Butterworth Heinemann

*Journal of Dentistry* Vol. 23, No 4, pp. 209–212, 1995

0300-5712/95 $10.00 + 0.00

# Salivary clearance of citric acid after an oral rinse

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## ABSTRACT

*Objectives:* Citric acid clearance from the oral cavity may be an important factor in the erosion of dental enamel. The aim of the present study was to investigate the clearance pattern of citric acid in normal subjects.
*Methods:* After determination of the unstimulated salivary flow rate and the residual volume of saliva after normal swallowing, 12 subjects rinsed with 95.2 mmol $l^{-1}$ citric acid for 5 s. At 1, 2, 5, 10 and 15 min after the rinse a small saliva sample was collected and the concentration of citric acid was determined enzymatically. For each subject three separate experiments on different occasions were performed with a citric acid solution of pH 2.1 and one experiment with a solution of pH 4.5.
*Results:* The salivary citric acid concentration declined biphasically: rapidly during the first 2 min, then more slowly. The elimination rate over the first minute was on average 87.7 mmol $l^{-1}$ $min^{-1}$ at pH 2.1 compared with 85.0 mmol $l^{-1}$ $min^{-1}$ at pH 4.5. The difference between individuals estimated from the areas under the clearance curve was strongly significant ($P < 0.001$). No significant relation was found neither between salivary flow rate and clearance, nor between residual volume after swallowing and clearance.
*Conclusion:* The clearance pattern of citric acid is an individual property.

KEY WORDS: Saliva, Citric acid, Enamel erosion, Clearance

*J. Dent.* 1995; **23**: 209–212 (Received 11 April 1994; reviewed 18 May 1994; accepted 6 July 1994)

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## INTRODUCTION

Enamel erosion may be defined as a mainly superficial loss of dental hard tissue by a chemical process that does not involve bacteria[1]. Besides dental caries, erosion of enamel is the main cause for loss of dental hard tissue. Due to the decreasing caries prevalence in most developed countries, the relative importance of enamel erosion to oral health is increasing. In Switzerland more than 7% of adults showed at least one tooth affected[2]. The prevalence of enamel erosion was found to be 3% in Virginia, USA[3].

The cause of enamel erosion may be attributed to acids contained in food or drinks[2,4,5], hydrochloric acid from the stomach[6,7] or from direct contact with acids in fumes[8]. The clinical appearance is dependent on the cause of the erosion[9]. When analysing the risk factors a considerable risk of erosion was found when citrus fruits were eaten more than twice a day, soft drinks were drunk daily, apple vinegar was ingested weekly, or sport drinks were drunk weekly[10]. The risk of erosion was also high in individuals with low unstimulated salivary flow rate[10].

The mechanism behind enamel erosion is basically that the degree of saturation of the fluid around the enamel with respect to calcium phosphates, mainly hydroxyapatite, is shifted to undersaturation[11] due to a decrease in the concentration of the tertiary orthophosphate ion caused by a decrease in pH. Also, some acids, like citric acid, are strong chelating agents, decreasing the free calcium ion concentration in the saliva[12]. Beverages containing erosive agents are strongly undersaturated with respect to apatites[13].

Every soluble substance taken into the oral cavity is eliminated from the oral cavity by an elaborate process modelled by Dawes[14]. Several important physiological factors have been identified with an effect on the clearance rate, e.g. the salivary flow rate and the volumes of saliva in the mouth before and after swallowing[15]. The oral clearance of citric acid has to our knowledge only been described previously in a single paper[16]. In that study, elimination of citric acid was studied in six subjects after ingestion of an orange or 100 ml orange juice. It was found that the acid was usually eliminated after 10 min, and that the elimination curves resembled those obtained for sugar in the saliva after carbohydrate ingestion.

In many clinical cases the aetiology for the lesions is obscure[1]. It seems likely that an unfavourable clearance pattern may be a part of an explanation for these cases. Therefore, the aim of the present investigation was to investigate the elimination of citric acid from the oral fluids after an exposure. More specifically we tested the hypotheses that:

1. Oral clearance rate is an individual property.
2. Salivary flow rate influences the clearance rate.
3. The residual volume after swallowing has an effect on the clearance pattern.

## MATERIALS AND METHODS

### Subjects

The test panel consisted of 12 subjects—eight females and four males—with ages ranging from 27 to 63 years (mean 45 years). All subjects declared themselves healthy and none were taking any drugs. No subjects wore removable dentures or had other extensive dental work. The study was approved by the Ethical Committee of the Karolinska Institute. The subjects were asked to refrain from eating or smoking 1 h before the experiment.

### Determination of unstimulated salivary flow rate

For each subject the resting salivary flow rate was estimated during a 15 min period. The subjects sat undisturbed in a relaxed position with the head slightly inclined forward, letting the saliva accumulate in the floor of the mouth. The saliva was passively transferred into a preweighed beaker. The measurements were repeated twice on each subject separated by several weeks.

### Determination of the residual volume after swallowing

The residual volume was determined as described by Lagerlöf and Dawes[17]. The volume of saliva left in the mouth after swallowing was measured by a dilution method. After collecting about 0.5 ml of unstimulated whole saliva, the subject swallowed, and immediately afterwards 5.0 ml of distilled water in a beaker was used to rinse the mouth for 5 s before expectoration into another container. The volume of water used was determined by the difference in weight of the beaker before and after rinsing. Five such trials were performed on each subject, during which he/she was asked to wait for three normal swallows to occur. The saliva and the expectorate were analysed for potassium by an ion-selective electrode (Kwik-Tip, World Precision Instruments Inc., Sarasota, FL, USA). The residual volume was calculated by the formula given by Lagerlöf and Dawes[17].

### Experimental procedure

The subject rinsed for 5 s with 10 ml 95.2 mmol $l^{-1}$(2%) citric acid/sodium citrate (Pro Analysi, Merck, Darmstadt, Germany) prepared fresh each week. The ratio citric acid/sodium citrate was chosen so the pH of the solution was either 2.1 or 4.5. A small saliva sample ($< 0.2$ ml) was collected at 1, 2, 5, 10 and 15 min after the rinse. Between the sampling the subject sat relaxed in a resting position. Each subject participated in three experiments using a citric acid solution of pH 2.1 and one experiment using a solution of pH 4.5, performed at different days but at approximately the same hour between 10.00 and 12.00 a.m.

### Analysis of citric acid in saliva

Citric acid was analysed using an enzymatic technique (Cat. No. 139 076, Boehringer, Mannheim, Germany). The principle behind the method is that citric acid is converted to oxaloacetate and acetate in the reaction catalysed by the enzyme citrate lyase. In the presence of the enzymes malate dehydrogenase and L-lactate dehydrogenase, oxaloacetate and its decarboxylation product pyruvate are reduced to L-malate and L-lactate, respectively, by reduced nicotinamide-adenine dinucleotide (NADH). The amount of NADH oxidized is stoichiometric with the amount of citrate. The 1 min sample was diluted 100 times, the 2 and 5 min samples 10 times. NADH was determined by means of its absorbance at 340 nm with spectrophotometer (UV-160A, Shimadzu Co., Kyoto, Japan). The precision of the method determined from 18 duplicate measurements was 8.4%.

### Statistical methods

Each clearance curve was integrated using the trapeze rule giving the area under the clearance curve (AUC). Differences between individuals were evaluated using analysis of variance (ANOVA). The effect of the residual volume and salivary flow rate on clearance AUC was estimated by correlation analysis. A statistical package (SPSS, SPSS Inc., Chicago, IL, USA) was used for the calculations.

## RESULTS

The clearance pattern for each individual, as described

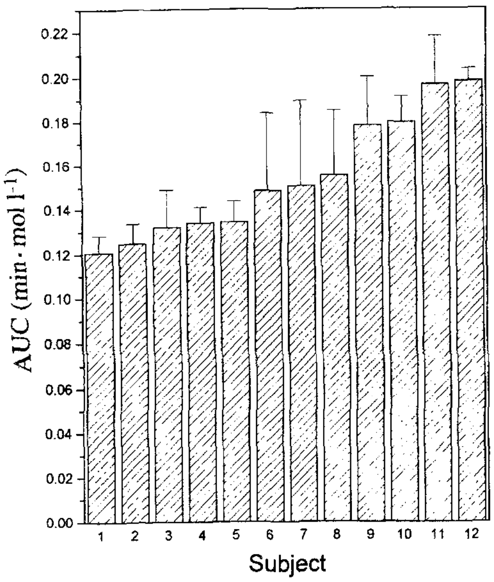


*Fig. 1.* The area under the clearance curve (AUC) after a rinse with 2% citric acid, pH 2.1. On each subject three separate experiments were performed. The vertical bars indicate the standard deviation.

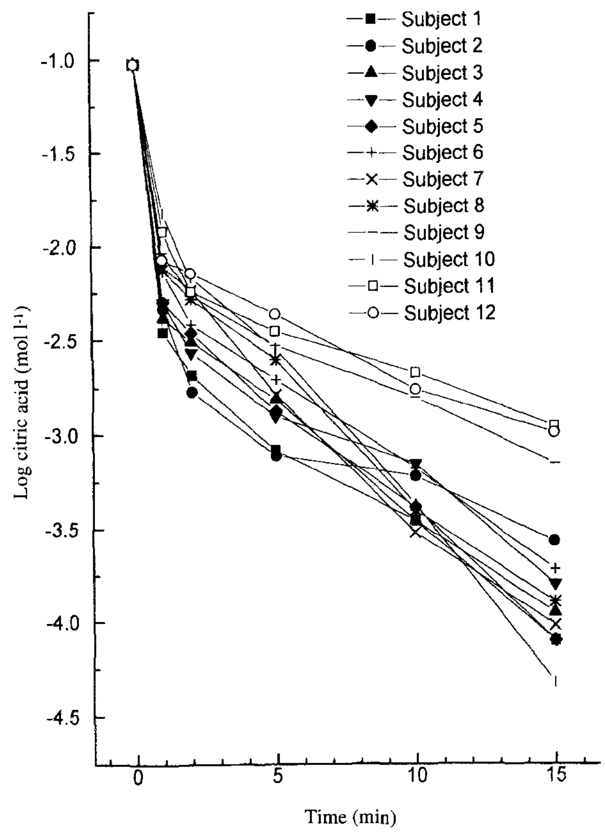


*Fig. 2.* The salivary clearance curve after a rinse with 2% citric acid, pH 2.1. The data are the means from three experiments on each subject.

by the area under the clearance curve (AUC) after a rinse with a citric acid solution, pH 2.1, is shown in *Fig. 1*. The mean AUC was $154.3 \pm 27.3$ min mol$^{-1}$ l$^{-1}$($\pm$S.D.). The difference between subjects was highly significant ($P < 0.001$), but not the difference between the three experiments on each subject ($P = 0.51$). The mean clearance curves after a rinse with a citric acid solution, pH 2.1, are shown in *Fig. 2* for each individual. The curves were bi-phasic, i.e. the citric acid concentration decreased rapidly during the first minutes, then slowly levelled out. The clearance rate during the first minute (means $87.7 \pm 0.72$ mmol l$^{-1}$ min$^{-1}$) differed signficantly between the individuals ($P < 0.05$) and was significantly correlated to AUC ($P < 0.05$), as was the slope between 1 and 2 min (mean $3.0 \pm 2.2$ mmol l$^{-1}$ min$^{-1}$). The mean AUC after a rinse with a citric acid solution, pH 4.5, was $162.2 \pm 51.1$ min mol$^{-1}$ l$^{-1}$ and was significantly correlated ($r = 0.71$, $P < 0.01$) with the AUC at pH 2.1. The slope between 0 and 1 min was on average $85.0 \pm 6.0$ mmol l$^{-1}$ min$^{-1}$, and between 1 and 2 min, $5.1 \pm 3.3$ mmol l$^{-1}$ min$^{-1}$. There was no statistically significant difference in the slope between the two pH levels.

The correlation between salivary flow rate and AUC was non-significant, which was also true for the correlation between the residual volume of saliva after swallowing and AUC.

## DISCUSSION

The present results indicate that the clearance pattern of citric acid from the oral cavity differs significantly between individuals, implying that citric acid clearance may be an individual property. Individual differences in citric acid clearance pattern have been suggested previously[16]. Studies on sugar clearance have shown that the clearance pattern is an individual property, with small variation over long time periods[18]. A relation between salivary flow rate and citric acid clearance has been suggested from observations made[16]. However, this relationship was not found in the present study.

The main cause for erosion is extrinsic acids, such as citric acid contained in a diversity of fruits and beverages[19]. However, there are many cases of erosions presenting clinically that are difficult to explain rationally—these are often called idiopathic erosions[1]. Several explanations of the clinical findings in these cases have been offered: an increase in the citric acid content of stimulated saliva[19], an increase in the mucin content of saliva[20] or local low pH in the vicinity of the eroded surfaces[21]. From *Fig. 2* it can be seen that the citric acid concentration after 10 min may differ with a factor of 10 between individuals. It means that the effect of low citric acid intake frequency may cause a comparatively high risk for erosion in some individuals. This may offer an alternative explanation to the clinical cases with idiopathic lesions, even if a hidden overconsumption of acid-containing food stuffs is not to be overlooked.

In this study the intraoral distribution of acid was not studied. There is much data available on sugar or fluoride distribution after an exposure[22,23], but to our knowledge, none on citric acid distribution in the oral cavity. It seems likely that the extrinsic acid will be retained in the upper incisive area, where erosion lesions have the highest prevalence[1]. It also seems likely that the movement of the salivary film[24] will have an influence on the local clearance pattern: the more prolonged acid clearance, the higher the risk for lesions. This topic has to be explored further.

Citric acid is a potent calcium-complexing agent. Normally about 10% of salivary calcium is bound to excreted citrate[16]. When citric acid is taken into the oral cavity it can be calculated that nearly all free calcium is complexed by the citrate ion. During the clearance process the degree of saturation with respect to calcium phosphate will first be low, presumably undersaturated, in particular since the pH of the solution may be as low as pH 2. During this period calcium and phosphate will leave the enamel surface because of the driving force for dissolution now present. The pH will after a certain period, dependent on several factors, increase mainly due to freshly secreted saliva. As can be seen from *Fig. 2*, the concentrations of citric acid are high for a long time after the ingestion. Since the concentrations of citric acid in these experiments in some of the subjects after 15 min were above 0.5 mmol $l^{-1}$, a considerable part of the calcium ions could be estimated bound[12]. It is not known how the degree of saturation with respect to calcium phosphates varies over time due to clearance of citric acid. However, in understanding erosion clinically this is an important topic and should be investigated.

## CONCLUSIONS

The clearance of citric acid from saliva after an oral rinse was very rapid during the first minute, eliminating more than 90% of the ingested acid. However, the clearance pattern was a highly individual property that was not significantly dependent on the unstimulated salivary flow rate or the residual volume of saliva after swallowing.

### Acknowledgements

This investigation was supported by grants from The Swedish Medical Research Council (project No. 7203) and The Swedish Patent Revenue Research Fund.

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