

# The *in vivo* effect of glucose solutions containing $\text{Cu}^{++}$ and $\text{Zn}^{++}$ on the acidogenicity of dental plaque

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A series of experiments were performed to evaluate the lowest concentration of copper or zinc effective in reducing the acidogenicity of dental plaque *in vivo* when applied simultaneously with a carbohydrate challenge. The effect of a combination of sub-effective concentrations of these metals was also determined. The results showed a significant reduction in acid production in plaque challenged with glucose solutions containing 0.25 mM  $\text{CuSO}_4$  or 5.0 mM  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2$  as compared to glucose solutions alone. Neither 0.1 mM copper sulphate nor 2.5 mM zinc acetate reduced the acidogenicity of plaque significantly, whereas a combination of these subeffective concentrations was effective. The low metal concentrations causing reduction in acid formation in this study may be explained by the inhibiting substances reaching the bacteria at the same time as the substrate. The synergistic effect of the combination of low concentrations of copper and zinc is consistent with the view that these metals effect dental plaque by the same mechanisms.

*Key-words:* Metal ions; preventive dentistry; pH measurements

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It has been shown that copper and zinc ions inhibit plaque formation *in vivo* (7, 16) and also reduce the acidogenicity of established plaque (13, 14). There are strong indications that these effects are caused by enzyme inhibition through oxidation of thiol groups in essential enzyme systems involved in carbohydrate metabolism (15). Oppermann and co-workers (13, 14) reported that 5 mM solutions of copper or 20 mM solutions of zinc inhibited acidogenicity when the metals and the sucrose challenge were applied in succession.

The aim of the present study was to find the minimum concentration of copper or zinc ions which could reduce the acidogenicity of dental plaque when these ions were included as a part of the carbohydrate solutions which constituted the challenge for acid formation. A further aim was to study whether zinc and copper had a synergistic or antagonistic effect if they were applied together in a system as described above.

## MATERIAL AND METHODS

*Test panel:* 3 subjects ranging in age from 18–25 years, full dentitions and healthy gingival conditions, volunteered for the experiment. Clinical and radiographic examinations showed that the participants had no carious lesions.

*Test agents:* To aqueous solutions of glucose (15% w/v) were added copper sulphate and zinc acetate, separately or in combination. The solutions were prepared immediately before use. The chemicals were purchased from Merck Laboratories (W. Germany).

*Experimental design:* Each experiment lasted for 4 days. The participants initially received a thorough scaling and polishing of the tooth surfaces in order to remove supragingival deposits. All mechanical oral hygiene was then suspended for the entire period of the experiment. During the 3 first days sucrose rinses were performed every fourth hour from 8 a.m. to 8 p.m. in order to enhance plaque formation (4). The participants discontinued the sucrose rinses and avoided further sucrose intake 12 hours prior to and throughout the period when the tests were performed.

*pH measurements:* Plaque pH was measured *in situ* with an Ingold flat surface glass electrode, model 203–M3, connected to an Orion Ionalyzer Model A 407. The electrode was calibrated prior to the readings against standard buffers at pH 7.0 and 4.0. Two contra-lateral teeth were selected for the measurements. In the morning of the 4th experimental day, the pH of plaque was measured immediately before and 5, 10, 15, 30, 45 and 60 minutes after rinsing for 1 minute with 10 ml of an aqueous solution of glucose (15% w/v). These data were regarded as the subjects' base-line acidogenicity. The same procedure was repeated when the pH of the plaque had returned to the original level, then using one of the test

solutions described above as mouthrinses.

*Control studies:* A series of control studies were performed in order to test the reliability of the method. The effect of glucose rinses on the pH of plaque was monitored at different occasions in the same day. Plaque pH measurements were also carried out after rinses with distilled water and aqueous solutions of sorbitol (30% w/v) or xylitol (30% w/v).

*Experimental series:* In the first series of experiments glucose solutions with  $\text{CuSO}_4$  concentrations of 0.5 mM, 0.25 mM or 0.1 mM were used. In a second series glucose solutions containing 5.0 mM or 2.5 mM of  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2$  were used. The effect of a combination of sub-effective concentrations of zinc and copper was tested in a third series. All participants tested all solutions. Several pilot studies were carried out to determine the concentration of metals to be used.

*Analysis of the results:* The pH drop ( $\Delta\text{pH}$ ) was expressed as the difference between initial pH value and the lowest pH value observed after a rinse. The statistical significance of the results was tested by an analysis of variance employing pooled standard deviation (1).

## RESULTS

*Control studies:* The minimum pH values following a glucose rinse dropped to a mean of 5.32 with a corresponding mean  $\Delta\text{pH}$  of 1.68 in our test panel. This value was reproduced following glucose rinses at two different occasions during the same day with a mean variation on  $\Delta\text{pH}$  of 0.08 pH units. Sorbitol, xylitol or distilled water did not cause pH levels below pH 6.1 and exhibited a maximum pH drop of 0.45 pH units. The pH values observed in the upper jaw were in general slightly lower than the corresponding values in the lower jaw. Since differences

Table 1. Mean  $\Delta pH^x$  values following a mouthrinse with glucose (15 % w/v) alone (base-line) or added a test agent (experiments)

AGENT		BASE-LINE	EXPERIMENTS	sp'
Copper sulphate	0.25 mM	1.96	0.95 <sup>XX</sup>	0.391
Zinc acetate	5.0 mM	1.46	0.78 <sup>XX</sup>	0.130
Copper sulphate	0.1 mM	1.91	1.64	0.384
Zinc acetate	2.5 mM	1.49	1.35	0.237
Copper sulphate	0.1 mM +	1.64	0.95 <sup>XX</sup>	0.270
Zinc acetate	2.5 mM			

<sup>x</sup> pH is the difference between pH value before and lowest pH value observed following a rinse.  
<sup>XX</sup> Indicate significant differences from the base-line values ( $p < 0.05$ ).

Pooled standard deviation.

were not significant, mean values are presented.

**Experiments:** The acid production in dental plaque following a glucose mouthrinse was significantly lower when 0.25 mM copper sulphate or 5.0 mM zinc acetate was added to the glucose mouthrinses. The differences in acid production in plaque following rinses with glucose solutions containing 0.1 mM Cu or 2.5 mM Zn and glucose alone were not significant. A combination of 0.1 mM copper sulphate and 2.5 mM zinc acetate inhibited acid production (Table 1).

The lowest pH value observed when zinc acetate (2.5 mM) was present was 5.48, and 5.15 when copper sulphate (0.1 mM) was in the mouthrinse. When a combination of Cu (0.1 mM) and Zn (2.5 mM) was used, the pH dropped to a mean of 6.04 with no single value observed below 5.9. The corresponding base-line values were 5.35 and 5.25. Figure 1 shows the mean pH values recorded during one hour after mouthrinses with the individual sub-effective concentrations and with their combinations.

## DISCUSSION

### Methodological considerations

Different *in vivo* and *in vitro* techniques have been described for measuring the

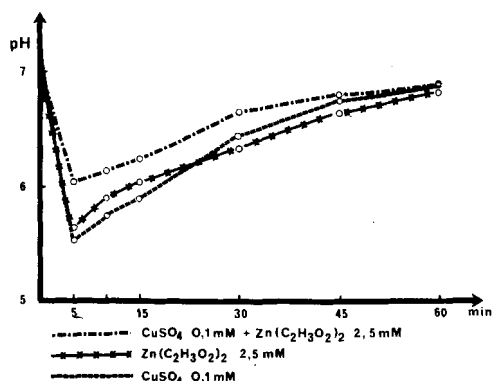


Fig. 1. Mean pH values recorded before and up to 1 hour after mouthrinses with three test solutions.

pH of dental plaque (2, 3, 5, 6, 8, 9, 17, 19). Svaton and Attramadad (20) and Oppermann (12) have discussed the different methods and concluded that a flat surface Ingold glass electrode gives results in accordance with more sophisticated methods like telemetry.

A mean difference in  $\Delta pH$  of 0.08 pH units was observed during repeated rinses with glucose. The response of dental plaque measured in the present study following rinses with H<sub>2</sub>O, xylitol or sorbitol are in agreement with previous studies of this kind (11). In addition the method was sensitive enough to demonstrate the small differences occurring in the pH of dental plaque from the upper and lower jaw after a glucose rinse as described in

previous studies (10, 18). The results of the control studies indicate that the method used in the present study has a good reproducibility. Consequently it was judged to be suitable for monitoring plaque pH changes under different experimental conditions.

Main objections against previous similar methods for pH measurements on the tooth surfaces have been that they were performed while the mouth was open and that the time during which pH of plaque was monitored was usually very limited. The experimental design in this study takes these factors into account. Each measurement required the mouth to be open for about half a minute. This means that the plaque where pH was measured was exposed to air only about 5% of the first 15 minutes. The significance of measurements beyond 5 minutes was investigated and seemed to be of little importance because the lowest pH values invariably were recorded 5 minutes after a challenge.

#### *Comments on the experiments*

Our results showed that a much smaller concentration of the metal ions was needed to achieve a reduction in acid production when these ions were applied simultaneously with the fermentable sugar, compared to the results of Oppermann and co-workers (13, 14) who used local applications of metal ions. These authors employed metal ions and sucrose applied in succession. Concentrations of 5 mM of Cu and 20 mM of Zn were needed to obtain a significant effect on the acidogenicity of plaque. In the present study the concentrations were only 0.25 mM and 5.0 mM respectively. This may to some extent be related to the dilution of sugar by saliva, but the fact that the inhibitory substances reach the bacteria at the same time as the substrate may

also be of importance. When a successive application of metal ions and sugar is used, a certain loss of metals before the carbohydrate challenge is applied seems conceivable.

The use of a combination of copper and zinc was thought to be of interest because it is known that zinc lowers the adsorption of copper in the digestive tract (21). Our results showed that copper and zinc when combined had a significantly better effect than the individual metals. This finding is consistent with the view that both metal ions influence the acidogenicity of dental plaque by the same mechanisms. Further investigations have to be performed, however, to establish if the synergism observed of these metals in plaque is purely additive.

The solutions containing zinc, or copper as described, tasted sweet and were well received by the participants of the study. A slight metallic after taste was reported in some cases.

#### REFERENCES

1. Brown, jr. B.W. & Holander, M. Statistics – A biomedical introduction. John Wiley & Sons, New York, 1977, 225 – 260
2. Frostell, G. Quantitative determination of the acid production from different carbohydrates in suspension of dental plaque material. *Acta Odontol. Scand.* 1964, 22, 457 – 475
3. Frostell, G. Dental plaque pH in relation to intake of carbohydrate products. *Acta Odontol. Scand.* 1969, 27, 3 – 29
4. Gjerme, P., Baastad, K.L. & Rølla, G. The plaque-inhibiting capacity of 11 antibacterial compounds. *J. Periodont. Res.* 1970, 5, 102 – 109
5. Graf, H. & Mühleman, H.R. Telemetry of plaque pH from interdental area. *Helv. Odontol. Acta.* 1966, 10, 94 – 101
6. Graf, R. & Graf, H. Simplified *in vivo* measurements of oral microbial deposits. *Helv. Odontol. Acta.* 1971, 15, 50 – 51
7. Hanke, T.H. Studies on the local factors in dental caries. I. Destruction of plaque and retardation of bacterial growth in the oral cavity. *J. Am Dent. Assoc.* 1940, 9, 1379 – 1393

8. Hassel, T.M. Construction of micro-antimony electrodes for use in telemetry of plaque pH. *Helv. Odontol. Acta.* 1971, 15, 50 – 51
9. Jenkins, G.N. & Kleinberg, I. Studies on the pH of plaque in interproximal areas after eating sweets and starchy foods. *J. Dent. Res.* 1956, 35, 964
10. Kleinberg, I. & Jenkins, G.N. The pH of dental plaques in different areas of the mouth before and after meals and their relationship to the pH and rate of flow of resting saliva. *Archs. Oral. Biol.* 1964, 9, 493 – 516
11. Mühlemann, H.R. Zuckerfreie, Zahn-schonende und nichtcariogene Bonbons und Süßigkeiten. *Schweiz. Monatsschr. Zahn-heilkd.* 1969, 79, 117 – 145
12. Oppermann, R.V. Effect of chlorhexidine on acidogenicity of dental plaque *in vivo*. *Scand. J. Dent. Res.* 1979, 87, 302 – 308
13. Oppermann, R.V. & Johansen, J.R. The effect of fluoride and non-fluoride salts of copper, silver and tin on the acidogenicity of dental plaque *in vivo*. *Scand. J. Dent. Res.* (in press)
14. Oppermann, R.V. & Rølla, G. The effect of some polyvalent cations on the acidogenicity of dental plaque *in vivo*. *Caries Res.* 1980 (in press)
15. Oppermann, R.V., Rølla, G. & Gjermo, P. The effect of metal ions on the acidogenicity of dental plaque *in vivo*. *AADR Abstracts* 1980 *J. Dent. Res.* 1980, 59, 453
16. Skjörland, K., Gjermo, P. & Rølla G. Effect of some polyvalent cations on plaque formation *in vivo*. *Scand. J. Dent. Res.* 1978, 86, 103 – 107
17. Stephan, R.M. Changes in hydrogen-ion concentration on tooth surfaces and in carious lesions. *J. Am. Dent. Assoc.* 1940, 27, 718 – 723
18. Stephan, R.M. Intra-oral hydrogen-ion concentrations associated with dental caries activity. *J. Dent. Res.* 1944, 23, 257 – 266
19. Straalfors, A. Investigations into the bacterial chemistry of dental plaques. *Odontol. Tidskr.* 1950, 58, 151 – 341
20. Svatun, B. & Attramadal, A. The effect of stannous fluoride on human plaque acidogenicity *in situ* (Stephan curve). *Acta Odontl. Scand.* 1978, 36, 211 – 218
21. Venugopal, B. & Luckey, T.D. *Metal toxicity in mammals.* Plenum Press, New York and London, 1978, 2, 24 – 32