluoride accumulates within bacterial cells while chloride does not. The tin moity may passively enter the cells coupled to the fluoride ion, and the large tin accumulation in SnF_2 treated plaque may be the most important antiplaque determinant of SnF_2.

In the slow release mechanisms phase of the experiment, we spent considerable time on incorporating fluoride into ZOE and IRM. It appears to us now that these two materials are not well suited for the addition of fluoride compounds due to their low compressive strengths. We have had very good results, however, with incorporating pulverized SnF_2 into polycarboxylate cement. Most remarkably, polycarboxylate cement with 70% SnF_2 still maintains a compressive strength of 9.7 K lbs/in. In our slow release studies, which we are currently performing, we have very encouraging results with SnF_2 incorporated into polycarboxylate cement.

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Summary

This contract deals with the incorporation and slow release of various fluoride compounds in temporary restorative materials to impart antiplaque and anticaries activity to the restorative material.

In the first phase of the study, we examined the effect of various fluoride compounds on bacterial growth, metabolism and attachment. We found that 10 ppm F^-, a concentration that is compatible with a slow release agent, was well below bacterial growth inhibition or bactericidal levels. However, all fluoride compounds at this concentration produced decreased acid production of plaque forming organisms. As well, Na_2SnF_2, NaF and SnF_2 appeared to affect the attachment mechanisms of S. mutans to enamel, with SnF_2 being the most effective. An increase in both alkali and water soluble glucans of the bacteria suggest that fluoride at low levels produces unbalanced growth. The large accumulation of tin found in those organisms treated with SnF_2 may account for the increased effectiveness of SnF_2. From this series of experiments, it appears that continuous exposure of SnF_2 (10 ppm F^-) has the most effect on S. mutans growth, metabolism and attachment.

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Effect of Continuous, Low Concentration of Fluoride Compounds on Bacterial Growth, Acid Formation, and Attachment

Introduction

It is now obvious that the fluoride ion effects not only enamel solubility as previously thought, but may also effect caries activity and plaque formation by altering bacterial growth and attachment. To date, most of the studies published used NaF to look at the fluoride ion effect on bacterial plaque. Few studies have compared the effects using one or more different fluoride compounds. In those that did, SnF_2 appeared more effective. Also, there have been only scattered studies or reviews on the effects of various fluoride compounds on bacterial growth, acid production, polysaccharide production, and mechanisms of attachment. Some of these studies have been performed at high enough concentrations of F- to be influenced by either a bactericidal or bacteriostatic effect of the test agent. It may be better to evaluate the efficacy of various antiplaque compounds under the continuous influence of low levels of F^- to determine the differences, if any, on bacterial antiplaque mechanisms.

The purpose of these studies were to determine the effect of continued exposure of low concentrations of NaF, SnF_4, Na_2SnF_6, TiF_4, and $SnCl_2$ on altering plaque formation by Streptococcus mutans NCTC 10449. Specific tests were performed:

1) to determine the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of the test agents.

2) to determine the effect continual exposure of the test agents at low concentrations on growth and acid production in order to assess any individual or combined fluoride ion, cation or pH effects of the test agents.

3) to determine the quantity of tin present in bacterial plaque after exposure to the test agents through atomic absorption spectrophotometry.

4) to evaluate the quantity of bacterial mass through DNA analysis of the bacteria attached to enamel and of the unattached bacteria after exposure to the various test agents or controls.

5) to evaluate the quantity of alkali and water soluble extracellular polysaccharide through glucan analysis and glucan/DNA ratios of the bacteria attached to enamel and of the unattached bacteria after exposure to the various test agents or controls.

If a specific fluoride compound, at levels compatible with a slow release delivery system, is found to either alter bacterial acid production, bacterial growth, or bacterial attachment; then we may possibly have an agent that is safe and effective to be incorporated in a restorative material which is slowly released to produce antiplaque and anticaries activity.

Materials and Methods

Enamel Specimen Preparation

Enamel sections from extracted bovine incisors, approximately 180 mm^2, were cut from the smooth surfaces using a diamond drill with water coolant. A hole was placed in each specimen so that 20 gauge nichrome wires could be used to suspend the enamel in the test tube (Figure 1). The enamel specimens were cleaned with a slurry of pumice to remove organic material, washed with deionized water in a ultrasonic cleaner, and then autoclaved. Wax (inlay casting wax, Kerr products, Emeryville, Co.) was used to cover the inner aspects of tooth leaving only the surface enamel exposed. The specimens were placed in 70% ethyl alcohol for 15 min. to disinfect the surfaces from microbial contamination during wax preparation and then placed in sterile deionized water for 10 minutes to remove residual alcohol. Care was taken to assure that the enamel cylinders were not dessicated during preparation.

Microorganisms, Growth Media, and Growth

A streptomycin resistant mutant of *Streptococcus mutans* NCTC 10449 (Bratthall serotype *c*) has been selected as the test organism since this organism has been noted to attach to enamel similarly as *in vivo* organisms (Tinanoff, et al., 1978); causes caries (Freedman and Tanzer, 1974; Tanzer, et al., 1974; Tanzer, et al., 1976; Tanzer, 1969), and is the most frequently found serotype in human population (Bratthall, 1972; Keene, et al., 1977). Stock cultures were maintained by monthly transfer in fluid thioglycolate medium (Difco) supplemented with meat extract (20% V/V) and excess $CaCO_3$. For MIC/MLC determinations, stock cultures were adapted to growth in trypticase soy medium (TSB; BBL). For all other experiments, stock culture was adapted to growth in Jordan's medium (Jordan, et al.,

FIGURE 1: Example of suspended bovine enamel section and adherent microorganisms after three days response to test agents or control in Jordan's medium with 5% sucrose. Organisms attached to the wire were used for tin analyses. DNA and glucan analysis were performed on organisms attached to the enamel, and on unattached organisms in the growth media.

1960) supplemented with 5% sucrose and 5 mg. % Na_2CO_3 (Figure 2). All experiments were performed at 37° C under aerobic conditions.

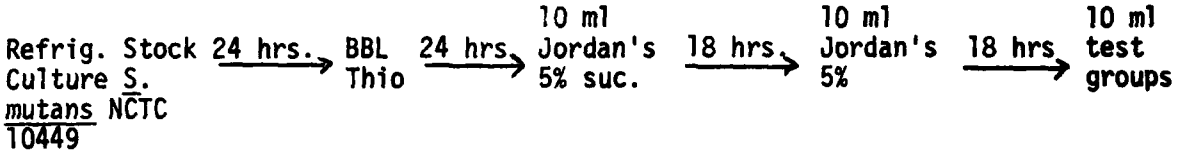

FIGURE 2. Procedure for adapting cultures of S. mutans to Jordan's medium supplemented into 5% sucrose. 0.1 ml of culture was transferred to uninoculated tubes each time.

Fluoride Compound and Controls

Fresh sterile solutions of the appropriate fluoride compounds or controls was added to the sterile test medium to obtain the proper fluoride or control dilution prior to inoculation of microorganisms. To obtain the appropriate dilutions, stock solutions of fluoride compounds were first prepared at 100 ppm F^-. These stock aqueous solutions NaF (0.022%W/v, pH 5.3), SnF_2 (0.041%, pH 3.8), Na_2SnF_6 (0.024%, pH 3.5), and TiF_4 (0.016%, pH 2.9) were added to Jordan's medium supplemented with 5% sucrose to produce fluoride concentrations of 10 ppm or 5 ppm F^-. Stock solution of $SnCl_2$ (0.05%, pH 2.9), which was equimolar to Sn^{++} in SnF_2 100 ppm F^-, was prepared and subsequently diluted into Jordan's

medium, served as a control for tin. Deionized water (pH 6.6) was added to the Jordan's medium at the same volume as the other aqueous solutions and this medium served as the control to which all solutions were compared.

For MIC/MLC testing, dilutions of the test agents or controls to the appropriate concentrations in medium required higher concentrations of test solutions to be made. Solutions of NaF, ranging from 50 to 5000 ppm F^-; SnF_2, from 0.5 to 250 ppm F^-; Na_2SnF_6 and TiF_4, from 100 to 1000 ppm F^-; and $SnCl_2$, from 50 to 1000 ppm Cl^- were prepared (Fig. 3).

Organic Binding of Free Fluoride

To rule out possible organic binding and to insure the accuracy of theoretical fluoride levels of the fluoride ion in the complex medium, the free fluoride was determined by fluoride analysis with a fluoride electrode (Orion 90-09A) connected to a digital electrometer. One ml aliquots of uninoculated and inoculated test media were evaluated immediately after addition of the fluoride agent and then after incubation of the test media for 24 hours at 37°C. This procedure entailed diluting a 1 ml aliquot of each media with 1 ml of TISAB (ORION) which stabilizes the pH and ionic strength of the sample. All samples were compared to NaF standards (ORION).

Purity Checks of Organisms

To insure purity and lack of contamination, the cultures at the beginning and end of each experiment were plated on blood and Mitis salivarius agar (Difco) and visually inspected for contaminants.

MIC/MLC Determination of Test Agents

To rule out the possibility that various fluorides or controls may have antiplaque properties at low concentrations due to their ability to kill or completely suspend growth of plaque forming bac-

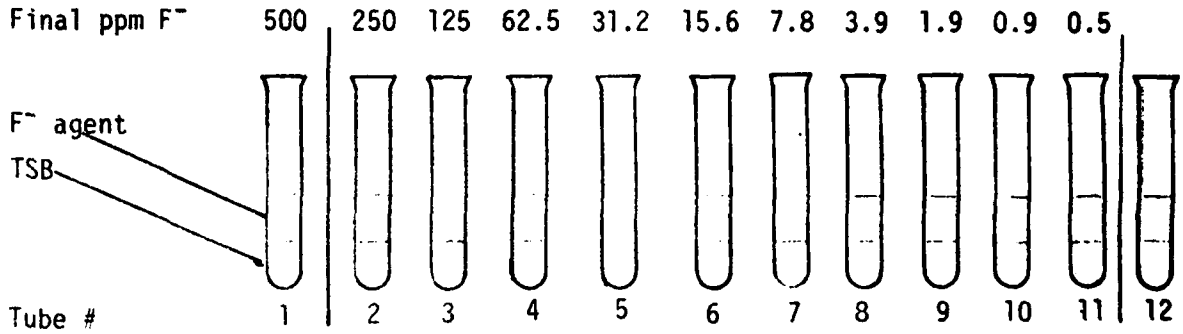

FIGURE 3. Example of a MIC and MLC dilution. First, 1.0 ml TSB is added to all tubes. Then, test agents are added to tube #1 and subsequently diluted with TSB starting with tube #2 and ending at tube #11. Tubes #1 and #12 are the uninoculated and inoculated controls. After serial dilution of the test agents, a 1.0 ml inoculum of standardized suspension is added to all tubes except #1.

teria; the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of these agents (Barry, 1976) was determined.

S. mutans NCTC 10449 was adapted to growth in trypticase soy broth (TSB; BBL) and 0.1 ml of the adapted strain was transformed to 10 ml of the medium. The turbidity of growth was standardized and diluted 1:200 to produce an inoculum containing approximately $5.0X10^5$ CFU/ml. A 1.0 ml aliquot of the ptoential antimicrobial agents, SnF_2, $SnCl_2$, TiF_4, Na_2SnF_6 and NaF was serially diluted in 1 ml of uninoculated TSB such that each test tube contained 1/2 the concentration of the agent from the previous tube (Figure 3). To these tubes containing the serial dilutions, 1 ml of the standardized cell suspension was added. The tubes containing the various dilutions of antimicrobial agent plus the inoculum were vortexed, incubated at 37° for 16-18 hours and read.

A large cluster of growth or definite turbidity was considered evidence that the agent failed to inhibit growth at that concentration. Also, due to the potential precipitation of some test agents the visual appearance of positive and negative control tubes was considered before determining the end points (MIC). The MLC was determined by transferring 0.1 ml of broth from each tube of broth showing inhibited growth to blood agar plates and to Mitis salivarius plates. The plates were inspected for growth of S. mutans after 2 days incubation at 37°.

Alteration in Bacterial Growth Due to Low Levels of Fluoride

To determine if the fluoride compounds or controls added to Jordan's medium affected the growth of S. mutans NCTC 10449, growth curves were established and compared to growth of this organism in the presence of NaF, SnF_2, Na_2SnF_6, TiF_4 at 10 and 5 ppm F^- added to this media. Additionally, $SnCl_2$ equimolar with respect to the tin in 10 and 5 ppm F^-

SnF_2 was used to observe any effect of the cation ion on growth. For this procedure duplicate tubes containing the appropriate compound were inoculated with 0.1 ml of the adapted organism, and growth rate was determined at 1 hr. intervals with a spectronic 20 spectrophotonater (Bausch and Lomb, Rochester, New York) at 600 mm. Cultures were maintained at 37° in a Thermomix 1420 shaker water bath (Braun, West Germany) oscilating at 60 cpm. The experiment was terminated at 24 hrs. because of the stability of O.D. readings recorded after this time. Also, initial and terminal pH readings were taken on all cultures. Controls for each agent tested were inoculated medium with the corresponding fluoride or control agent at the same concentration. The test agents and controls were compared for changes in doubling time and final growth of S. mutans.

Alteration in Bacterial Acid Production Due to Low Levels of Fluoride or Controls

Into tubes containing 25 ml of Jordan's medium supplemented with 5% sucrose, NaF, SnF_2, Na_2SnF_6 and TiF_4 was added to produce 10 or 5 ppm F^-, respectively. $SnCl_2$ (Sn equal to Sn^{++} in SnF_2 at 10 and 5 ppm F^-) was also tested to observe the effect of the tin ion alone on bacterial acid production. An appropriate volume of deionized water was added to another tube containing growth medium to serve as the control. The tubes were inoculated with the adapted S. mutans strain. Culture tubes were then incubated in a shaker bath (37°C, 70 cpm). Aliquots were removed from the tubes for pH measurements at intervals during a 48 hr. growth period. The mean pH of duplicate samples for each test agent was plotted against time. Samples were compared for differences in drop in pH and terminal pH.

DNA/Glucan Analysis

Jordan's medium supplemented as previously described with 10 ppm F^- of NaF, SnF_2, Na_2SnF_6, TiF_4 or 10 ppm Cl^- of $SnCl_2$ (Sn = Sn in 10 ppm F^- SnF_2) were placed into tubes containing the suspended enamel cylinders and inoculated with 0.1 ml of an adapted S. mutans culture. Sterile deionized water served as the control agent. The enamel specimens were transferred after 24 hrs. to fresh Jordan's medium supplemented with 5% sucrose and containing the appropriate fluoride agent or control (Figure 4).

After 3 day growth in the appropriate media, the wax was peeled off each enamel slab leaving only the attached bacteria to the surface enamel. The enamel specimens were then sonicated (Bronson Model w 185) with a microprobe tip in deionized water for 30 sec. at 50 watts with the output at 4. This procedure separates the plaque from the enamel and disrupts bacterial chains (Liljemark and Schauer, 1977).

After washing and centrifugation (9.000xG, 10 min. 0°C) of the bacterial sample three times, the sample was resuspended in a known volume of deionized water. An aliquot of the suspended sample and the spent supernatant was saved for glucan analysis (Figure 5). The remained of the suspended pellet was added to 0.5 N hot perchloric acid (70°C, 30 min.) to extract the bacterial DNA (Ogur and Rosen, 1950). The DNA analysis enables quantitation of bacterial mass. Also, the media from which the enamel specimens were removed after the final enamel specimen transfer was prepared to analyze DNA and glucan content as described above.

After neutralization with KOH, DNA was analyzed (Burton, 1956) using a 2-deoxy-D-ribose as a standard. DNA analysis involved centrifuging the sample as described. The supernatant containing the extracted

DNA was then removed for analysis. From the sample, a 1 ml aliquot was combined with 2 ml of diphenylamine reagent, vortexed, covered, and incubated at 37°C overnight. Two-Deoxy ribose standards and blanks were also treated in a like fashion. Optical densities of the samples and standards were read at 600 nm with a Gilford Microsample 300 Spectrophotometer, and converted to µg/ml DNA. All samples and standards were analyzed in duplicate. In conjunction with DNA analysis, all samples were prepared to determine glucan production (Figure 5). Alkali and water soluble glucan (µg/ml) was determined at 410 nm with Glucostat reagent (Worthington Biochemicals, Freehold, NJ) dissolved in 500 mM tris phosphate buffer (pH 7) after incubation for 60 min. at 37°C. Hydrolized commercial dextran ranging from 0 to 50 µg/ml served as standards (Freedman and Tanzer, 1974; Freedman and Coykendall, 1975). All samples and standards were analyzed in duplicate.

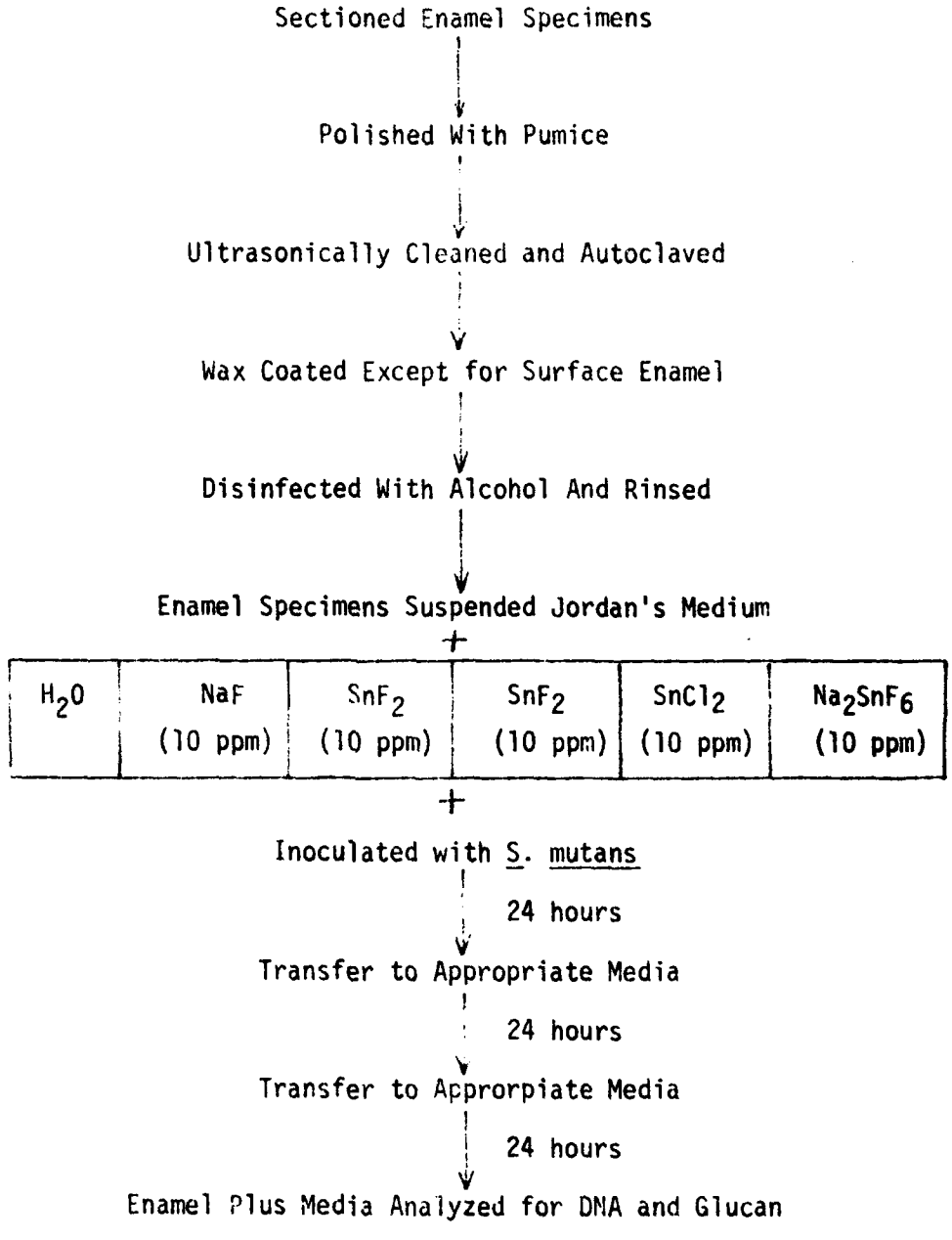

FIGURE 4. Experimental design for DNA and Glucan Analysis.

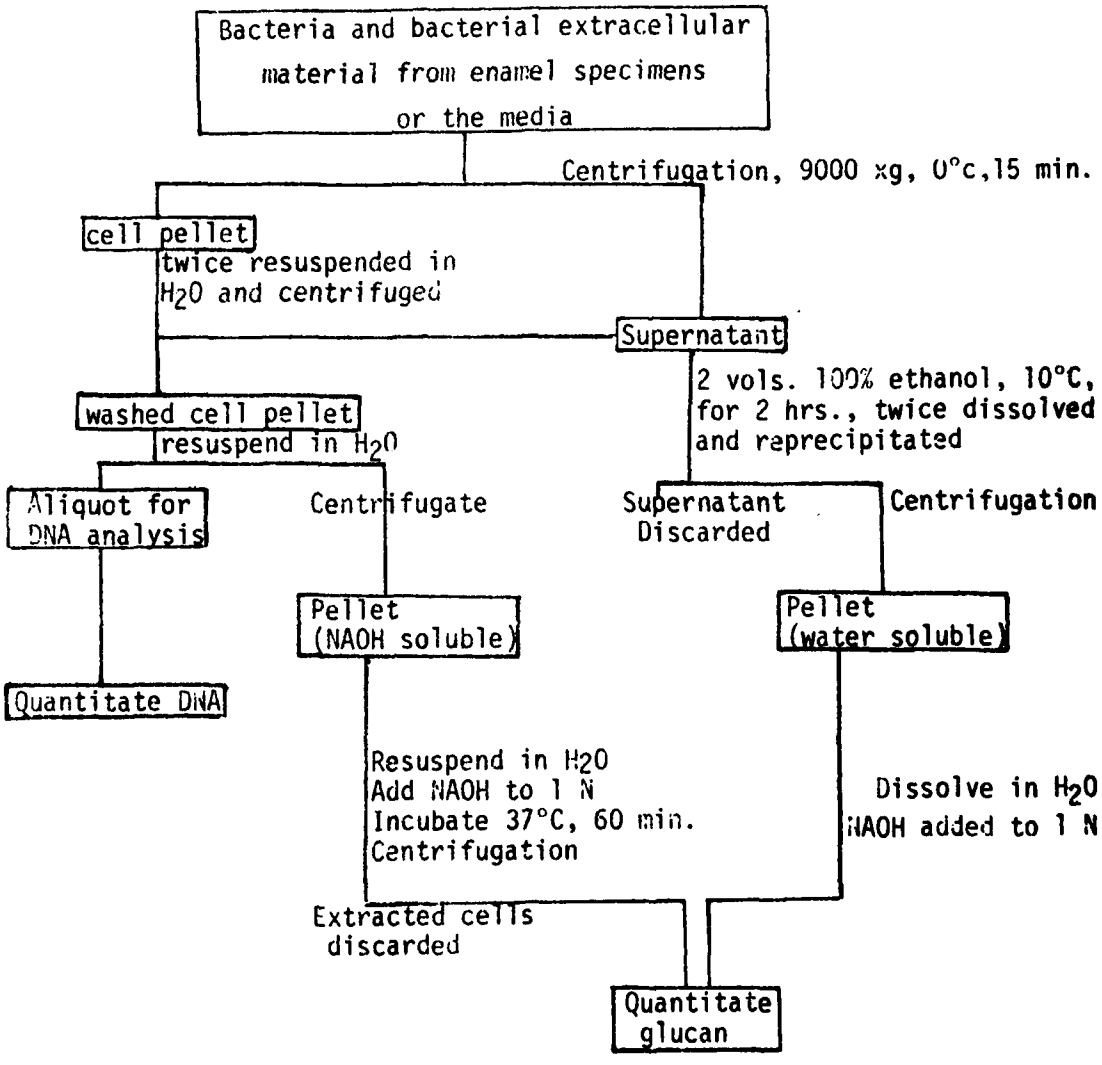

Figure 5. Flow diagram of the preparation of cells and culture liquor for glucose and DNA quantitations. All centrifugations: 9000 xg, 10 min. 0°C.

Determination of Enamel Surface Area

The surface area of the enamel slabs exposed to the various test agents or controls was calculated by placing black and white photographic negatives, determined to be a 1:1 representation of the enamel specimens, over mm^2 blocked graph paper. The number of mm^2 blocks contained within the outline of the enamel specimen negative is equivalent to the surface area of the specimen. The surface area of the enamel exposed to each test agent was used in determining μg DNA/mm^2 enamel and μg Glucan/mm^2 enamel ratios.

Atomic Absorption Spectrophotometry

After 3 days growth, the bacteria on wires of each treatment group (Figure 1) were pooled into one pre-weighed centrifuge tube, pelleted by centrifugation, and excess water removed. Samples were dried for 3 days at 70° C and the tubes were re-weighed. After the dry weights of the harvested cells are calculated, the samples were suspended in known quantities of 10% HCl. Tin standards ($SnCl_2$, Alfa Division, Danvers, Ma.) were prepared at 0.1, 0.5, 1.0, 10.0, 25.0, 35.0, and 50.0 ppm by dilution with 10% HCl. Tin in the plaque samples and in the standards were measured in triplicate and compared using an atomic absorption spectrophotometer (Perkin-Elmer, Model 403) equipped with a graphite furnace (AGA-74). A deuterium discharge lamp was used to correct for non-atomic absorption signals.

Statistical Analysis

For statistical verification of DNA and Glucan Test results, the mean and standard deviation of the second DNA/Glucan experiment (3 samples, test or control group) was calculated. An analysis of variance indicated statistically significant differences ($p < .01$). Individual comparisons

of the test agents on controls was performed using the Scheffe procedure (Scheffe, 1953) to establish homogeneous subsets at the .01 level.

Determination of Fluoride Levels in Growth Median

Aliquots of the media supplemented with the various fluoride compounds at 10 ppm F^- (W/V) were analyzed for fluoride on activity immediately after the additions of the fluoride compounds, after inoculation of the media for one day, and after one day growth of S. mutans cultures in the media.

The water and $SnCl_2$ supplemented media showed no fluoride ion present. NaF, Na_2SnF_6 and SnF_2 supplemented media had 10 ppm F^- present in fresh medium. After 24 hours incubation, however, all three of these agents showed a decrease of approximately 1 ppm F^- in both inoculated and uninoculated medium. TiF_4 was the only compound tested which did not show fluoride electrode measurements as being equal to theoretical levels. A theoretical level of 10 ppm F^- displayed a fluoride ion measurement of only 2.3 ppm F^- in fresh medium and after 24 hours incubation of inoculated and uninoculated medium (Table 1).

MIC/MLC Determinations

The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) tests showed that SnF_2 had the lowest MIC and MLC, 60 ppm F^- and 125 ppm F^- respectively (Tables 2 and 7). TiF_4 had a MIC at 525 ppm F^- and a MLC at 575 ppm F^- (Tables 3 and 7); NaF had MIC at 300 ppm F^- and a MLC at 3000 ppm F^- (Tables 4 and 7); Na_2SnF_6 had a MIC at 600 ppm F^- and MLC at 675 ppm F^- (Tables 5 and 7). $SnCl_2$ was found to have a MIC at 200 ppm Cl^- and a MLC at 225 ppm Cl^- (Tables 6 and 7).

With respect to the tin ion concentration of the compounds tested, SnF_2 had a MIC of 180 ppm Sn while $SnCl_2$ and Na_2SnF_6 both had a MIC of 600 ppm Sn, and SnF_2 had a MLC of 375 Sn while $SnCl_2$ and Na_2SnF_6 had a MLC of 675 ppm Sn (Table 7).

	ppm F		
	Fresh uninoculated medium	Uninoculated medium after incubation for 24/hrs at 35° C	Inoculated medium after 24/hrs growth of S. mutans NCTC 10449 at 35°C
Control	> 0.3	> 0.3	> 0.3
$SnCl_2$	> 0.3	> 0.3	> 0.3
TiF_4	2.3	2.3	2.3
NaF	10.0	9.0	9.0
Na_2SnF_6	10.0	8.8	8.8
SnF_2	10.0	9.0	9.0

Table 1. Parts per million fluoride ion in inoculated and uninoculated Jordan's medium supplemented with fluoride components or controls.

MIC/MLC for SnF_2

ppm F^-	Culture Vial (MIC)	MSS plate (MLC)	BA plate (MLC)
250	NG, P	NG	NG
125	NG, P	NG	NG
100	NG, P	<G	<G
90	NG, P	<G	<G
80	NG, P	<G	<G
70	NG, P	<G	<G
60	NG, P	G	G
50	G, P	G	G
40	G, P	G	G
30	G, P	G	G
20	G, P	G	G
10	G, P	G	G
5	G, P	G	G
2.5	G, NP	G	G
1.8	G, NP	G	G
0.9	G, NP	G	G
0.5	G, NP	G	G

Key: NG = No Growth
<G = Less Growth
G = Growth
P = Precipitate
NP = No Precipitate

Table 2: Dilutions of SnF_2 ranging from 0.5 to 250 ppm F^- were evaluated for a minimum inhibitory concentration (MIC) and a minimum lethal concentration (MLC) against S. mutans NCTC 10449. A MIC for SnF_2 of 60 ppm F^- was determined by the culture vial of the lowest dilution showing no growth; a MLC for SnF_2 of 125 ppm F^- was determined by the lowest concentration on BA and MSS plates showing no growth. None of the control vials or plates exhibited growth and contaminants were noted. A milky white precipitate was present in both the control and test dilutions at 5 ppm F^- and above.

MIC/MLC for TiF_4

ppm F^-	Culture Vial (MIC)	MtS plate (MLC)	BA plate (MLC)
1000	NG, P	NG	NG
900	NG, P	NG	NG
800	NG, P	NG	NG
700	NG, P	NG	NG
650	NG, P	NG	NG
625	NG, P	NG	NG
600			
575±25	NG, P	NG	NG
550±25	NG, P	G	G
525			
500	G, P	G	G
475	G, P	G	G
450	G, P	G	G
400	G, P	G	G
300	G, P	G	G
200	G, P	G	G
100	G, P	G	G

Key: NG = No Growth
G = Growth
P = Precipitate

Table 3: Dilutions of TiF_4 ranging from 100 to 1000 ppm F^- were evaluated for MIC and MLC against S. mutans. In all test runs, the MIC and MLC was found within the 525 to 600 ppm F^- range. The MIC of TiF_4 was 550±25 ppm F^-; the MLC of TiF_4 was 575± 25 ppm F^-. None of the control vials or plates exhibited growth and no contamination was noted on culture plates of test dilutions. A cloudy gray-white precipitate was present in all test and control dilutions.

MIC/MLC for NaF

ppm F^-	Culture Vial (MIC)	MSS plate (MLC)	BA plate (MLC)
5000	NG	NG	NG
4500	NG	NG	NG
4000	NG	NG	NG
3500	NG	NG	NG
3250	NG	NG	NG
3000	NG	NG	NG
2750	NG	<<<G	<<<G
2500	NG	<<<G	<<<G
2000	NG	<<G	<<G
1500	NG	<<G	<<G
1000	NG	<G	<G
900	NG	<G	<G
800	NG	<G	<G
700	NG	<G	<G
600	NG	<G	<G
500	NG	<G	<G
400	NG	G	G
350	NG	G	G
300	NG	G	G
250	G	G	G
200	G	G	G
150	G	G	G
100	G	G	G
50	G	G	G

Key: NG = No Growth
<G = Less Growth
G = Growth

Table 4: Dilutions of NaF ranging from 50 to 5000 ppm F^- were evaluated for MIC and MLC against S. mutans. The MIC for NaF was 300 ppm F^-; the MLC for NaF was 3000 ppm F^-. Plated dilutions of NaF ranging from 500 to 2750 ppm F^- exhibited decreased growth on BA and MSS plates after 24 hours incubation at 35° C. None of the control vials or plates exhibited growth. No contamination was noted on culture plates of test dilutions.

MIC/MLC for Na_2SnF_6

ppm F^-	Culture Vial (MIC)	MHA plate (MLC)	BA plate (MLC)
1000	NG	NG	NG
[illegible]	[illegible]	[illegible]	[illegible]
800	NG	NG	NG
700	NG	NG	NG
675	NG	NG	NG
650	NG	<G	<G
625	NG	<G	<G
600	NG	G	G
575	<G	G	G
550	G	G	G
525	G	G	G
500	G	G	G
475	G	G	G
400	G	G	G
300	G	G	G
200	G	G	G
100	G	G	G

Key: NG = No Growth
<G = Less Growth
G = Growth

Table 5: Dilutions of Na_2SnF_6 ranging from 100 to 1000 ppm F^- were evaluated for MIC and MLC against S. mutans. The MIC of Na_2SnF_6, was 600 ppm F^-; the MLC of Na_2SnF_6, was 675 ppm F^-. None of the control vials or plates exhibited growth. No contamination was noted on culture plates of test dilutions.

MIC/MLC for $SnCl_2$

ppm Sn*	Culture Vial (MIC)	MSS plate (MLC)	BA plate (MLC)
1000	NG, P	NG	NG
900	NG, P	NG	NG
800	NG, P	NG	NG
700	NG, P	NG	NG
600	NG, P	NG	NG
500	NG, P	NG	NG
400	NG, P	NG	NG
300	NG, P	NG	NG
250	NG, P	NG	NG
200	NG, P	G	G
175	<G, P	G	G
150	G, P	G	G
125	G, P	G	G
100	G, P	G	G
75	G, P	G	G
50	G, P	G	G

Key: NG = No Growth
<G = Less Growth
G = Growth
P = Precipitate

*ppm equivalent to Sn^{++} in a SnF_2 solution of equal concentration

Table 6: Dilutions of $SnCl_2$ ranging from 50 to 1000 ppm Cl^-, were evaluated for MIC and MLC. The MIC of $SnCl_2$, was 200 ppm Cl^-; the MLC of $SnCl_2$, was 225 ppm Cl^-. None of the control vials or plates exhibited growth. No contamination was noted on culture plates of test dilutions. A cloudy yellow-white precipitate was present in all control and test dilution vials.

Test Compound	MIC		MLC	
	ppmF^-	ppmSn	ppmF^-	ppmSn
SnF_2	60	180	125	375
$SnCl_2$	(200 ppmCl^-)	600	(225 ppmCl^-)	675
Na_2SnF_6	600	600	675	675
NaF	300		3000	
TiF_4	550 ± 25		575 ± 25	

Table 7: Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of different fluoride compounds or controls on S. mutans NCTC 10449.

Alterations in Bacterial Acid Production Due to Low Levels of Fluoride

Acid production of S. mutans incubated in medium supplemented with various fluoride compounds at 10 and 5 ppm F^- or controls were performed in order to observe the effect of these agents at concentrations well below the levels determined to inhibit growth (MIC).

SnF_2, NaF, and Na_2SnF_6 appear to be equally effective in altering the terminal pH of the S. mutans cultures both at 5 ppm F^- and 10 ppm F^- concentrations (Figures 6 and 7). At 5 ppm F^- concentration of these compounds, the terminal pH of SnF_2, NaF and Na_2SnF_6 supplemented media was approximately pH 4.8 whereas the $SnCl_2$ supplemented media and water control had a terminal pH of 4.3. When the media was supplemented with 10 ppm SnF_2, NaF, or Na_2SnF_6 the terminal pH only reached 5.0.

TiF_4 at both 5 and 10 ppm F^- showed little or no effect on acid production. This may be due to the fact that the actual levels of F^- in solution for this compound were found to be much lower than the theoretical levels.

All experiments were performed in duplicate runs and slight variations were averaged. Note that the addition of the test compounds did not produce an initial pH change of the media when compared to the control medium.

Alteration in Bacterial Growth Due to Low Levels of Fluoride or Controls

Alterations in growth rate of S. mutans in medium supplemented with various fluoride compounds at 10 and 5 ppm F^- or controls were evaluated in order to observe the effect of these agents at concentrations well below the levels determined to inhibit growth (MIC).

The results of the growth curves performed in duplicate suggest little change in the doubling time of the organisms exposed to the test

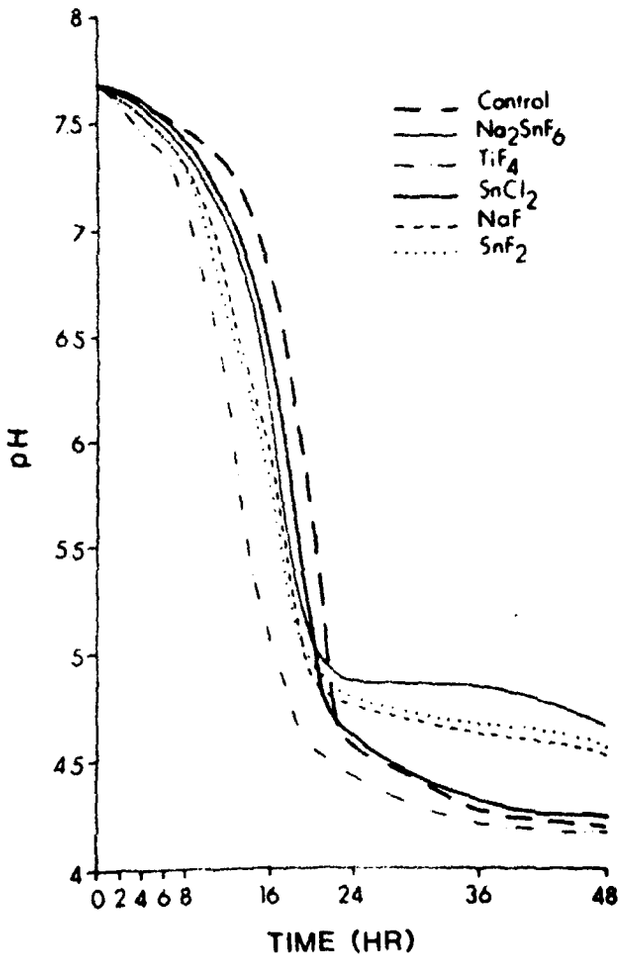

Figure 6: Acid production by S. mutans NCTC 10449 in medium supplemented with various fluoride compounds (5 ppm F^-).

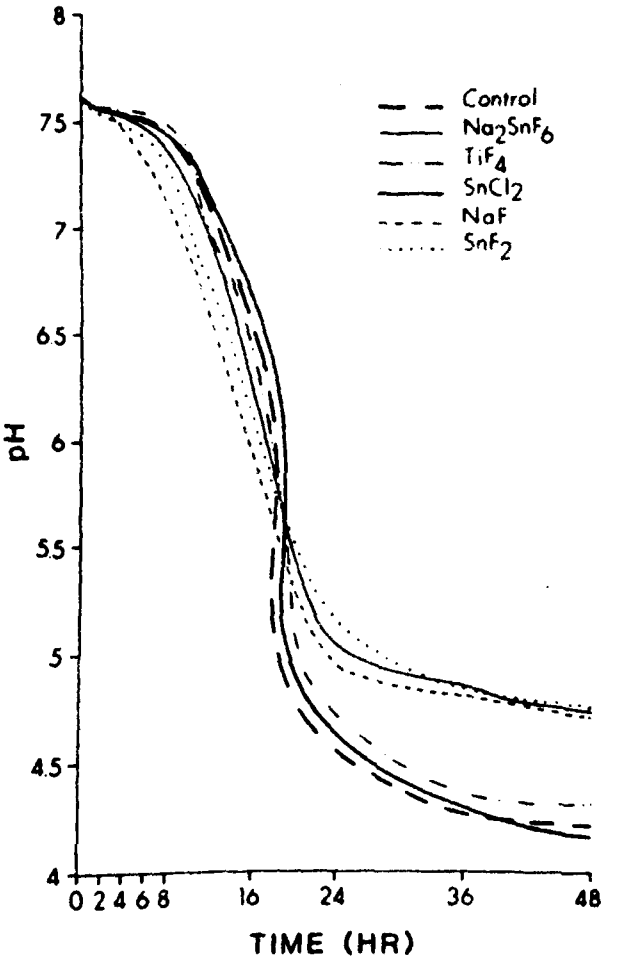

Figure 7: Acid production by S. mutans NCTC 10449 in medium supplemented with various fluoride compounds (10 ppm F^-).

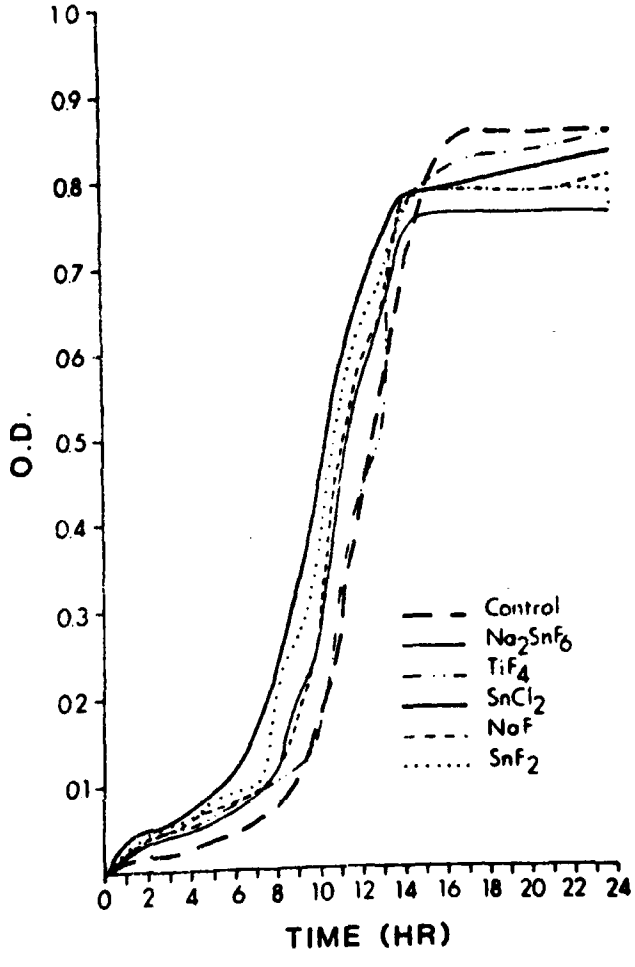

Figure 8: Growth of S. mutans NCTC 10449 in medium supplemented with various fluoride compounds (5 ppm F^-).

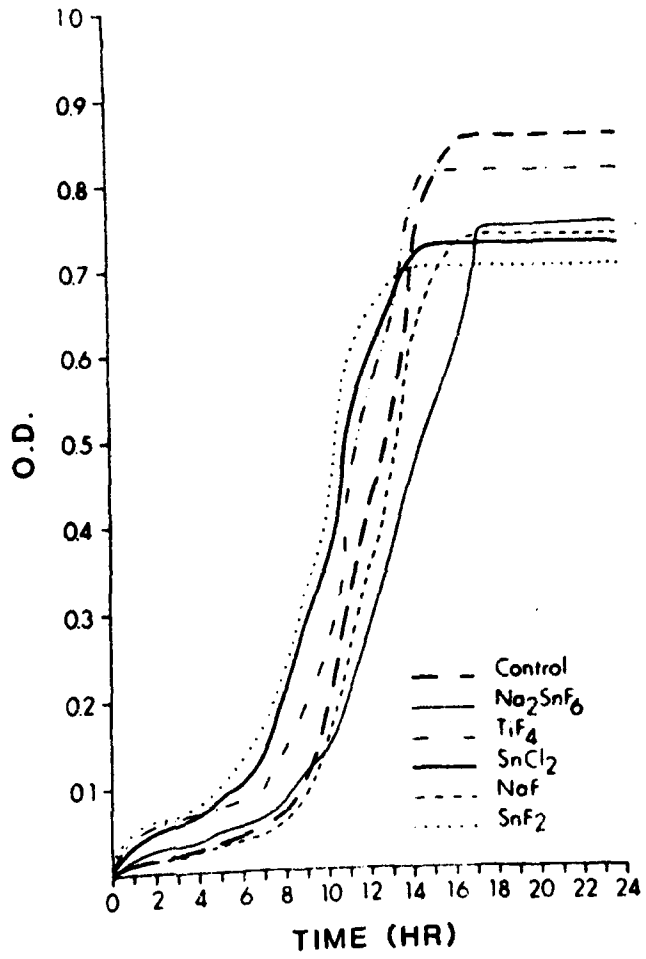

Figure 9: Growth of S. mutans NCTC 10449 in medium supplemented with various fluoride compounds (10 ppm F^-).

agents at 5 or 10 ppm. At 5 ppm, no distinct difference in final bacterial growth were seen between the control and the test compounds, which exhibited greater reduction in growth compared to the control (Figure 8). However, at 10 ppm, the final bacterial growth appears less in all media supplemented with either fluoride or tin compounds (Figure 9) with SnF_2 showing the greatest effect.

DNA/Glucan Analysis

DNA and glucan analysis were performed on bacteria attached to suspended enamel specimens and bacteria present in the surrounding media after three days growth in media supplemented with 10 ppm fluoride or controls. This series of experiments was performed twice. Initially, testing was done on samples run in duplicate. In order to verify these results and to allow a statistical appraisal using subset grouping through analysis of variance with the Scheffé procedure, these experiments were repeated using three samples per test group.

Results in both experiments show a decrease in the amount of DNA and alkali soluble glucan (ASG) attached to enamel for SnF_2, Na_2SnF_6, and NaF compared to other compounds tested, with SnF_2 showing the greatest decrease (Table 8,12); yet no significant difference in µg ASG/µg DNA suggests that the lower glucan production found in the fluoride test groups is due to fewer bacteria in these groups (Table 12).

When the total amount of DNA per ml medium (attached and unattached bacteria) of the various test groups was compared, all fluoride test agents had less DNA/ml medium than the H_2O control, with the SnF_2 supplemented medium producing the greatest decrease in bacterial quantity (Table 9, 13). When total µg ASG/µg DNA ratios were evaluated, it was found that SnF_2, Na_2SnF_6 and NaF exhibited an increase in alkali soluble

glucans compared to other test groups. As well, the water soluble glucan (µg WSG/µg DNA) showed the greatest increase in those tubes supplemented with SnF_2, NaF or Na_2SnF_6 (Table 9,13). By looking at just the ASG present in the culture tube, there is further evidence that increased ASG production of the unattached bacteria by S. mutans, under the influence of SnF_2, NaF, or Na_2SnF_6, occurs (Table 10, 14).

Tin Analysis

After 3 days growth, the bacteria on the wires of each treatment group (Figure 1) were analyzed for tin using atomic absorption spectrophotometry. As expected, no tin was detected in the control, TiF_4, and NaF treatment groups. The bacteria exposed to compounds containing tin were found to have tin present. The plaque incubated in SnF_2 supplemented media had more tin per mg. dry plaque than those specimens cultured in $SnCl_2$ or Na_2SnF_6 supplemented media (Table 11, 15).

µg DNA/mm^2 enamel*		µg ASG/mm^2 enamel		µg ASG/µg DNA	
Control	0.18 ± .03	Control	1.02 ± .01	NaF	7.06 ± .64
$SnCl_2$	0.18 ± .003	TiF_4	0.99 ± .05	TiF_4	6.31 ± .06
TiF_4	0.16 ± .02	$SnCl_2$	0.97 ± .12	SnF_2	5.79 ± .15
Na_2SnF_6	0.14 ± .02	NaF	0.86 ± .19	Na_2SnF_6	5.77 ± .27
NaF	0.12 ± .02	Na_2SnF_6	0.84 ± .07	Control	5.67 ± .30
SnF_2	0.09 ± .01	SnF_2	0.55 ± .06	$SnCl_2$	5.19 ± .73

*Mean of 2 samples

Table 8. Experiment 1. Amount of bacteria (DNA) and Alkali Soluble Glucan (ASG) adherent to enamel after three day's incubation of S. mutans NCTC 10449 in medium supplemented with various fluoride compounds (10 ppm F^-) or controls.

Total µg DNA/ml*	
$SnCl_2$	4.6 ± 0.5
Control	3.7 ± 0.7
NaF	3.2 ± 0.1
Na_2SnF_6	3.1 ± 0.6
TiF_4	3.0 ± 0.6
SnF_2	2.3 ± 0.3

*Mean of 2 samples ± S.D.

Total µg ASG/ml		Total µg ASG/µg DNA	
NaF	47.0 ± 6.8	NaF	14.8 ± 1.7
Na_2SnF_6	44.2 ± 0.9	Na_2SnF_6	14.4 ± 2.4
$SnCl_2$	35.8 ± 1.0	SnF_2	13.3 ± 0.7
TiF_4	31.7 ± 3.5	TiF_4	10.9 ± 0.9
SnF_2	31.4 ± 1.6	Control	8.3 ± 0.6
Control	30.7 ± 3.3	$SnCl_2$	7.8 ± 0.04

Total µg WSG/ml		Total µg WSG/µg DNA	
SnF_2	28.9 ± 1.9	SnF_2	12.3 ± 2.0
Na_2SnF_6	22.4 ± 0.6	Na_2SnF_6	7.3 ± 1.5
NaF	20.9 ± 1.3	NaF	6.6 ± 0.2
TiF	5.3 ± 1.1	TiF	1.8 ± 0.01
$SnCl_2$	3.9 ± 2.3	$SnCl_2$	0.9 ± 0.05
Control	2.3 ± 1.2	Control	0.6 ± 0.2

Table 9. Experiment 1. Total amount of bacteria (DNA), Alkali Soluble Glucan (ASG), and Water Soluble Glucan (WSG) adherent to enamel and the culture tube after three day's incubation of S. mutans NCTC 10449 in medium supplemented with fluoride compounds (10 ppm F^-) or controls.

	µg ASG/ml*
Na_2SnF_6	30.5 ± 2.1
NaF	28.6 ± 2.7
SnF_2	21.4 ± 0.4
TiF_4	14.7 ± 0.4
$SnCl_2$	14.1 ± 3.3
Control	11.7 ± 0.5

*Mean of 2 samples ± S.D.

Table 10. Experiment 1. Alkali Soluble Glucan (ASG) present in the culture tube after three days incubation of S. mutans NCTC 10449 in medium supplemented with various fluoride compounds (10 ppm F^-) or controls.

	Plaque Dry Weight (mg)	Sn^0/Total Sample (ppm)	Sn^0/mg Plaque (µg)
Control	0.7	N.D.	N.D.
Na_2SnF_6	0.7	19	13
TiF_4	1.3	N.D.	N.D.
$SnCl_2$	0.8	42	26
NaF	0.7	N.D.	N.D.
SnF_2	0.7	66	48

*Pooled plaque from 2 samples.

Table 11. Experiment 1. Bacteria harvested from the wires suspending the enamel specimens in each treatment group. Samples were pooled, dried, and analyzed for tin using Atomic Absorption Spectrophotometry.

	μg DNA / mm² Enamel*	Subsets**		μg ASG / mm² Enamel*	Subsets		μg ASG / μg DNA	Subsets
Control	0.17 ± .02		TiF_4	1.35 ± .43		TiF_4	9.02 ± 1.24	
$SnCl_2$	0.15 ± .00		$SnCl_2$	1.19 ± .05		$SnCl_2$	7.42 ± 0.85	
TiF_4	0.14 ± .03		Control	1.10 ± .16		NaF	7.18 ± 0.70	
Na_2SnF_6	0.12 ± .02		NaF	0.83 ± .19		SnF_2	6.72 ± 1.28	
NaF	0.11 ± .02		Na_2SnF_6	0.62 ± .04		Control	6.39 ± 0.59	
SnF_2	0.05 ± .00		SnF_2	0.32 ± .09		Na_2SnF_6	5.37 ± 0.95	

* Mean of 3 samples ± S.D.
** Homogeneous subsets using Analysis of Variance with Scheffe procedure ($p \leq .01$)

Table 12. Experiment 2. Amount of bacteria (DNA) and Alkali Soluble Glucan (ASG) adherent to enamel after 3 days incubation of S. mutans NCTC 10449 in medium supplemented with various fluoride compounds (10 ppm F^-) or controls.

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Total μg DNA/ml *		Subsets **
Control	3.56 ± .41	
NaF	2.87 ± .86	
Na_2SnF_6	2.72 ± .75	
TiF_4	2.66 ± .57	
$SnCl_2$	2.52 ± .20	
SnF_2	1.27 ± .11	

Total μg ASG/ml		Subsets
Na_2SnF_6	58.1 ± 12.0	
NaF	55.2 ± 2.2	
SnF_2	55.1 ± 13.0	
TiF_4	33.0 ± 7.9	
Control	32.9 ± 3.8	
$SnCl_2$	21.7 ± 2.6	

Total μg ASG/μg DNA		Subsets
SnF_2	33.2 ± 3.6	
Na_2SnF_6	23.3 ± 10.7	
NaF	20.2 ± 5.3	
TiF_4	12.4 ± 0.45	
Control	9.3 ± 1.3	
$SnCl_2$	8.6 ± 0.81	

Total μg WSG/ml		Subsets
NaF	57.5 ± 7.8	
SnF_2	56.6 ± 10.5	
Na_2SnF_6	47.2 ± 8.3	
TiF_4	17.7 ± 4.8	
Control	15.5 ± 0.9	
$SnCl_2$	10.9 ± 4.0	

Total μg WSG/μg DNA		Subsets
SnF_2	45.0 ± 11.3	
NaF	21.4 ± 7.2	
Na_2SnF_6	14.1 ± 3.9	
TiF_4	6.7 ± 1.6	
$SnCl_2$	4.2 ± 1.3	
Control	4.0 ± 0.48	

* Mean of 3 samples ± S.D.

** Homogeneous subsets using Analysis of Variance with Scheffe procedure ($p \leq .01$)

Table 13. Experiment 2. Total amount of bacteria (DNA), Alkali Soluble Glucan (ASG), and Water Soluble Glucan (WSG) adherent to enamel and the culture tube after 3 days incubation of S. mutans NCTC 10449 in medium supplemented with various fluoride compounds (10 ppm F^-) or controls.

	μg ASG/ml*	Subsets**
Na_2SnF_6	47.9 ± 11.8	|
NaF	42.3 ± 1.3	|
SnF_2	35.8 ± 3.7	
TiF_4	11.2 ± 3.6	|
Control	11.2 ± 5.0	|
$SnCl_2$	3.1 ± 0.8	

* Mean of 3 samples ± S.D.

** Homogenous subsets using Analysis of Variance with Scheffe procedure ($p \leq .01$)

Table 14. Experiment 2. Alkali Soluble Glucan (ASG) present in the culture tube after three day's incubation of S. mutans NCTC 10449 in medium supplemented with various fluoride compounds (10 ppm F^-) or controls.

	Plaque Dry Weight (mg)	Sn^{+}/ Total Sample (ppm)	Sn^{+}/ mg. plaque (μg)
Control	1.8	N.D.	N.D.
Na_2SnF_6	1.5	6	4
TiF_4	3.0	N.D.	N.D.
$SnCl_2$	2.4	48	20
NaF	1.7	N.D.	N.D.
SnF_2	1.4	47	34

Table 15. Experiment 2. Bacteria harvested from the wires suspending the enamel specimens in each treatment groups. Samples were pooled, dried, and analyzed for tin using Atomic Absorption Spectrophotometry.

Discussion

Fluoride electrode measurements confirmed that the Jordan's medium, water, and $SnCl_2$ controls were essentially free of fluoride ion. Also, NaF, SnF_2 and Na_2SnF_6 had initial fluoride levels as expected according to theoretical formulations but that after 24 hours incubation, fluoride levels of these agents dropped approximately 1 ppm F^- in both inoculated and uninoculated medium. This may indicate that some of the free fluoride ion present may become organically bound. Unexpectedly, TiF_4 solutions did not equal the theoretical F^- levels. Possible release of HF when this compound is added to water or the hydroscopic nature of this compound may account for the difference between theoretical and actual F^- levels. The low fluoride levels of this compound in this study limits its value as a test agent.

The MIC/MLC determinations were performed to rule out the possibility that various fluorides may have antiplaque properties at low concentration (10 ppm) due to their ability to kill or suspend growth of plaque forming bacteria. SnF_2 had the lowest fluoride and tin ion concentration needed for both the minimum inhibitory and minimum lethal effect. When the test compounds are compared with respect to ppm F^-, SnF_2 had a MIC of 60 ppm F^- and a MLC of 125 ppm F^-. All other fluoride compounds exhibited a MIC of greater than 300 ppm F^- and a MLC greater than 575 ppm F^-. Confirmatory experiments were consistent for all compounds except TiF_4 which may be due to the instability of this agent. With respect to the tin ion concentration of the compounds tested, SnF_2 had an MIC of 100 ppm Sn while $SnCl_2$ and Na_2SnF_6 both had a MIC of 600 ppm Sn; and SnF_2 had a MLC of 375 ppm Sn while $SnCl_2$ and Na_2SnF_6 had a MLC of 675 ppm Sn. Since the lowest inhibitory concentration of the agents tested

was found to be 60 ppm F^-, other findings to be presented cannot be ascribed to the ability of these fluoride compounds to kill or suspend the growth of S. mutans.

An interesting observation was that NaF had a wide range between bacteriostatic and bactericidal values; whereas, the other compounds tested, all of which contained heavy metals, had a much narrower range between inhibition and lethality. The greater bactericidal effect of metal containing compounds on bacterial cells is compatible with those effects noted for heavy metals (Salle, 1968).

The high bacteriostatic and bactericidal activity observed for SnF_2 cannot be explained by the separate action of tin alone or fluoride alone, since when this agent is compared to $SnCl_2$, NaF, or Na_2SnF_6 either as fluoride or tin concentration, SnF_2 is apparently more potent. The greater potency of the compound SnF_2 as compared to NaF and SnF_2 has been observed previously (Tinanoff et al., 1976; Tinanoff and Camosci, 1980b) and the mechanism for these differences will be explained by other aspects of this study.

The alteration of acid production by S. mutans seems to be due to the free fluoride ion released from the test agent rather than from the effect of other elements in the compound or from the combined effect of the fluoride ion with other elements in the compound. All of the fluoride agents tested except TiF_4 showed an effect on acid production at 10 ppm F^- and a lesser effect at 5 ppm F^-. Since TiF_4, had one-third the expected F^-, this fluoride agent was consequently less effective.

The effect of sodium fluoride on bacterial metabolism has been known for some time and is relatively well understood. Inhibition of acid production by salivary and plaque bacteria in vitro has been demonstrated with less than 1 ppm F^- (Bibby and von Kesteren, 1940; Wright

and Jenkins, 1954). Furthermore, plaque collected from subjects living in fluoridated areas has shown smaller increases in acid production with sucrose than has plaque from subjects living in nonfluoridated areas (Jenkins et al., 1969). These findings may be explained by the observation that fluoride alters the bacterial enzyme, enolase, which is essential for the degradation of simple sugars to lactic acid and is also essential for the transport of sugars across the bacterial cell membrane (Hamilton, 1977). The inactivation of enolase is the result of fluoride binding with the magnesium component of this enzyme (Warburg and Christian, 1942). Fluoride ions acting in this manner could reduce bacterial-acid production and might account for some of the caries inhibition noted for this agent.

Fluoride at 10 ppm was noted to alter the terminal pH by approximately 0.7 pH units. Since the critical pH needed for enamel dissolution is thought to be in the range of 4.5-5.5 (for review, see Fitzgerald, 1976), it is conceivable that this alteration in pH by fluoride may change the environment from enamel demineralization to remineralization.

In the growth curve experiments, bacterial growth appears less in all media supplemented with either fluoride or tin compounds except for TiF_4 which, as stated previously, is not a reliable test agent because of its hygroscopic nature. This decrease may be due to a fluoride effect on growth for all fluoride compounds tested and a tin effect on growth for $SnCl_2$, and SnF_2, the compound with the greatest decrease in growth, may have a combined tin-fluoride effect.

Miller (1974, 1976) and Kashket et al. (1977) examined how various concentrations of fluoride alter both acid production and glucose uptake in S. faecalis and S. mutans. Results show a decrease in bacterial acid production as low as 0.5 ppm F^-. However, there was no inhibition of

glucose transport across the cell membrane until at least 10 ppm F^- was used. In the present experiments, adverse effect of the agents tested on growth at 10 ppm F^- may be due to the decreased metabolic activity of S. mutans as a result of decreased carbohydrate uptake.

Furthermore, heavy metals, such as tin, are known to be "germicides" because of their ability to precipitate proteins (Salle, 1968), and tin, itself, may be metabolically disruptive accounting for the decreased growth at low concentrations. The apparent combined tin-fluoride effect on growth of S. mutans when subjected to low concentrations of SnF_2 has been suggested by Tinanoff and Camosci (1980) as being a result of tin entering passively into the cell couple with fluoride. This may be one factor relating to the increased antiplaque effects of SnF_2 at low concentrations.

Most studies evaluating the effect of fluoride on bacterial extracellular polysaccharide (EPS) production have observed decreases in EPS synthesis by bacteria under the influence of fluoride ranging from 10 to 70 ppm F^- (Loesche et al., 1973 and 1975; Bowen and Hewitt, 1974). Recently, Treasure and Handelman (1980) looked at EPS/bacterial mass ratios of several strains of S. mutans under the influence of 25 to 50 ppm F^-. In contrast to the earlier studies, they reported increased EPS synthesis under the influence of fluoride. The results of the present study concurs with those of Treasure and Handleman. The apparent conflict may be due to the fact that in the latter two studies the extracellular polysaccharide is reported as EPS/mg bacteria or ug EPS/ug DNA instead of total amount of extracellular material. With the data expressed as a ratio, a better understanding of EPS production due to various agents can be achieved. With regard to the amount of alkali soluble glucan adherent to enamel, this study does not find any differences in the amount of ASG/DNA between test groups or controls

even though there was a decrease in overall bacterial material present on enamel subjected to Na_2SnF_6, NaF, or SnF_2. However, when the total (unattached and attached bacteria) ASG/DNA ratios and ASG/ml (unattached bacteria) ratios are assessed an increase in ASG in medium supplemented with SnF_2, Na_2SnF_6 and NaF ($p < .01$) can be noted. This increase in ASG could be due to either: (1) increased ASG production in the bacteria present in the test tube that was not attached to the enamel specimens, or (2) the ASG produced by the bacteria attached to the enamel did not remain associated with these attached bacteria. Since enamel specimens were transferred daily into fresh uninoculated media, it is interesting to speculate that those bacteria not able to maintain attachment to the enamel specimens had increased ASG production. This is supported by the fact that there was very little unattached bacterial mass present in the control tubes.

Also, there was an overall increase in total water soluble glucan (WSG)/DNA ratios for those test groups exposed to SnF_2, NaF, and Na_2SnF_6. Since this extracellular polysaccharide is not cell associated, it is not possible to determine if increased WSG production is primarily from the unattached or attached bacteria.

The high levels of tin found by atomic absorption in those plaques treated with SnF_2 may be explained by several theories. Rölla (1976) and Svatun et al. (1977) have suggested that tin ions may compete for acidic groups on the bacterial surface, thus allowing accumulation of this cation on the cell wall. Further indirect evidence exists as to the possible reason for the increased tin uptake of SnF_2 vs. $SnCl_2$ intracellularly. Fluoride has been noted to be concentrated in bacterial cells (Jenkins and Edgar, 1969); yet, chloride apparently is not concentrated in bacteria (Mitchell and Moyle, 1959; Schultz et al., 1962). Therefore, an increased accumulation of tin found in bacterial plaque

exposed to SnF_2 may be explained by tin passively entering the cells coupled to fluoride (Tinanoff and Camosci, 1980a). Recently, Tinanoff and Camosci (1980b) have observed tin accumulation in or on cells exposed to SnF_2 as low as 5 ppm F^-. However, these observations were not noted with $SnCl_2$ or Na_2SnF_6. In the present study, there was increased tin uptake with SnF_2 exposure but not with $SnCl_2$ or Na_2SnF_6 exposure and these findings are compatible with previous findings and theories. It should also be noted that whereas $SnCl_2$ and SnF_2 were adjusted for equimolar Sn concentrations, SnF_2 and Na_2SnF_6 were adjusted for equimolar F^- concentrations, not Sn concentrations. This may, in part, account for the decreased tin uptake of Na_2SnF_6 compared to SnF_2.

Summary and Conclusions

From the literature it was apparent that fluoride affects not only enamel solubility, as previously thought, but also affects bacterial growth and attachment. Unfortunately, most of the studies published used only NaF, and only a few reports have compared these antimicrobial effects using different fluoride compounds. In those studies that did compare various fluoride compounds, SnF_2 appeared the most effective agent against bacteria. Furthermore, most of the studies showing antibacterial effects of fluoride have been performed at concentrations of fluoride which could be either bactericidal or bacteriostatic.

To study the effect of low levels of fluoride which could be slowly released into the oral environment, this study investigated the effect the several fluoride compounds on bacterial plaque formation when these compounds were present continuously in the environment. After establishing, through MIC and MLC testing, that 10 ppm F^- of the test agents was well below bacterial growth inhibition or bactericidal levels, the effect of these agents on growth, acid production, extracellular polysaccharide

production, bacterial mass, tin uptake, and attachment to enamel by S. mutans was studied. The results of this series of studies indicate:

1. MIC and MLC values of SnF_2 and the other compounds tested do not account entirely for the effectiveness of these compounds at much lower concentrations.
2. As seen from growth and acid production studies, S. mutans has decreased growth when exposed to all test agents.
3. Although there was a decrease in total bacterial mass (attached and unattached) in the test groups continuously exposed to 10 ppm F^-, antiplaque mechanisms other than bacterial growth inhibition by these agents were suggested.
4. The increased effectiveness of SnF_2 over NaF or $SnCl_2$ as seen in the MIC, MLC, growth curves and DNA results suggest a combined tin-fluoride interaction.
5. The large accumulation of tin found in those organisms treated with SnF_2 may account for the increased effectiveness of SnF_2.
6. Tin uptake by bacteria exposed to $SnCl_2$ may partially explain the bacteriostatic effects of this compound.
7. Even though fluorides, especially SnF_2, affect the amount of bacteria attached to enamel (µg DNA), these attached bacteria showed no change in glucan production (ASG) due to the test agents.
8. An increase in ASG and WSG of the unattached bacteria found for SnF_2, NaF and Na_2SnF_6 test groups suggests that these bacteria have unbalanced growth.
9. Na_2SnF_6, NaF and especially SnF_2 appears to effect the attachment mechanisms of S. mutans under continuous exposure to 10 ppm F^-.

10. SnF_2 appears to be the most effective fluoride compound when present in the environment at low concentrations.

From these quantitative and qualitative in vitro tests of the four fluoride agents and the $SnCl_2$ control, it appears that the continuous exposure of SnF_2 (10 ppm F^-) has the most effect on S. mutans growth, metabolism and attachment. Further in vivo and in vitro investigations to determine if slowly released SnF_2 will prevent plaque formation and pathogenicity is necessary to set the stage for human clinical trials.

Besides using fluorides only for their physicochemical effect on enamel mineral solubility. These studies suggest that a slow release fluoride agent may prove to be an effective method of plaque control.

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Quantitation of Tin in Dental Plaque Exposed to SnF_2 and $SnCl_2$

Introduction

SnF_2 was first noted in 1959 to have antiplaque effects when König reported dramatic plaque inhibition on rat molars with once a day application of a 250 ppm SnF_2 solution. These results were not pursued, possibly due to the belief that the plaque inhibition was due solely to the fluoride ions. However, we and others have shown that SnF_2 is superior to the other fluoride compounds in reducing plaque quantity and in reducing the number of organisms adherent to enamel surfaces *in vitro* (Tinanoff and Camosci, 1980).

There has been some speculation that the antiplaque mechanisms of SnF_2 may be the result of the divaltent cation, tin, reacting with negatively charged components of plaque and thus interfering with adhesion or cohesion (Rölla, 1976). However, because our past *in vitro* studies showed a significantly greater antiplaque effect of SnF_2 over $SnCl_2$, we have felt that the "surface charge theory" could not entirely explain the antiplaque and/or antibacterial properties of SnF_2.

Electron micrographs of bacteria exposed to SnF_2 have shown numerous electron dense granules within the cells and subsequent electron microprobe analysis have confirmed that these granules contained tin.

The amount of tin within the cells appears to be related to the antiplaque activity of SnF_2 and $SnCl_2$. Therefore, the purpose of the present study was to further explore the antiplaque mechanisms of SnF_2 and $SnCl_2$ and to quantitate those findings which shows tin deposits ultrastructurally in those plaques treated with SnF_2.

In addition to measuring the amount of tin deposited within the cells, the following measurements were also included: 1) bacterial acid production, 2) size of plaque deposit and 3) the weight of the plaque deposit.

Materials and Methods

A streptomycin-resistant mutant of Streptococcus mutans NCTC 10449, Bratthall serotype c, known to produce heavy plaque in vitro (Tinanoff, Tanzer and Freedman, 1978), to be cariogenic in rats (Tanzer, J. M., 1979) and to be representative of the most frequently found human serotype in Europe and in the U.S. (Bratthall, 1972; Shklair and Keene, 1973) was used in the experiments.

Stock cultures were maintained by monthly transfer in fluid thioglycollate medium (Difco) supplemented with meat extract (20% v/v) and excess $CaCo_3$. For the experiments, the cultures were adapted to growth in the complex medium of Jordan et al. (1960), (pH 7.5) supplemented with 50 mg of Na_2CO_3/l and containing 5% sucrose.

The following fluoride solutions with appropriate controls, were used to determine their ability to alter colonization of S. mutans on stainless steel wires: SnF_2, 250 ppm F^- (0.10%) pH 3.8; $SnCl_2$ (0.12%) equimolar to Sn in SnF_2 at 250 ppm F^-; NaF, 250 ppm F^- (0.055%) pH 5.3; and H_2O (deionized) pH 6.0.

In one experiment, the pH of SnF_2 and NaF were adjusted to compare the possible effect of undisassociated HF during plaque formation.

Stainless steel wires (0.030") were suspended by rubber stoppers into test tubes containing Jordan's medium. The medium was then inoculated with 0.1 ml of S. mutans and incubated aerobically at 37° C. After one hour of incubation, the tubes were vortexed to ensure even distribution of the organisms on the wires. Eleven hours later, the wires were exposed for one minute to their appropriate agents simulating a mouthrinse regimen (Figure 10). The samples were placed in a standing rinse of deionized water for one minute, and then transfered into fresh medium.

This procedure was repeated every 12 hours for 2 days. After two days, the thickness of the plaques were visually scored by the method of McCabe et al. (1967), and the last transfer broth was measured for bacterial acid production using the pH meter. The plaques were then removed by scraping the deposits off the wires with a platinum loop and depositing each sample into a pre-weighed centrifuge tube. The tubes were centrifuged at 3,000 g for 15 minutes, washed 3 times in deionized water, dried at 70° for 3 days and reweighed.

After the weights of the harvested plaque were calculated, the samples were suspended in known quantities of 10% HCl prior to tin analysis. Tin standards ($SnCl_4$, Alfa Div., Danvers, MA) were prepared at 1.0, 5.0, 10.0, 20.0, 30.0, 40.0 ppm Sn by dilution with 10% HCl. Tin in the plaque samples and in the growth medium was compared to standards using an atomic absorption spectrophotometer (Perkin-Elmer, Model 403) equipped with a grahite furnace (HGA-74). A deuterium discharge lamp was used to correct for non-atomic absorption signals. Preliminary experiments had indicated that the coefficient of variation for tin determinations was $\pm$ 3%. The Sn/mg plaque was calculated by dividing the Sn/total sample by the dry plaque weight.

References for tin analysis seem to be nonexistent, therefore the following chart describes the settings which were developed and used for atomic absorption analysis:

	Dry	Char	Atomize
Ramp time (sec.):	10	20	None
Temperature (C°):	100	600	2700
Length of time (sec.):	32	20	9

Wavelength: 225 nm
Slit width: 4
Argon flow: 70 mm/Hg
Sample volume: 25 µl
Recorder voltage: 10 mv
Recorder scale: 1A
Recorder sensitivity: 2
Auto high temp.: use for organic samples
Switch settings: Absorbance; repeat; 10 ave.

Figure 10: Experimental Design - Initial Plaque Formation

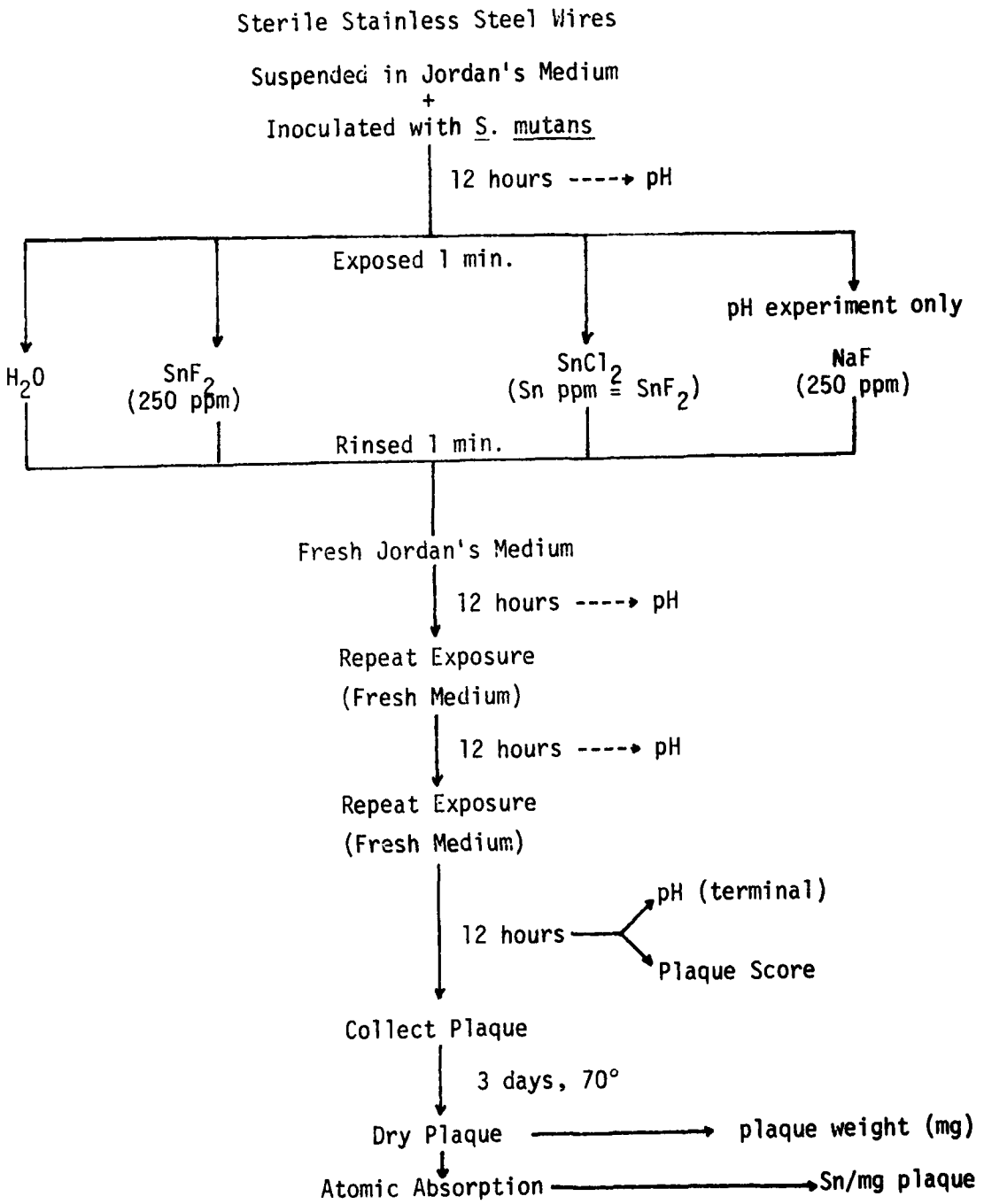
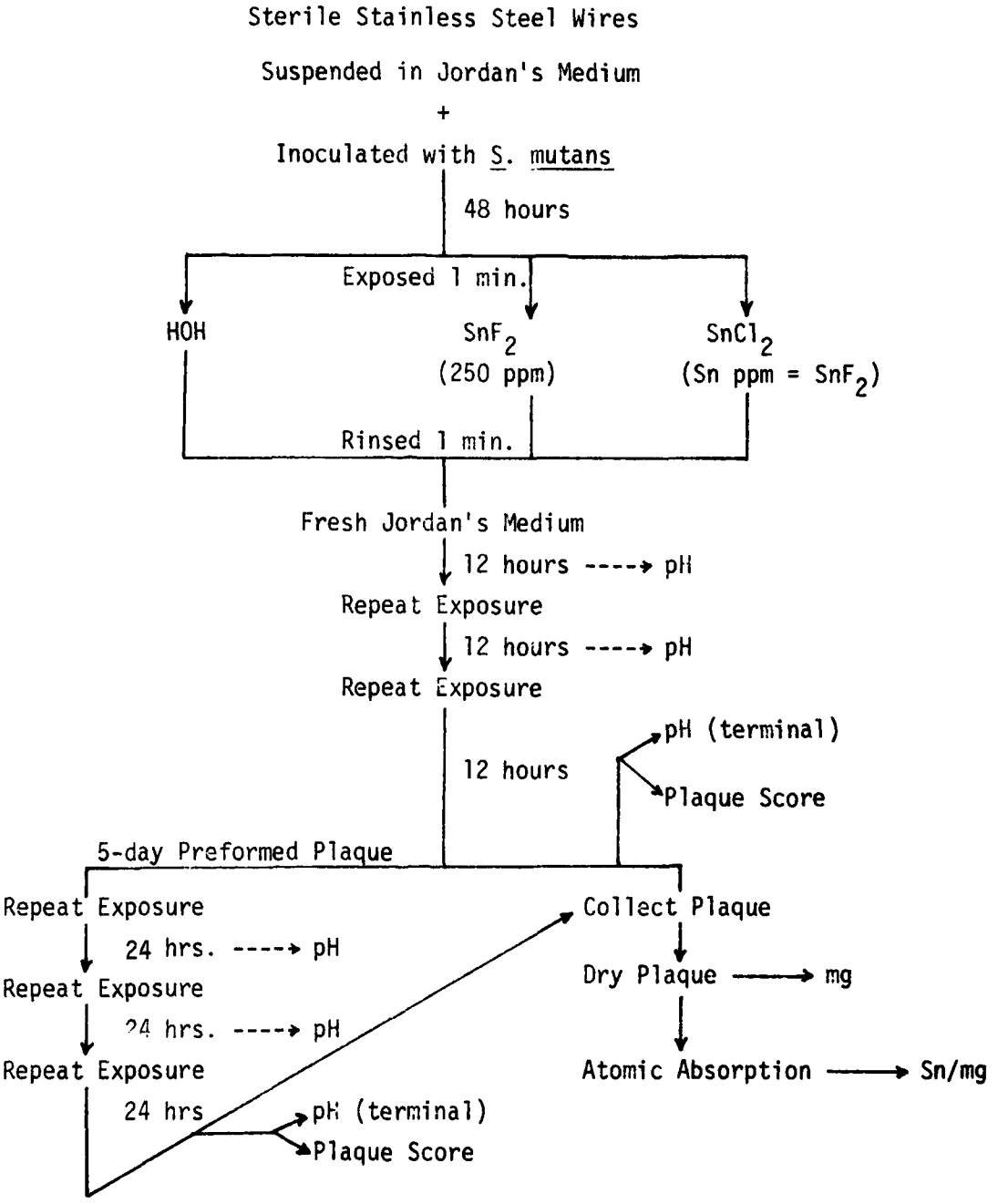


Figure 11: Experimental Design - Preformed Plaque



Results

The first experiment which examined initial plaque formation in the presence of intermittent exposures of SnF_2, $SnCl_2$, or HOH, showed that after 2 days there was very little plaque formation on those specimens exposed to SnF_2 (Table 16). Some variation of plaque thickness was noted on those specimens treated with $SnCl_2$.

These visual plaque scores are numerically converted to <1 for SnF_2, variable thickness for $SnCl_2$ and a score of 4 for the H_2O control. No difference in acid production was found when comparing $SnCl_2$ and H_2O treatments. When the plaque was exposed to SnF_2, the almost non-existent plaque formation prevented any pH change from occurring. Plaque exposed to $SnCl_2$ had 60% less dry weight than the H_2O control. The Sn/total sample is shown to verify the ppm readings recorded by the spectrophotometer. When the amount of tin is divided by the plaque weight, we notice 11 times more tin in those samples exposed to SnF_2 than exposed to $SnCl_2$.

In the second experiment (Table 17), where plaques were performed on the wires and then exposed 1 minute, twice a day to the same agents as described in the previous experiment, we can see no difference between the amounts and consistencies of the plaques in the $SnCl_2$ and H_2O groups. However, a difference was noted in the SnF_2 exposed plaques, both by its final plaque thickness and greater tenacity observed during the removal of plaque from the wires.

The terminal pH measurements from the H_2O and $SnCl_2$ exposed plaques reveal the same rate of acid production, whereas acid production in the SnF_2 exposed plaque is reduced. Again, we see 10 times more Sn/mg plaque in those plaques exposed to SnF_2 when compared to $SnCl_2$.

The third experiment utilizes the same protocol as the second experiment except the intermittent exposures to the plaques continue for 3 more days (once a day for 2 minutes) (Table 18). There were no differences in the terminal pH measurements (24 hrs. growth period), plaque scores, or dry plaque weights between any of the agents or the control in this experiment. However, again we see 6 times more Sn/mg plaque in those plaques expsoed to SnF_2 when compared to $SnCl_2$.

There has been some speculation concerning the effects of fluoride compounds at different pH's. One theory suggests that antiplaque effects of fluorides are due to hydrogen fluoride concentrations. To test this hypothesis, we balanced the amount of hydrogen fluoride by adjusting the pH of the test solutions. The results show the pH of NaF or water had little effect of plaque formation or plaque dry weight (Table 19). Again, only SnF_2, at both low and high acidic pH's, markedly affected plaque formation.

In the initial plaque formation experiment and the pH experiment (first and last experiments in this phase), the plaque samples in each group were pooled in order to increase the detection of tin in the samples, and therefore, no statistical analyses could be performed. In the other two experiments, preformed plaque and the 5-day preformed plaque experiment, the amount of Sn/mg plaque between the SnF_2 and $SnCl_2$ groups were significantly different ($p \leq .01$).

Discussion

The present series of experiments, designed to further examine the antiplaque determinants of SnF_2, have shown that SnF_2 has impressive antiplaque properties with initial plaque formation but with lesser effects noted on preformed plaque. The decreased effectiveness of SnF_2 noted

with preformed plaque suggests that one of the antiplaque mechanisms may be associated with adhesion/cohesion altering properties. These findings are consistent with electron microscopic observations showing bacterial separation from enamel and from each other when plaque is treated with intermittent exposure to SnF_2 (Tinanoff et al., 1976; Tinanoff and Camosci, 1980).

Tin may be effective in altering bacterial attachment by competing with calcium for acidic groups on the bacterial surface or acidic groups on the pellicle or on the tooth itself (Svatun et al., 1977; Rölla, 1976). Additionally, others have noted that SnF_2 reduces the surface tension at the air-water interface of enamel (Glantz, 1969). These adhesion reducing properties of SnF_2 may contribute to the noted diminished plaque formation.

Previous studies have suggested that individual ion species of SnF_2 are not by themselves as effective as when these ions are in combination. Neither stannous ions as $SnCl_2$, nor fluoride ions as NaF appeared as potent as SnF_2 in decreasing the amount of plaque (Tinanoff et al., 1976). The present results which also show more substantial antiplaque properties for SnF_2 provide additional support for the previous findings. The reason for the increased effectiveness of SnF_2 may be explained by results of the atomic absorption analyses in the present study which shows consistently higher levels of tin in those cells exposed to SnF_2 than to $SnCl_2$.

The difference in bacterial cell uptake of fluoride and chloride ions may partially explain the observations of tin deposition in plaque treated with SnF_2 but not $SnCl_2$. Many authors have reported higher concentrations of fluoride in dental plaque than in the surrounding oral fluids (Hardwick and Leach, 1962; Dawes et al., 1965; Jenkins and Edgar,

1969). This fluoride accumulates only within the bacterial cells (Jenkins and Edgar, 1969). However, chloride apparently is not concentrated in bacteria (Schultz et al., 1962; Mitchell and Moyle, 1959). Hence, the large accumulation of tin found in the bacterial cells exposed to SnF_2 could possible be explained by the transport and accumulation of fluoride in the cells, with tin passively entering the cells coupled to the fluoride ions.

An important feature of presently known antiplaque agents is their ability to bind to oral structures, which aids in oral retention. The presence of tin on and within plaque bacteria exposed only briefly to dilute solutions of SnF_2 suggests that tin accumulates and is retained in plaque. The large tin accumulation in SnF_2 treated plaque may be the most important antiplaque determinant of SnF_2.

INITIAL PLAQUE FORMATION

Pooled Samples (7)	Terminal pH of medium	Plaque score [α]	Dry Plaque Weight (mg)	Sn/ Total Sample (ppm)	Sn/ mg Plaque (μg)
H_2O	4.6	4	88.4	ND [β]	ND
$SnCl_2$ (Sn^{++} equimolar to SnF_2)	4.7	4 [γ]	32.7	58	1.8
SnF_2 (250 ppm F^-)	7.6	< 1	0.6	12	20.0

α Scored by McCabe method

β None Detected

γ Variable in thickness

Table 16 S. mutans NCTC 10449 was grown on wires and exposed every 12 hours for 1 min. to various agents. After 2 days, the plaque thickness on 7 wires in each treatment group was scored, then pooled, dried, weighed and analyzed for tin.

PREFORMED PLAQUE

N = 4	Terminal pH of medium	Plaque Score[α]	Dry Plaque Weight (mg)	Sn/ Total Sample (ppm)	Sn/ mg Plaque (μg)
H_2O	5.3	5	8.3 ± 1.0[β]	N.D.	N.D.
$SnCl_2$ (Sn^{++} equimolar to SnF_2)	5.5	5	8.8 ± 0.8	10.5 ± 3.4	1.2 ± 0.3
SnF_2 (250 ppm F^-)	6.2	3	12.2 ± 0.7	135.8 ± 37.2	11.3 ± 3.2

α Scored by McCabe method

β Mean of 4 sample ± S.D.

N.D. None Detected

Table 17: S. mutans NCTC 10449 was grown on wires for 2 days and exposed thereafter every 12 hrs. for 1 min. to various agents for the next 2 days. Each wire was scored for plaque, then collected, dried, weighed and analyzed for tin.

PREFORMED PLAQUE (5-days)

N = 3	Terminal pH of medium	Plaque Score[α]	Dry Plaque Weight (mg)	Sn/ Total Sample (ppm)	Sn/ mg Plaque (μg)
H_2O	4.29	4	18.9 ± 0.2[β]	N.D.	N.D.
$SnCl_2$ (Sn^{++} equimolar to SnF_2)	4.28	4	22.6 ± 4.7	67.0 ± 1.4	3.1 ± 0.7
SnF_2 (250 ppm F^-)	4.62	4	15.7 ± 0.8	~ 300	19.2 ± 1.0

α Scored by McCabe method

β Mean of 3 sample ± S.D.

N.D. None Detected

Table 18 S. mutans NCTC 10449 was grown on wires for 2 days and then exposed every 12 hrs. for 1 min. to various agents for the next 2 days. The intermittent exposures to the wires continued for 3 more days, once a day for 2 min. Each wire was scored for plaque, then collected, dried, weighed and analyzed for tin.

pH EFFECT

Pooled Samples (3)		Terminal pH of medium	Plaque score [a]	Dry Plaque Weight (mg)	Sn/ Total sample (ppm)	Sn/ mg plaque (μ g)
H_2O	pH 3.8	4.9	2	20.2	N.D.	
	pH 5.3	5.0	2	20.2	N.D.	
SnF_2	pH 3.8	7.5	< 1	0.2	1	5
	pH 5.3	7.0	2[β]	4.5	105	23.3
NaF	pH 3.8	5.1	2	16.0	N.D.	
	pH 5.3	5.0	2	20.1	N.D.	

[a] Scored by McCabe method

[β] Variable thickness

N.D. None Detected

Table 19: S. mutans NCTC 10449 was grown on wires and exposed every 12 hrs. for 1 min. to various agents balanced in the amount of HF by adjusting the pH. After 2 days, the 3 wires in each treatment group was scored, then pooled, dried, weighed and analyzed for tin.

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Slow Release of Fluoride From Temporary Restorative Materials

Introduction

The scope of work set out in our contract called for incorporating fluoride compounds into Intermediate Restorative Material (IRM) so that a slow release of fluoride, 1-2 mg F^-/day, could be achieved over at least a months period. It became obvious from *in vivo* and *in vitro* studies that stannous fluoride was the fluoride compound with the greatest antiplaque potential, so experiments with slow release were limited to this compound.

In last years' annual report, we presented results of trials measuring the slow release of SnF_2 from IRM and zinc oxide-eugenol (ZOE). In these trials, circular holes 16.0 mm^2 x 5 mm in depth were prepared in the buccal surface of human molars. IRM and ZOE mixed to 30% or 50% SnF_2 (weight F^-/weight powder) was used to fill the cavities. After a 2 day setting period, each tooth was placed in 1 liter of normal saline which simulated the volume of saliva secreted per day (saliva secretion is approximately 800 ml/day (J. Dent. Res. 53:246-266, 1974). The flasks were then placed in a shaker water bath at 37°C and agitated at slow speed. After each 24 hour period, a 1 ml aliquot was removed from the flasks and set aside for fluoride analyses. After collection of the sample, new saline was added to the test specimen. These procedures were repeated for 30 days. The results of this experiment showed that in all cases, IRM released all fluoride after 10 days of exposure to the saline. The ZOE material showed the greatest promise in having extended the release to 25 days; yet, the fluoride levels after 12 days may have been below therapeutic levels (0.35 ppm F^-). Besides the acceptable long term release of fluoride from these compounds, we also subjectively noted that when SnF_2 was incorporated into these two materials, they appeared to be not "set" completely.

From these results we decided that the initial concepts of incorporating

SnF_2 into IRM and using only the criteria of fluoride release may have been insufficient. We decided that other dental cements needed to be tested for fluoride release but first we would have to examine the comprehensive strength of the materials prior to fluoride release experiments.

The following changes in the protocol were initiated:

(1) Test other dental cements, i.e., zinc phosphate and polycarboxylate cement, besides IRM and ZOE.

(2) Reduce the grain size of SnF_2 (30 mesh) by pulverizing SnF_2 into a fine powder. This reduction of the size of the SnF_2 would consequently reduce the pore spaces in the cements producing less effect on the properties of the cement.

(3) Performing compressive strength tests on all ratios of fluoride to powder for all cements prior to continuation of further slow release studies.

Materials and Methods

ZOE (generic), IRM (Caulk), zinc phosphate (Penwalt, S.S. White), and polycarboxylate (Durelon, Premier) powders were combined with the pulverized SnF_2 to produce mixtures of 0, 20, 40, 60, and 70% SnF_2 (weight/weight). Each cement at each ratio was then mixed with its respective cement as per manufacturer's instructions using either glass slab or paper slab. The cements were placed in cylindrical molds, 11 mm and 4 mm in diameter. After a 2 day set, each specimen was removed from the mold and the specimens were ground flat and parallel to a standard height of 7.3 mm.

Compressive strength testing (the maximum stress that a material can withstand) for each specimen was performed in duplicate with an Instron universal testing machine and then compared to ADA specifications.

Results

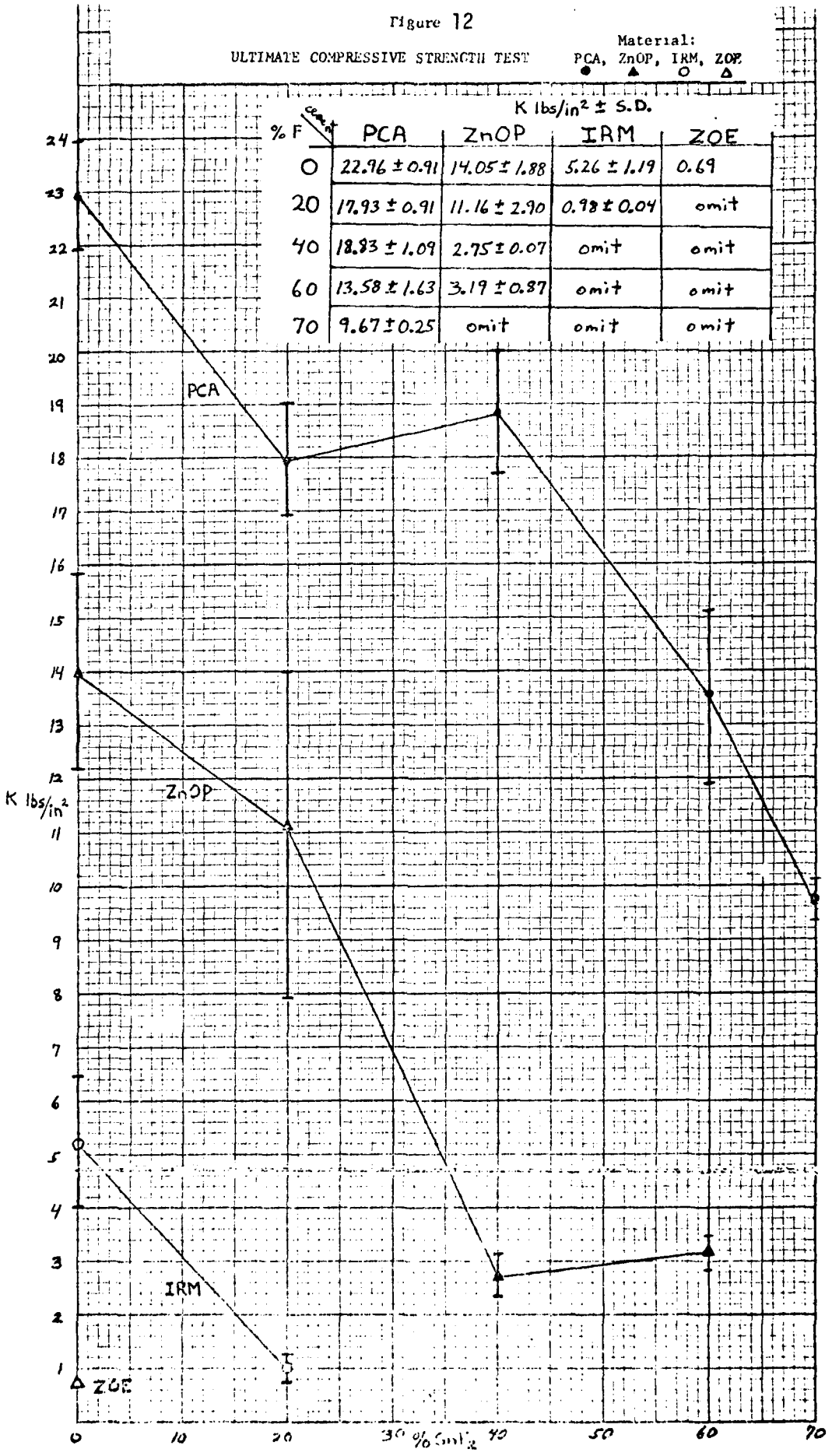
Ultimate compressive strengths of polycarboxylate cement without addition

Figure 12

ULTIMATE COMPRESSIVE STRENGTH TEST

Material: PCA, ZnOP, IRM, ZOE

% F (cement)	K lbs/in² ± S.D. PCA	ZnOP	IRM	ZOE
0	22.96 ± 0.91	14.05 ± 1.88	5.26 ± 1.19	0.69
20	17.93 ± 0.91	11.16 ± 2.90	0.98 ± 0.04	omit
40	18.83 ± 1.09	2.75 ± 0.07	omit	omit
60	13.58 ± 1.63	3.19 ± 0.87	omit	omit
70	9.67 ± 0.25	omit	omit	omit



of SnF_2 was 22.9±0.9 K lbs/in^2. This compares very favorably to the ADA specification for polycarboxylate cement of 10.2 K lbs/in^2. This cement held together well in the compressive strength tests with the addition of SnF_2 (Figure 12). Even at 70% SnF_2 incorporated into the polycarboxylate cement, the compressive strength was 9.6 K lbs/in^2 which is almost equal to the ADA specification for this material alone.

Zinc phosphate cement had a compressive strength of 14.0±1.9 K lbs/in^2 without the addition of any fluoride. This is comparable to the ADA specification of 17.0 K lbs/in^2 for this cement. The addition of SnF_2 dramatically altered the compressive strength of the cement. With the 40% SnF_2/cement ratio, the compressive strength was a low 2.8±.07 K lbs/in^2.

Both IRM and ZOE failed to produce a firm set with the addition of SnF_2 and for IRM compounds above 20%, SnF_2/cement were not even tested. Only the basic ZOE was tested due to the fact that the ZOE with SnF_2 remained soft. The unmodified IRM had a compressive strength of 5.3±1.2 K lbs/in^2 which is comparable to the ADA specifications of 7.3. The unaltered ZOE had a compressive strength of 0.7 which is comparable to the ADA specification of 1.2.

Discussion

After much time expended with the slow release of fluoride from either ZOE or IRM, it appeared that these agents are not appropriate as a slow release agent. Both IRM and ZOE initially have a very low compressive strength and with the addition of SnF_2, these materials remain soft and probably would not be an acceptable temporary dental material. It is possible that these materials would actully work better *in vivo* than in our tests because moisture aids in their setting properties. In the *in vitro* testing, however, the addition of water is such an uncontrollable variable that it is not practical.

One big breakthrough was pulverizing SnF_2 prior to incorporating it into the cement powders. By pulverizing SnF_2, the "holes" in the cement are made

smaller and more evenly dispersed. Hence, the incorporation of SnF_2 has less impact on the mechanical properties of the cement.

The polycarboxylate cement was quite remarkable in its ability to have large quantities of SnF_2 incorporated without deleterious effects on its compressive strength. Most remarkably, polycarboxylate cement with 70% SnF_2 still maintained a compressive strength of 9.7 K lbs/in^2. This is approximately the same strength as zinc phosphate cement with only 20% fluoride incorporated into it.

We are presently performing the slow release trials with all the various ratios of SnF_2/cement powder for polycarboxylate cement, zinc phosphate cement, IRM, and ZOE. If we subsequently find that polycarboxylate cement releases the SnF_2 at consistent levels over a 1 month period, we may truly have a slow release system that has great potential in the future animal studies and in clinical trials.

Appendix A

List of Publications and Presentations
Supported by U.S. Army Contract DAMD 17-78-C-8066

Papers published:

1. Tinanoff, N., Hock, J., Camosci, D., and Helldén, L. The Effect of Stannous Fluoride Mouthrinse on Dental Plaque. J. Clinical Periodontology 7:232-241, 1980.

2. Tinanoff, N. and Weeks, D. Current Status of SnF_2 as an Antiplaque Agent. Pediatric Dentistry 1:199-204, 1979.

3. Hock, J. and Tinanoff, N. Resolution of Gingivitis in Dogs Following Topical Applications of 0.4% Stannous Fluoride and Toothbrushing. J. Dent. Res. 56:1652-1653, 1979.

4. Tinanoff, N. and Camosci, D. A. Microbiological, Ultrastructural and Spectroscopic Analyses of the Anti-Tooth-Plaque Properties of Fluoride Compounds In Vitro. Arch. Oral Biol. 25:531-543, 1980.

Papers submitted:

1. Camosci, D. A. and Tinanoff, N. Competitive Attachment Between Streptococcus mutans and Streptococcus sanguis in Glucose or Sucrose Supplemented Media. J. Dent. Res.

2. Fisher, J. G., Tanzer, J. M., Tinanoff, N. and Paulakis, V. Plaque formation by LTA-deficient oral streptococci. Infect. Immun.

Papers in preparation:

1. Ferretti, G. A., Tanzer, J. M., and Tinanoff, N. The Effect of Various Fluoride Compounds on Bacterial Growth, Metabolism and Attachment. Caries Res.

2. Tinanoff, N. and Camosci, D. A. Intracellular Electron-Dense Granules Associated with SnF_2 Treated Plaque. J. Perio.

Presentations:

1. Tinanoff, N., Camosci, D. A. and Gross, A. Intermittent Exposure of Fluorides on Bacterial Colonization of Enamel In Vitro. Proc. 57th Ann. Meet. Int. Assoc. Dent. Res., New Orleans, 1979.

2. Tinanoff, N. and Tanzer, J. M. Electron Microscopy of Pellicle Formed by Enamel-Adherent Organisms. Proc. 57th Ann. Meet. Int. Assoc. Dent. Res., New Orleans, 1979.

3. Tinanoff, N., Hock, J., Camosci, D. and Helldén, L. Clinical Trial to Test Antiplaque Effect of SnF_2 Mouthrinse. Proc. 57th Ann. Meet. Int. Assoc. Dent. Res., New Orleans, 1979.

4. Camosci, D. A., Tinanoff, N. and Gross, A. Intermittent Exposure of Fluorides and Bacterial Colonization of Enamel In Vitro. Ann. Soc. Microbiol., Conn. Valley Branch, Storrs, CT, 1979

5. Tinanoff, N. and Camosci, D. A. Intracellular Electron-Dense Granules Associated with SnF_2 Treated Plaque. Proc. 58th Ann. Meet. Am. Assoc. Dent. Res., Los Angeles, 1980.

*6. Camosci, D. A. and Tinanoff, N. Quantity of Tin in Dental Plaque Exposed to SnF_2 and $SnCl_2$. Proc. 58th Ann. Meet. Am. Assoc. Dent. Res., Los Angeles, 1980.

*7. Ferretti, G. A., and Tinanoff, N. Bacteriostatic and Bactericidal Effects of Different Fluoride Compounds on S. Mutans. Proc. 58th Ann. Meet. Amer. Assoc. Dent. Res., Los Angeles, 1980.

*8. Young Pavlakis, V., Tanzer, J. M. and Tinanoff, N. Plaque formation by LTA-deficient Oral Streptococci. Proc. 58th Ann. Meet. Amer. Assoc. Dent. Res., Los Angeles, 1980.

9. Tinanoff, N. Antiplaque properties of SnF_2. The Album Society, Philadelphia, Penn., April 18, 1980.

10. Tinanoff, N. Influence of Metal Ions on Microbial Structure. European Research Group of Oral Biology, Merseyside, England, April 25-27, 1980.

11. Tinanoff, N. Stannous Fluoride as an Antiplaque Agent. The University of Liverpool, Dental School, England, April 25, 1980.

12. Tinanoff, N. The Antiplaque Properties of SnF_2 Mouthrinse. The University of Bern Dental School, Switzerland, April 30, 1980.

13. Camosci, D. A. Quality of Tin in Dental Plaque Exposed to SnF_2 and $SnCl_2$. Am. Soc. Microbiol., Connecticut Valley Branch, Bridgeport, Connecticut, 1980.

*Abstracts attached in Appendix B

Appendix B

Published Abstracts of Papers Presented in 1980

FORM A
INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
and
AMERICAN ASSOCIATION FOR DENTAL RESEARCH
ABSTRACTS MUST BE RECEIVED BY OCTOBER 16, 1979

Complete: Items 1-7 below and type abstract within box, following instructions on reverse side.
Submit: Form A — Original and 4 Xerox copies; Form B card — 1 original (complete blank 3x5 card for each co-author, in same style as Form B).

1. Full name and address of author who will present paper.

David A. Camosci
Department of Pediatric Dentistry
Univ. of Conn. Health Center
Farmington, CT 06032

2. I wish my paper considered for (check one) IADR (Japan) ☐ AADR (Los Angeles) ☒

3. (For NIDR Grantees ONLY.)
I wish to apply for a Travel Assistance Grant for the IADR meeting in Japan. (See separate instructions.)
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4. Mode of presentation:

☒ oral presentation only
☒ read by title acceptable
☐ poster presentation only
☐ oral or poster mode acceptable

5. Do you wish to withdraw your paper if it is placed in a mode not of your choosing?
yes ☒ no ☐

6. **GROUP CLASSIFICATION**

☐ Behavioral Sciences
☐ Cariology
☐ Craniofacial Biology
☐ Dental Materials
☒ Microbiology, Immunology
☐ Mineralized Tissue
☐ Neuroscience
☐ *Oral & Maxillofacial Surgery*
☐ Periodontal Research
☐ Pharmacology, Therapeutics & Toxicology
☐ Prosthodontics
☐ Pulp Biology
☐ Salivary Research

7. **SUBJECT CLASSIFICATION**

☐ Anatomy
☐ Biochemistry
☐ Cell Biology
☐ Chemistry
☐ Education
☐ Embryology
☐ Endocrinology
☐ Endodontics
☐ Enzymology
☐ *Epidemiology-Biostatistics*
☐ Genetics
☐ Health Services
☐ Histology
☐ Nutrition
☐ Oral Medicine
☐ Orthodontics
☐ Pathology
☐ Physics
☐ Physiology
☒ Preventive Dentistry
☐ Radiology, Radiobiology
☐ Restorative Dentistry
☐ *Temporomandibular Joint*
☐ Tissue Culture
☐ Ultrastructure
☐ Other _____

TYPE PERFECT ORIGINAL OF ABSTRACT HERE
(Do not type beyond outline of box.)

Quantity of Tin in Dental Plaque Exposed to SnF_2 and $SnCl_2$. D. A. Camosci* and N. Tinanoff, Univ. of Conn. Health Center, Farmington, CT

Quantitation of tin in plaque samples by atomic absorption spectrophotometry was used to further investigate the antiplaque mechanisms of SnF_2 and $SnCl_2$. In these experiments 42 wires suspended by rubber stoppers (7/treatment group) were exposed initially to either SnF_2 (100 & 250 ppm F^-), $SnCl_2$ (Sn^{++} = Sn^{++} in 100 & 250 ppm SnF_2), or H_2O; and then washed in H_2O. Next the wires were placed in 10 ml of Jordan's medium supplemented with 5% sucrose and inoculated with a 0.1 ml adapted S.mutans culture. During plaque growth the wires were exposed 2x/day to their respective treatments with transfer after 24 hr to fresh media. At 48 hr (12 hr after the last treatment), the thickness of the plaque was visually scored and the pH of the media from the tubes were measured. The plaque from the 7 wires of each group was then pooled, dried, and weighed. Tin in the samples was measured using an atomic absorption spectrophotometer equipped with graphite furnace. Plaque accumulation on the wires exposed to SnF_2 was barely visible after 2 days; whereas, large accumulations were apparent on the wires treated with $SnCl_2$ and on the H_2O control wires. Acid production was greatly reduced in those specimens exposed to SnF_2. At the 100 ppm conc. the SnF_2 treated samples had 14x less adherent plaque (dry wt.) than the $SnCl_2$ samples. yet, the SnF_2 samples had 8x more Sn^{++}/mg plaque. For 250 ppm, SnF_2 samples had 55x less plaque, but 11x more Sn^{++}/mg plaque. The results suggest that SnF_2 is more effective than $SnCl_2$ as an antiplaque agent with higher concentrations showing the greatest difference. These findings may be explained by the higher tin levels found in those samples treated with SnF_2.

Supported by U.S. Army contract DAMD 17 78 C 8066.

8. Reviewer's Ratings:
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

9. Disposition:
☐ O ☐ P ☐ T ☐ R ☐ W

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Complete: Items 1-7 below and type abstract within box, following instructions on reverse side.
Submit: Form A — Original and 4 Xerox copies; Form B card — 1 original (complete blank 3x5 card for each co-author, in same style as Form B).

1. Full name and address of author who will present paper.
Gerald A, Ferretti, D.D.S.
Department of Pediatric Dentistry
University of Connecticut Health Ctr.
Farmington, Ct. 06032

2. I wish my paper considered for (check one) IADR (Japan) ☐ AADR (Los Angeles) ☒

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- ☐ Mineralized Tissue
- ☐ Neuroscience
- ☐ Oral & Maxillofacial Surgery
- ☐ Periodontal Research
- ☐ Pharmacology, Therapeutics & Toxicology
- ☐ Prosthodontics
- ☐ Pulp Biology
- ☐ Salivary Research

7. **SUBJECT CLASSIFICATION**
- ☐ Anatomy
- ☐ Biochemistry
- ☐ Cell Biology
- ☐ Chemistry
- ☐ Education
- ☐ Embryology
- ☐ Endocrinology
- ☐ Endodontics
- ☐ Enzymology
- ☐ Epidemiology-Biostatistics
- ☐ Genetics
- ☐ Health Services
- ☐ Histology
- ☐ Other _____
- ☐ Nutrition
- ☐ Oral Medicine
- ☐ Orthodontics
- ☐ Pathology
- ☐ Physics
- ☐ Physiology
- ☒ Preventive Dentistry
- ☐ Radiology, Radiobiology
- ☐ Restorative Dentistry
- ☐ Temporomandibular Joint
- ☐ Tissue Culture
- ☐ Ultrastructure

TYPE PERFECT ORIGINAL OF ABSTRACT HERE
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Bacteriostatic and Bactericidal Effects of Different Fluoride Compounds on S. Mutans.
G.A. FERRETTI* and N. TINANOFF, U. of Conn. Health Ctr., Farmington, Ct.

Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of NaF, TiF_4, Na_2SnF_6, SnF_2, and $SnCl_2$ (Sn^{++} = Sn^{++} in SnF_2) were performed in order to determine the lowest concentrations of these agents which either retarded growth or killed S. mutans NCTC 10449. For these tests, 1 ml of the appropriately diluted test agent was added to tubes containing 1 ml of tryptocase soy medium which had been previously inoculated with an adapted, log phase, S. mutans culture ($5X10^5$ CFU/ml media). These tubes, plus controls of diluted test agents in uninoculated medium, were incubated for 18 hrs. at 35° C. The MIC of each agent was determined by the lowest dilution showing no visual growth after incubation. The MLC of each agent was determined by the lowest concentration plated on blood agar showing no growth.

The results showed that SnF_2 had the lowest MIC and MLC, 60 ppm and 125 ppmF^-, respectively. TiF_4 had a MIC at 525 ppm and a MLC at 575 ppmF^-; NaF had a MIC at 300 ppm and a MLC at 3000 ppmF^-; Na_2SnF_6 had a MIC at 600 and a MLC at 675 ppmF^-. $SnCl_2$ was found to have a MIC at 200 ppm Cl^- and a MLC at 225 ppm Cl^-. The low bacteriostatic and bactericidal activity observed for SnF_2 on S. mutans cannot be explained by the separate action of tin and fluoride. Also, the MIC and MLC values of SnF_2 cannot entirely account for the noted anti-plaque effect of this agent at lower concentration.

Research supported by U.S. Army Contract DAMD 17 78 C 8066.

8. Reviewer's Ratings:
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

9. Disposition:
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Complete: Items 1-7 below and type abstract within box, following instructions on reverse side.
Submit: Form A — Original and 4 Xerox copies; Form B card — 1 original (complete blank 3x5 card for each co-author, in same style as Form B).

1. Full name and address of author who will present paper.
Virginia Young Pavlakis
Department of Oral Diagnosis
University of Connecticut Health Center
Farmington, CT 06032

2. I wish my paper considered for (check one) IADR (Japan) ☐ AADR (Los Angeles) ☒

3. (For NIDR Grantees ONLY.)
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4. Mode of presentation:
☒ oral presentation only
☐ *read by title acceptable*
☐ poster presentation only
☐ oral or poster mode acceptable

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☐ Behavioral Sciences	☐ Oral & Maxillofacial Surgery
☐ *Cariology*	☐ *Periodontal Research*
☐ Craniofacial Biology	☐ Pharmacology, Therapeutics & Toxicology
☐ Dental Materials	
☒ Microbiology, Immunology	☐ Prosthodontics
☐ *Mineralized Tissue*	☐ Pulp Biology
☐ Neuroscience	☐ *Salivary Research*

7. **SUBJECT CLASSIFICATION**

☐ Anatomy	☐ Nutrition
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☐ Cell Biology	☐ Orthodontics
☐ Chemistry	☐ Pathology
☐ Education	☐ Physics
☐ Embryology	☐ Physiology
☐ *Endocrinology*	☐ *Preventive Dentistry*
☐ Endodontics	☐ Radiology, Radiobiology
☐ *Enzymology*	☐ Restorative Dentistry
☐ Epidemiology-Biostatistics	☐ Temporomandibular Joint
☐ Genetics	☐ Tissue Culture
☐ *Health Services*	☐ *Ultrastructure*
☐ Histology	
☒ Other Microbiology	

TYPE PERFECT ORIGINAL OF ABSTRACT HERE
(Do not type beyond outline of box.)

Plaque Formation by LTA-deficient Oral Streptococci. V. YOUNG PAVLAKIS*, J. M. TANZER and N. TINANOFF. University of Connecticut School of Dental Medicine, Farmington, CT 06032

Lipoteichoic acids (LTA) have been implicated as mediating the adhesion of oral streptococci to surfaces. Some strains of Streptococcus mitis have been reported to lack LTA. Therefore, we studied the ability of these and other S. mitis strains, as well as presumptive LTA-producing strains to form plaque in vitro on stainless steel and enamel surfaces, and to make LTA. The following strains were studied: S. mitis 9811 and ATCC-10557 (formerly identified as S. sanguis), and 171A-1 and 176A, isolated from the teeth of our colony of Osborne-Mendel rats; S. mutans NCTC-10449 and 6715-13-33; and S. faecalis 9790. Ability to adhere and to form tenacious plaque were evaluated both visually and electron-microscopically. It was observed that the S. mitis strains and S. mutans 10449, but not S. mutans 6715-13-33 or S. faecalis 9790, adhered to enamel and stainless steel in vitro. However, both of the S. mutans strains and the S. faecalis cell extracts reacted with anti-LTA serum but similar extracts of the four S. mitis strains did not. Therefore, LTA production cannot mediate adhesion of the four S. mitis strains. Also, the S. faecalis strain and S. mutans 6715-13-33 which both produce LTA fail to adhere. These data thus do not support the hypothesis that LTA mediates the adhesion of these oral streptococci to solid surfaces such as metal and enamel.

Work was supported by U.S.P.H.S. grant DE-03758 from the National Institute of Dental Research and U.S. Army Contract DAMD-17-78-C-8066.

8. Reviewer's Ratings: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

9. Disposition: ☐ O ☐ P ☐ T ☐ R ☐ W

Appendix C

Copy of Paper Submitted to Journal of Dental Research

Competitive Attachment between *Streptococcus mutans* and *Streptococcus sanguis* in Glucose or Sucrose Supplemented Media

D. A. Camosci and N. Tinanoff

An *in vitro* model consisting of nichrome wires, suspended in culture tubes inoculated with either pure cultures or mixed cultures of *S. mutans* NCTC 10449 (serotype *c*) and *S. sanguis* ATCC 10558 was used to study competitive bacterial adhesion on wires. Pure cultures of *S. mutans* formed plaque in complex media supplemented with either 5% sucrose or 5% glucose. However, when the *S. mutans* and *S. sanguis* cultures were mixed for competitive attachment in either glucose or sucrose supplemented media, *S. mutans* predominated on the wires as demonstrated by enumeration of colony-forming units (CFU) and colonial morphology. Macroscopically, plaque formation was observed on all wires except for the pure cultures of *S. sanguis* in glucose supplemented medium. *S. mutans* plaques cultured in glucose supplemented medium appeared less tenacious than those grown in sucrose; yet enumeration of organisms on wires revealed similar CFU of organisms from both glucose and sucrose supplemented media.

Research supported by U.S. Army Contract #DAMD 17-78-C-8066

INTRODUCTION

The ability of microorganisms to attach to tooth surfaces, to proliferate while localized there and to cohere are presently felt to be some of the essential factors in colonization of tooth surfaces and subsequent pathogenicity (9, 18).

Two organisms shown to colonize teeth *in vivo* are *S. mutans* and *S. sanguis* (4, 12). The appearance of *S. sanguis* in the oral cavity is closely correlated with the eruption of the first teeth supporting the concept that hard surfaces favor colonization of this species (1, 25). Even though this organism is found in high numbers in plaque (12), it is generally considered non-pathogenic (7, 8). In contrast, studies have shown that *S. mutans* possess a rather feeble ability to attach to teeth relative to such organisms as *S. sanguis* and *S. mitis* (17, 21, 26); however, *S. mutans* is most implicated in the carious process (2, 5, 20).

Besides this antithesis in the ability to attach and pathogenicity, studies furthermore have suggested that *S. sanguis* and *S. mutans* may compete for colonization sites on enamel; while others have suggested that those organisms have mutual interactions which enhance colonization. Schachtele, *et al.* has demonstrated the adherence of *S. mutans* to *S. sanguis* layered on glass in the presence of sucrose, was approximately twice as rapid as compared to the adherence of *S. mutans* to glass alone (22). In contrast, de Stoppelaar, *et al.* has shown that in samples collected from human teeth, an inverse relationship existed between *S. mutans* and *S. sanguis* or a variable percent of *S. sanguis* was present when *S. mutans* was not detected (6). The proportional increase of *S. sanguis* found in plaque samples during the carbohydrate-free period also suggested that sucrose was not the prime requisite for the colonization of *S. sanguis* on the tooth surface (6).

Because of the uncertainty of the interaction between S. mutans and S. sanguis in early plaque formation, this investigation was performed to explore the competitive relationship of these two species in a sucrose and in glucose rich in vitro system.

MATERIALS AND METHODS

Wire Preparation

Cleaned, autoclaved 20 gauge nichrome wires, fixed to culture tube caps were used to study the adherence of the microorganism. The length of each wire was placed into an 18 x 150 mm culture tube so that each wire ended 1 cm from the bottom of the tube. A standard length of 1 cm was then identified at the end of each wire by a 20^{0} angle bend.

Microorganisms, media and growth

A streptomycin-resistant mutant of S. mutans NCTC 10449 (serotype c) known to be a good plaque former in vitro (24) and known to be cariogenic (Tanzer, J. M., personal comm.) and S. sanguis ATCC 10558, a species found in high numbers in the human oral civity and considered non-cariogenic (7, 8) were studied.

Stock cultures were maintained by monthly transfer in fluid thioglycollate medium (Difco) supplemented with meat extract (20% v/v) and excess $CaCO_3$. For experiments, cultures were adapted to growth in the complex medium of Jordan, et al. (15), supplemented with 50 mg of Na_2CO_3 per liter and containing either 5% sucrose or 5% glucose. After establishing log phase of growth in both adapted cultures, they were vigorously vortexed and optical densities at 600 nm were determined (Gilford Instrument Lab., Model 260 spectrophotometer, Oberlin, Ohio). Equal volumes of equal optical densities of S. mutans and S. sanguis were combined and again vortexed before transferring 1.0 ml of the mixed cultures to 10 tubes, 5 containing 10 ml of complex medium supplemented with sucrose and 5 containing complex medium supplemented with glucose. Non-competitive attachment of S. mutans and S. sanguis was studied by inoculating 0.5 ml of the pure isolates separately into 2 tubes in complex medium containing 5% sucrose or complex medium con-

taining 5% glucose. Immediately after inoculation, the nichrome wires were placed in each tube and the culture tubes were incubated aerobically at 37°. After 24 hr., the wires were transferred to fresh media containing the appropriate 5% sucrose or 5% glucose (24).

Plate counts

After 48 hrs., the wires were gently lifted from the broth and a sterile wire cutter was used to cut the 1 cm portion of wire into a vial of 2.0 ml of 0.01 M phosphate buffer (pH 7.0) containing 0.05% w/v yeast extract (BBL). Each sample was sonicated for 30 sec. at 50 watts with the output setting at 4 (Bronson Sonifier, Model W185 equipped with microprobe, Heat Systems-Ultrasonic Inc., Plainview, N.Y.). This procedure separates the plaque from the wire and disrupts virtually all bacterial chains (19). Immediately, 1.0 ml of the sonicated sample was serially diluted to 10^{-4}, 10^{-5} and 10^{-6} org/ml and then spread in duplicate using 0.1 ml inoculum on Mitis salivarius agar (MS) and Mitis salivarius agar containing 100 µg/ml of streptomycin sulfate (Sigma Chem., St. Louis, MO) (MSS). All plates were incubated for 2 days in a 5% CO_2 environment at 35°. Plates containing 30-300 colonies were counted with the aid of a darkfield Quebec colony counter (American Optical Co., Boston, MA).

RESULTS

Visual plaque formation, i.e., dense white adherent deposits on wires, was evident in all the S. mutans controls and the mixed cultures irrespective of the supplemental carbohydrate. However, the S. mutans plaques grown in glucose containing media appeared less tenaciously attached to the wires as noted by the tendency for some plaque to be dislodged readily from the wires.

The enumeration of CFU from the sonicated plaque samples of S. mutans controls showed no difference between glucose grown and sucrose grown plaques (Table 1). S. sanguis controls showed substantial CFU present on the wires, with less organisms found in the glucose supplemented broth. The mixed cultures containing S. mutans and S. sanguis showed the same number of CFU on the MSS agar as well as on the MS agar in both sucrose and glucose broths suggesting that the plaque on those wires consisted of only the streptomycin resistant S. mutans organism. Furthermore, no S. sanguis was observed by colonial morphology on any of the MS agar plates, yet both organisms were shown to be present in the initial inoculum and after 24 hours of growth as determined by colony morphology.

Variation of the surface area of the cut wires used for enumeration purposes were determined indirectly assuming the weight of the wire is proportional to the surface area. The coefficient of variation of gravimetric measurements was calculated to be 5.8%.

Table 1. Number of colony-forming units (CFU) of S. mutans NCTC 10449 (streptomycin resistant mutant) and S. sanguis ATCC 10558 adherent to nichrome wire cultured in complex medium containing 5% sucrose or 5% glucose.

Carbohydrate	Plate media	N[f]	S. mutans	N	S. sanguis	N	S. mutans + S. sanguis
Sucrose	MS[a]	2	$1.48 \times 10^9 \pm .54$[c]	2	$5.85 \times 10^7 \pm 4.4$	5	$1.71 \times 10^9 \pm .55$[e]
	MSS[b]	2	$1.43 \times 10^9 \pm .46$	2	NG[d]	5	$1.78 \times 10^9 \pm .68$
Glucose	MS	2	$1.47 \times 10^9 \pm .46$	2	$3.36 \times 10^6 \pm 4.1$	5	$1.30 \times 10^9 \pm .16$[e]
	MSS	2	$1.47 \times 10^9 \pm .54$	2	NG	5	$1.32 \times 10^9 \pm .13$

a Mitis salivarius agar.

b MS + 100μg/ml streptomycin.

c Standard deviation.

d No growth.

e No S. sanguis detected by colonial morphology.

f Total number of observed values

DISCUSSION

In vitro microbiological studies generally use one microorganism and expose the organism to different variables. *In vivo*, however, many organisms are associated with many variables and are constantly competing for survival. In an unbalanced condition, one organism may gain a competitive advantage so that it outgrows other organisms, predominates the ecosystem, and produces pathology. This initial investigation examined one possible variable, the type of carbohydrate associated with the proportional attachment of two common plaque forming organisms, *S. mutans* and *S. sanguis*.

The results of this study suggest that *S. mutans* NCTC 10449 (serotype c) when cultured in complex media supplemented with excess sucrose or glucose competes successfully against *S. sanguis* ATCC 10558 for attachment to smooth surfaces. Unexpected, however, was the complete absence of *S. sanguis* from the wires in the mixed cultures grown in both sucrose and glucose supplemented broths.

As suggested by de Stoppelaar (7), the colonization of *S. mutans in vivo* varies inversely with the number of *S. sanguis* implying that these organisms may compete. Furthermore, it has been established that frequent ingestion of sucrose increases the number of *S. mutans* colonizing tooth surfaces in experimental animals (14, 16) and man (15). Results from the present *in vitro* experiment suggest other variables besides sucrose may influence the proportions of these two organisms with *S. mutans* predominating.

S. mutans NCTC 10449 Bratthall (serotype c) was initially chosen for this study because it is known that this serotype is common in Europe and the United States (3, 23). Recent data have also shown that *S. mutans* serotype *c* and *e* form plaque in growth media supplemented with glucose, as well as sucrose (24). However, lower plaque scores, most evident in those organisms cultured in chemically defined medium supplemented with glucose, suggest

that plaque may be less tenacious in glucose medium. The present study confirms these earlier findings of S. mutans serotype c plaque formation. However, since all experiments in the present study were performed in complex media, only slight differences of plaque growth were observed. The differences of tenacity of attachment for S. mutans in complex media and clinically defined media suggest that factors in complex media other than glucan may foster tenacity of attachment.

Enumeration of S. mutans CFU on wires in glucose and sucrose complex medium reveal similar number of organisms. Other data show no difference in bacterial DNA when S. mutans NCTC 10449 is cultured in chemically defined glucose or sucrose supplemented media. Yet, plaque production, as shown by plaque scores, were not similar (24). It is conceivable that plaque quantity may thus not necessarily be proportional to the number of organisms present.

Since S. mutans growth in glucose is similar to that in sucrose and S. mutans metabolizes glucose as readily as sucrose, it is apparent that for at least S. mutans serotype a, b, and d dependence on attachment is related to sucrose (11).

It is interesting to speculate that if S. mutans (serotype c) in vitro has the ability to adhere although less tenaciously in the absence of sucrose; in vivo, S. mutans (serotype c) might also adhere in the absence of sucrose, yet mechanical abrasion and salivary flow could prevent proliferation and virulence. Organisms not dislodged, however, could conceivably proliferate and become virulent when conditions are more favorable, i.e., when sucrose is ingested.

Further studies using this model system are indicated to explore whether: 1) coating wires with saliva; 2) decreasing carbohydrate in medium; 3) altering the proportions of the inocula; and/or 4) using different bacterial strains would enhance the ability for S. sanguis to compete for attachment against S. mutans.

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Appendix D

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