The effect of stannous fluoride on human plaque acidogenicity in situ (Stephan curve)

BJARNE SVATUN & AUDUN ATTRAMADAL

Department of Pedodontics, Dental Faculty, University of Oslo, Oslo, Norway

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A system employing an Ingold glass electrode was shown to give reliable measurements of pH drops in dental plaque *in situ* (Stephan curve readings). The system was used to demonstrate that mouthrinses of 0.2 per cent aqueous solutions of stannous fluoride reduced the pH drops markedly for at least seven hours. A reduction of the stannous fluoride concentration increased the pH drops and decreased the duration of the inhibiting effect. A commercial toothpaste containing stannous fluoride and stannous pyrophosphate had an effect similar to the 0.2 per cent mouthrinse.

It was shown that tin accumulated in dental plaque after application of solutions containing stannous fluoride. About 40 per cent of the amount of tin present in the plaque immediately after the mouthrinse was still retained seven hours later.

It is suggested that the reduction in acid formation may be caused by stannous ions adsorbing to the bacterial cell wall thus disturbing membrane transport mechanisms, or through inhibition of enzyme systems essential in the fermentation of sugars.

The observed effect may be a part of the mechanism involved in the caries preventive function of stannous fluoride.

Key-words: Preventive dentistry; pH changes; tin

Bjarne Svatun, Department of pedodontics, Dental Faculty, Geitmyrsveien 71, Oslo 4, Norway

It is generally accepted that the production of acid by microorganisms is a dominant factor in the development of dental caries. Drops in the pH of dental plaque exposed to various dietary sugars are thought to reflect the cariogenic challenge at the tooth surface. Consequently, a great deal of research has been done in the effort to find substances which would prevent or retard the rate of acid formation in plaque. Among the substances which have been recommended are urea (33, 39), ammonium salts (22), quaternary ammonium salts (34), vitamin K (5) and penicillin (40). The effect of various metallic salts upon acid production in saliva containing sucrose has also been studied (9). Recently Sialin has received much attention (23).

It has been suggested, furthermore, that the relatively large amounts of fluoride introduced into the mouth through mouthwashes or dentifrices could inhibit acid production. Although there is a decreased lactic acid formation in saliva collected shortly after rinsing the mouth with a solution of 0.5 per cent sodium fluoride, this effect disappears after 20–30 minutes (8) and the effect of rinsing with 0.1 per cent sodium

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fluoride lasts only for 10 to 15 minutes (19). In contrast to saliva, appreciable amounts of fluoride may accumulate in plaque (17). Although it is not known in what form this fluoride is present, there are reasons for believing that only a minor fraction is ionized. Calcium and phosphate are known to accumulate rapidly in plaque material (2, 7), and to form inorganic salts increasing with time (31). Since less than 1 ppm of fluoride will cause precipitation of fluorapatite from the concentrations of calcium and phosphate present in plaque, it is unlikely that fluoride inhibition of bacterial acid production in plaque is of major importance (4).

Stannous fluoride has been reported to inhibit bacterial acid production by salivary sediment more than sodium fluoride (26). A similar inhibition was found when stannous fluoride was used as a mouthwash (27). The inhibitory effect was shown to be unrelated to the fluoride content and was proportional to the tin concentration. Another *in vitro* study suggested that the stannous fluoride had an antienzymatic effect and that the stannous ion was responsible (21).

The aim of the present investigation was to study the influence of stannous fluoride on pH changes in dental plaque *in vivo* after the use of stannous fluoride mouthrinses or a commercial stannous fluoride toothpaste. A further purpose was to measure the uptake of tin and its retention in the plaque after introducing stannous fluoride in the mouth and to approach the mechanisms involved.

MATERIAL AND METHODS

Three dental students, characterized as heavy plaque formers (13) volunteered for the study. In order to collect plaque, all oral hygiene measures were suspended four days prior to the experiments. The pH of the plaque was measured *in situ* on two different teeth with an Ingold flat surface glass electrode, model 203-M3, connected to an Orion Ionalyzer Model A 407. In order to test the reliability of the Ingold electrode system, the pH was measured when sucrose was applied on clean tooth surfaces and in plaque when distilled water was added. Similar experiments were conducted with a 30 per cent sorbitol solution and with milk. The electrode was calibrated against standard buffers at pH 4 and pH 7 every morning before use.

Fifty μl 15 per cent w/v of a sucrose solution was applied on the site of the plaque where the measurements were performed and the pH was recorded every 15 seconds for five minutes. The students then rinsed with 10 ml of a 0.2 per cent stannous fluoride solution for one minute, and the same recordings were repeated after ten minutes, two, seven and 24 hours. The same procedure was used after rinsing with 0.1 per cent or 0.02 per cent stannous fluoride solutions, but pH recordings were discontinued when the inhibitory effect was no longer measurable. All stannous fluoride solutions were made immediately prior to use. The commercial dentifrice containing stannous fluoride and stannous pyrophosphate was applied in cap splints, each splint containing approximately 1.5 g of paste. They were kept *in situ* for two minutes and after application of sucrose the pH was recorded as described. The students were permitted three days of habitual oral hygiene between each experiment when the agent tested showed an inhibitory effect.

The students also rinsed with a 0.11 per cent sodium fluoride solution, a 0.24 per cent stannous chloride solution, and a 20 mM acetate buffer, pH 3.6.

The uptake and retention of tin was determined in dental plaque from three dental students who abstained from all oral hygiene measures for four days. A plaque sample was collected from each student, weighed and placed in two ml of

distilled water. The students then rinsed for one minute with a 0.2 per cent stannous fluoride solution and plaque samples were taken instantly, and after two and seven hours. The content of tin was determined by means of atomic absorption spectroscopy at the Central Institute for Industrial Research, Oslo. Two different atomic absorption spectrophotometers (Perkin Elmer models 303 and 503) were used for the measurements. The analysis technique has been described in detail elsewhere (36).

RESULTS

No pH drop occurred when sucrose was applied on clean tooth surfaces or when distilled water was added to plaque. Sorbitol and milk both caused pH drops, but markedly less than sucrose. When milk was used, the pH never decreased below 5.7. The lowest pH value observed after application of a 30 per cent sorbitol solution was 5.6.

Fig. 1 shows the pH drop in plaque of one individual after sucrose application, and the effect of stannous fluoride on this drop. When a 15 per cent sucrose solution was applied to the plaque, a pH drop from 6.5 to 4.6, *i.e.* a drop of 1.9 pH units was observed. Extention of the pH measurements beyond five minutes exhibited no or only negligible continuation in the pH drop. When sucrose was applied ten minutes after a rinse with stannous fluoride solution, there was a pH change of only 0.2 pH unit. Two, seven and 24 hours after stannous fluoride rinses the pH changes were 0.5, 0.9 and 1.1 pH units respectively.

The same experiment was repeated with a 0.1 per cent stannous fluoride solution, as shown in Fig. 2. This concentration also had a clear effect but not as marked as the 0.2 per cent stannous fluoride solution, and it was of shorter duration. Seven hours after the rinsing the pH dropped with 1.9 units to 5.1.

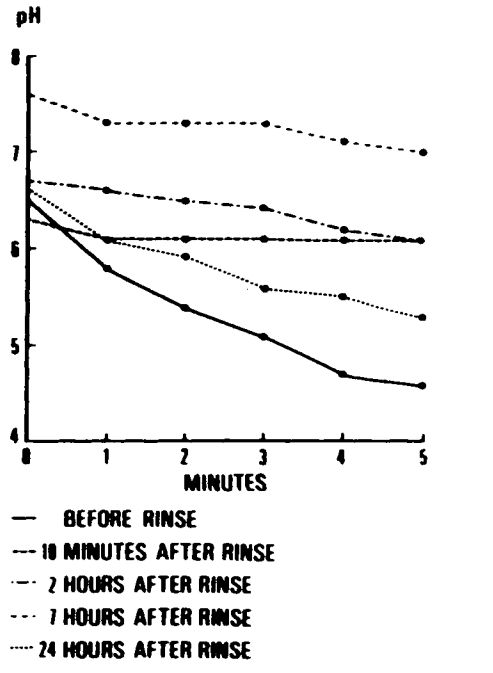

Fig. 1. Inhibition of pH changes in dental plaque after oral rinsing with a 0.2 per cent stannous fluoride solution.

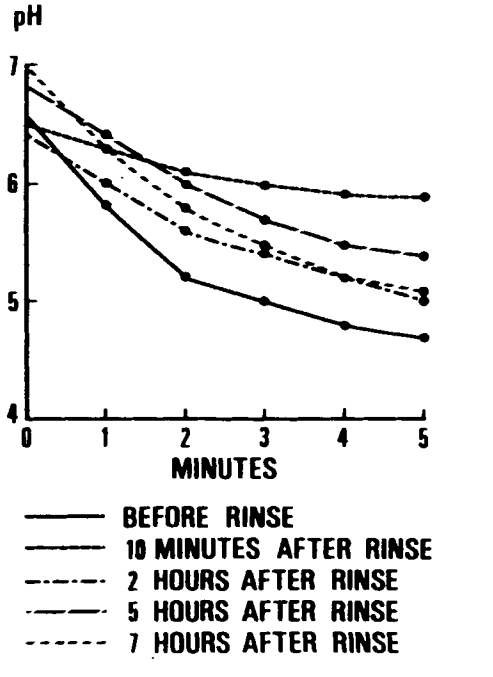

Fig. 2. Inhibition of pH changes in dental plaque after oral rinsing with a 0.1 per cent stannous fluoride solution.

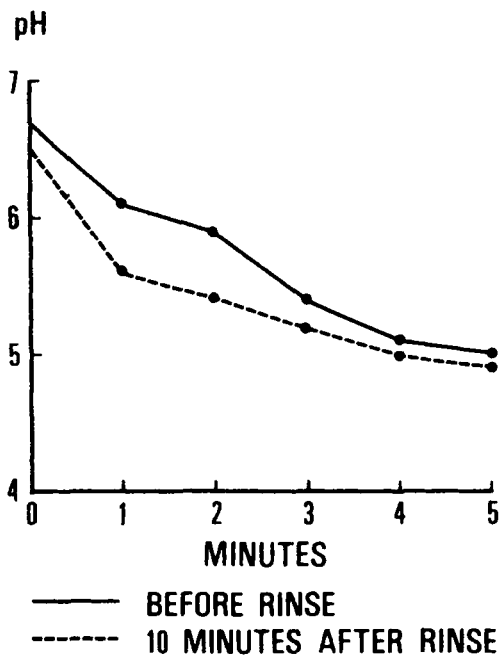

Fig. 3. Inhibition of pH changes in dental plaque after oral rinsing with a 0.02 per cent stannous fluoride solution.

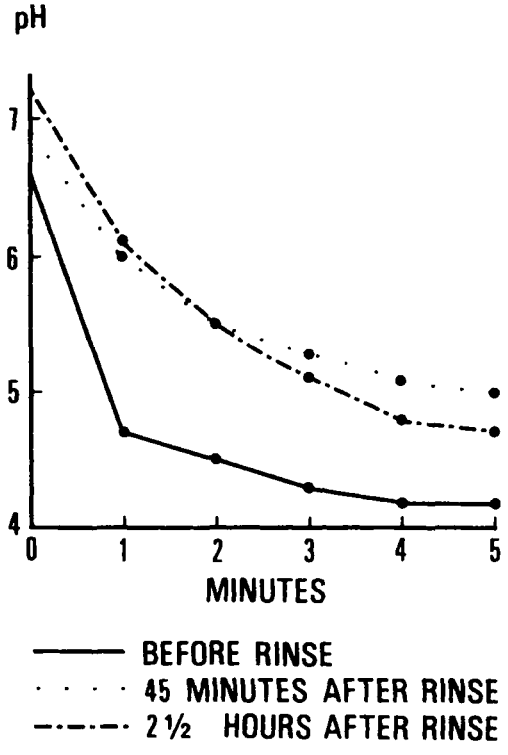

Fig. 4. Inhibition of pH changes in dental plaque after oral rinsing with a 0.11 per cent sodium fluoride solution. (Equalling the fluoride content of 0.2 per cent stannous fluoride.)

Fig. 3 demonstrates that a 0.02 per cent stannous fluoride solution did not inhibit the acid formation. Ten minutes after rinsing the pH dropped from 6.5 to 4.9.

Fig. 4 illustrates the effect of a 0.11 per cent sodium fluoride solution. The inhibitory effect was only slight and virtually ceased after 2 1/2 hours, showing then a pH drop from 7.2 to 4.7.

Fig. 5 shows the inhibitory effect of a stannous fluoride/stannous pyrophosphate dentifrice on the pH drop. The data presented in this figure are similar to those found when the test person used 0.2 per cent stannous fluoride solutions. The results shown in Fig. 1–5 are all from the same test person.

The mean pH changes of all test persons, measured in pH units, before and after the rinsing with a 0.2 per cent stannous fluoride solution or application of the dentifrice are given in Tables 1 and 2 respectively. Before the application of either of these agents all pH readings decreased below 5.0 except for one (pH 5.4). Seven hours after the application the lowest pH value observed was 5.4.

The 0.24 per cent stannous chloride solution had only a slight inhibitory effect, and a mean pH drop of one unit was found already ten minutes after the rinsing. No inhibition was observed after rinsing with acetate buffer, pH 3.6.

Table 3 shows the concentration and retention of tin in the plaque after rinsing with a 0.2 per cent stannous fluoride solu-

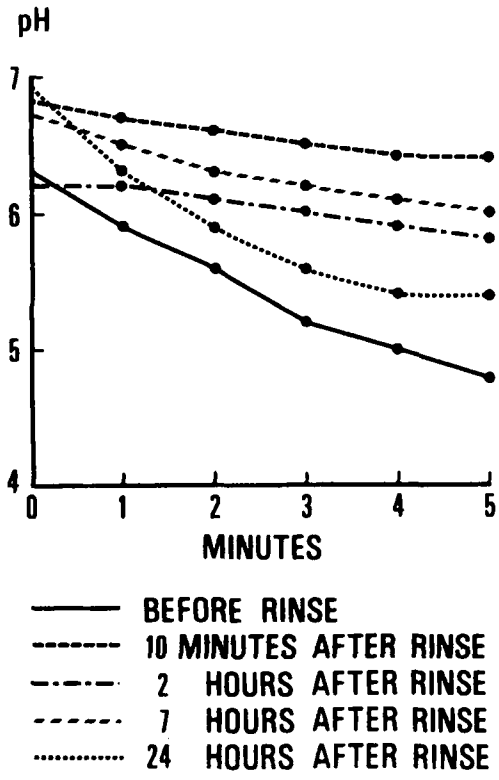

Fig. 5. Inhibition of pH changes in dental plaque after application of a stannous fluoride/stannous pyrophosphate dentifrice in cap splints.

tion. 41 per cent of the amount of tin present immediately after the rinsing was still retained seven hours later.

DISCUSSION

Multiple approaches have been made towards the measurement of plaque pH (10, 11, 12, 14, 15, 18, 20, 29, 32, 35). Objections have been raised to many of the methods used. It has been argued that: 1. The pH electrode may disarrange the structure and thus the diffusion rate in the plaque. 2. When the pH is measured after removing the plaque from the tooth surface, this may influence the results. 3. Measurements performed with the mouth open may not reflect physiological conditions. 4. The experimental time is too limited. 5. The antimony electrodes used for the measurements may not give accurate pH values (30). A major question is, however, whether or not the observed pH changes also occur at the plaque enamel interface.

The most reliable but also the most complicated method is probably the telemetry technique developed by Graf & Mühlemann (14). This method has been declared mandatory by the Swiss Federal Health Authorities for the testing of carbohydrate-containing products. The pH changes observed in the present study were in accordance with those found when the same agents were tested by telemetry (28) as a marked difference was seen in the pH drops caused by sucrose and sorbitol. Furthermore, sucrose on a clean tooth surface or distilled water on plaque induced no pH changes. Milk and sorbitol caused similar pH changes, and a limited effect by sodium fluoride could be observed. The close correlation between the present experiments and those obtained in telemetry studies indicate that the Ingold flat surface electrode is a reliable instrument for measuring the influence of stannous fluoride on the acidogenicity of dental plaque.

The present study showed that the acid formation from fermentation of sucrose in dental plaque was strongly inhibited by a 0.2 per cent stannous fluoride mouthrinse and also by the stannous fluoride/stannous pyrophosphate dentifrice. Seven hours after the rinsing or application of the dentifrice the pH changes were still greatly reduced. Sodium fluoride with an equivalent fluoride concentration had only a slight and brief effect. This supports the view that the inhibitory effect is mostly related to the tin and not to the fluoride (21, 27). The slight effect of stannous chloride in the system could on the other hand indicate that the fluoride ions were essential. However, the

moderate effect was probably due to the rapid hydrolysis of stannous chloride in water. It has been suggested that three times as much stannous chloride as stannous fluoride is necessary to obtain the same degree of inhibition (26). Higher concentrations were, however, not tested in the present study.

Bonesvoll and Rölla (3) showed a prolonged retention of tin in saliva after mouthrinses with stannous fluoride. The observations in the present study that large amounts of tin were retained in the plaque for many hours after a mouthrinse also supports the concept that the stannous ion may play an important role in the inhibitory mechanism.

It has been shown that stannous fluoride prevents plaque formation (16, 36, 38), conceivably by interfering with the surface potential of the bacteria (36). It seems possible that tin bound to the surfaces of the bacteria may block the passage of sucrose into the cell and hence inhibit the acid formation. An influence of tin on cellular enzyme systems involved in the metabolism of sucrose is also conceivable. It is generally known that bacteria are more susceptible to fluoride at low pH (24, 25). Some of the effect observed could thus be caused by the combination of fluoride and low pH present in stannous fluoride preparations. However, acidulated fluoride solutions have not proved to reduce the caries increment in clinical experiments more than neutral preparations (1). The acid formation was not influenced by the acetate buffer (pH 3.6); acidity alone had seemingly no effect on the metabolism of the bacteria.

Significant caries reduction has been demonstrated in numerous clinical trials when stannous fluoride has been tested (6). The mode of action has generally been believed to relate to its fluoride content. The result of the present and previous studies (16, 36, 37, 38) indicate that the plaque inhibiting effect of stannous fluoride and its interference with the acidogenicity of plaque should be considered.

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Table 1. *Mean pH changes, measured in pH units, in dental plaque after rinsing with a 0.2 per cent stannous fluoride solution and application of a 15 per cent w/v sucrose solution*

Test person	Before rinse	10 min. after rinse	2 hours after rinse	7 hours after rinse	24 hours after rinse
U.B.	1.9	0.2	0.5	0.9	1.1
H.P.	1.2	0.4	0.4	0.8	1.8
K.R.	2.2	0.8	1.2	1.3	1.9
Mean	1.8	0.5	0.7	1.0	1.6

Table 2. *Mean pH changes, measured in pH units, of dental plaque after application of stannous fluoride/stannous pyrophosphate dentifrice and addition of a 15 per cent w/v sucrose solution*

Test person	Before application	10 min. after application	2 hours after application	7 hours after application	24 hours after application
U.B.	1.9	0.5	0.6	0.9	1.5
H.P.	2.2	0.7	0.7	1.3	1.8
K.R.	1.7	0.6	0.4	1.1	1.8
Mean	1.9	0.6	0.6	1.1	1.7

Table 3. *Concentration and retention of tin in dental plaque, measured in ppm, after rinsing with a 0.2 per cent stannous fluoride solution*

Test person	Before rinse	Immediately after rinse	2 hours after rinse	7 hours after rinse
U.B.	1760	4900	3700	2020
H.P.	403	4830	1910	1945
R.G.	52	5420	3890	2270

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