Oral retention and discoloration tendency from a chlorhexidine mouth rinse

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Individual differences in tooth discoloration have been observed after using chlorhexidine mouth rinses. To study the correlation between retention of chlorhexidine and the discoloration tendency, two groups, 'stainers' and 'non-stainers', were selected. The following parameters were tested: initial retention of chlorhexidine in vivo, retention of chlorhexidine to saliva-coated hydroxyapatite in vitro, and prolonged release of chlorhexidine in vivo. The initial oral retention of chlorhexidine was identical for the two groups, whereas in vitro retention of hydroxyapatite and prolonged release of chlorhexidine in vivo were higher among 'stainers'. □ *Hydroxyapatite; tooth discoloration; in vivo and in vitro retention*

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Chlorhexidine is a potent plaque-preventive agent, although investigations and clinical experience have demonstrated individual differences in its effectiveness. This variation seems to be related to the retention and availability of chlorhexidine in the oral cavity (6). There are also marked differences in the individual tendency to develop extrinsic dental staining after using chlorhexidine as a mouth rinse (4, 5). Heyden (7) found a direct relationship between the presence of extrinsic staining and local high concentrations of chlorhexidine in the oral cavity.

A recently concluded investigation on two selected groups of dental students demonstrated consistently lower plaque scores among 'stainers' than 'non-stainers' after using a 0.2% chlorhexidine mouth rinse (9). It therefore seemed relevant to perform a more specific analysis of the oral retention of chlorhexidine in 'stainers' and 'non-stainers'. Three parameters were chosen: initial in vivo retention and release of chlorhexidine, in vitro retention of chlorhexidine to saliva-coated hydroxyapatite, and prolonged (12 h) release of chlorhexidine in vivo.

Materials and methods

The test subjects were six selected dental students, three classified as 'stainers' and three as 'non-stainers' on the basis of 10 days' use of 0.2% chlorhexidine acetate (CH) mouth rinse twice daily (9).

Initial retention and release of chlorhexidine in vivo

To estimate the oral retention of chlorhexidine, the participants rinsed with 10 ml 0.2% CH (3.2 mmol/l chlorhexidine) for 1 min. The fluid was then expectorated and collected in the same plastic test tube that had contained the test solution. Three 1-min after-rinses with deionized water then followed with 10-sec intervals, and the expectorates were collected. Two series were performed for each participant with a 3-week interval.

The chlorhexidine content of the expectorates was measured with a colorimetric method described by Holbrook (8). The chlorhexidine extraction was modified in accordance with Dolles et al. (3). Ten milliliters 2 N HCl was added to the expectorates. The first expectorate was then diluted to 100 ml, the others to 25 ml, and they were filtered with a Schleicher & Schüll Weissband filter. The chlorhexidine absorbance of the filter was also measured. Only 10 ml of the first filtrate was used; in the others all the filtrate had to be used. OD_{480} absorption

was measured in a Cecil CE 595 spectrophotometer to determine the final concentration of chlorhexidine.

In vitro retention of chlorhexidine by saliva-coated hydroxyapatite

In the in vitro investigation the affinity of chlorhexidine to hydroxyapatite coated with saliva proteins from each of the 'stainers' and 'non-stainers' was studied. Samples of 10 mg hydroxyapatite (Bio-Gel, Bio-Rad, USA) were washed with 5 ml 1 mmol/l phosphate buffer, pH 6.8, for 5 min and then shaken with 5 ml fresh saliva for 5 min.

After being washed with the phosphate buffer until the supernatants were free from protein, the apatite samples were shaken with either 0.2% CH (3.2 mmol/l) or 0.001% CH (16 μmol/l). The use of the lower concentration was based on the observation that after a conventional mouth rinse chlorhexidine is found in saliva at concentrations falling from 89 μmol/l to 0.3 μmol/l during a 24-h period. This is due to release from depots bound to anionic groups, mainly phosphates, on surface structures of the oral cavity (2).

After centrifugation the OD_{253} of the supernatants were read in a spectrophotometer and the adsorption of chlorhexidine to the saliva-coated apatite calculated by using a standard curve (3). The measurements were performed in duplicate at four different occasions for each subject to determine the individual retention values.

The release of chlorhexidine from saliva-coated hydroxyapatite soaked in a 0.2% CH solution was studied by uisng five consecutive after-rinses of 10 ml distilled water each. The OD_{253} of the after-rinses was read in the spectrophotometer and the release of chlorhexidine calculated. This procedure was repeated twice for each of the six participants.

Prolonged (12 h) release of chlorhexidine in vivo

The six students rinsed for 1 min with 0.2% CH. Three milliliters of unstimulated saliva was collected after $\frac{1}{2}$, 1, 2, 4, 8, and 12 h. One half milliliter 2 N HCl was added to the saliva-coated apatite calculated by blank control and the samples were then centrifuged for 10 min at 4500 rpm.

The samples were analyzed by high-performance liquid chromatography, using external calibration. The chromatograph was operated at a flow rate of 0.8 ml/min, corresponding to about 11 MPa (1600 psi). The mobile phase was 0.1 mol/l NH_4Cl in water. Twenty microliters of supernatant was filtered through a membrane filter, injected through a syringe loading loop injector, and separated on a 5-μm octadecylsilane (ODS) column (2.1 mm × 100 mm). Chlorhexidine was detected by a filter photometer (OD_{253}) equipped with a calculating integrator.

It should be noted that the mobile phase is aggressive towards silica, and column life is short. The chromatographic condition is therefore *not* recommended for general use.

Results

Initial in vivo retention

The results of the initial chlorhexidine retention measurements demonstrated small intraindividual but substantial interindividual variations. The highest initial retention was 48% and the lowest was 21% of the administered dose of the 0.2% CH solution, both from 'non-stainers'. The average retention values were calculated to 30% for the 'stainers' and 34% for the 'non-stainers'. The release of chlorhexidine measured from the three consecutive water after-rinses showed a release of about 20% of the initially retained chlorhexidine (Table 1).

In vitro retention

The results of the adsorption studies in vitro are given in Fig. 1. Hydroxyapatite (HA) coated with saliva from 'stainers' retained 0.1 μmol chlorhexidine/mg HA, whereas the apatite coated with saliva from 'non-stainers' retained 0.07 μmol chlorhexidine/mg HA on the average, after using the 0.2% CH solution. A continuous decrease could be observed from the consecutive after-rinses. The amount of chlorhexidine adsorbed to saliva-coated hydroxy-

Table 1. Oral retention of chlorhexidine from a 10-ml 0.2% chlorhexidine acetate mouth rinse. Oral retention was measured by means of spectrophotometry at OD_{480}, and the values given are the average of two tests

	Retention (% of adm. dose)		Release into water after rinses (% of initial retention)			
Subject		x̄	1st	2nd	3rd	x̄
1 'Stainer'	26		15	5	3	
2 'Stainer'	34	30	12	4	4	22
3 'Stainer'	31		14	5	3	
4 'Non-stainer'	22		12	4	2	
5 'Non-stainer'	21	34	17	5	2	21
6 'Non-stainer'	48		11	5	4	

apatite was dependent on the concentration in the surrounding medium. Thus, the coated apatite retained only 0.002 µmol chlorhexidine/mg HA using the 0.001% CH solution. A tendency to greater chlorhexidine retention associated with saliva from 'stainers' than 'non-stainers' could be observed. Owing to the limited number of participants a statistical analysis has not been performed.

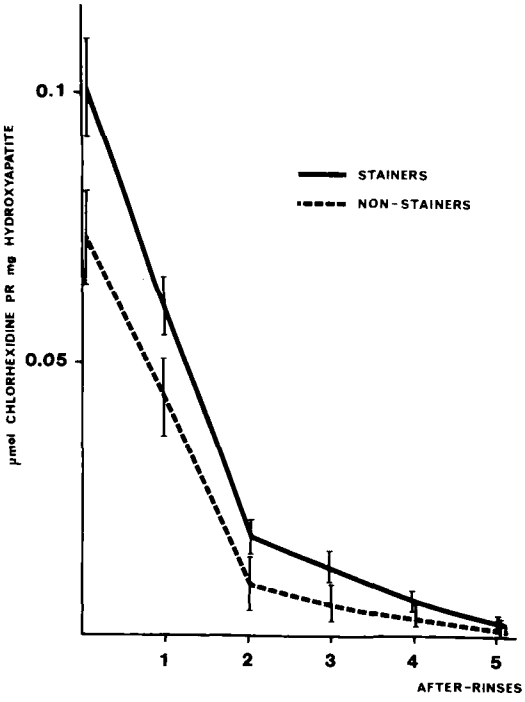

Fig. 1. Retention and release of chlorhexidine from hydroxyapatite coated with saliva from 'stainers' or 'non-stainers'. Chlorhexidine concentrations were measured spectrophotometrically and are given in µmol/mg hydroxyapatite. The standard deviation of the measurements is indicated with vertical lines.

Release in vivo

The results of the prolonged (12 h) release of chlorhexidine after a 0.2% CH mouth rinse are presented in Fig. 2. The 'stainers' had consistently higher chlorhexidine levels in their saliva ½ h to 8 h after a 0.2% CH rinse. After 12 h only one subject, a 'stainer', had detectable amounts of chlorhexidine in saliva. The interindividual variations in chlorhexidine levels were again found to be substantial.

Discussion

Previous investigations indicate that the plaque-preventive capacity of chlorhexidine is dependent on the level of retention in the oral cavity (2, 6). It has further been shown that extrinsic discoloration of teeth appears in connection with locally high concentrations of chlorhexidine (7). In accordance with these results, a study of two selected groups demonstrated better plaque-preventive capacity of chlorhexidine among 'stainers' than 'non-stainers' (9). The present investigation aimed at obtaining more information about the mode of retention and release of chlorhexidine and the etiology of chlorhexidine-induced extrinsic discolorations.

When studying the immediate retention and release of chlorhexidine, marked indi-

vidual variations could be observed. These variations could not be related to the individual discoloration tendency (Table 1). The release of chlorhexidine by water after-rinses was almost identical for the two groups, adding up to about 20% of the initially retained chlorhexidine after three consecutive rinses. The results are in good agreement with those presented by Bonesvoll et al. (1).

The in vitro model is standardized, with the saliva used for coating as the only variable. The results from the in vitro retention of chlorhexidine to saliva-treated hydroxyapatite show higher retention values from 'stainers' than 'non-stainers'. This may indicate that retention to hydroxyapatite—that is, dental retention—more than total oral retention may be of importance for the differences between the two selected groups.

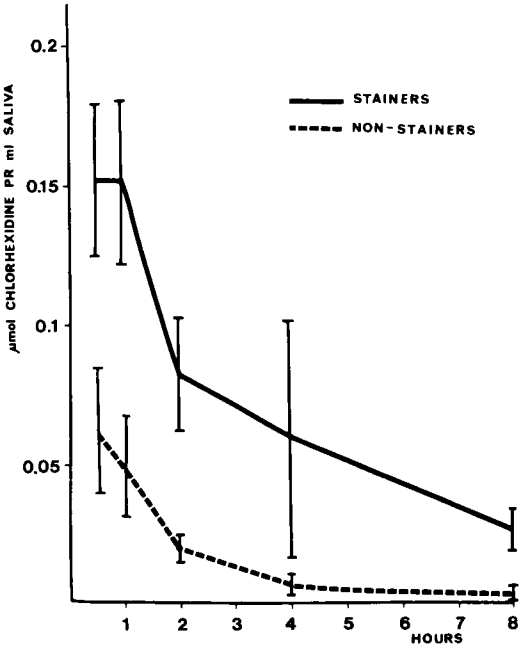

Fig. 2. In vivo release of chlorhexidine after a 0.2% mouth rinse. Saliva concentrations were measured spectrophotometrically in intervals from ½–12 h, and the chlorhexidine concentrations detected are given in µmol/ml saliva. The standard deviation of the measurements is indicated with vertical lines.

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The interindividual variations in retention values were, however, substantial.

When studying the long-term release of chlorhexidine in the oral cavity, marked differences between 'stainers' and 'non-stainers' were observed. Consistently higher oral levels of chlorhexidine were detected in the 'stainer' group ½ h to 8 h after an oral rinse (Fig. 2). This prolonged release and availability of chlorhexidine may explain the increased staining tendency and the consistently lower plaque scores among the 'stainers'. For more detailed discussions about the mechanisms of extrinsic discoloration of teeth, see Refs. 3, 4, and 10.

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