

# Analysis of Buffer Value of Bicarbonate In Saliva

## SUMMARY

Saliva has a buffer capacity which neutralizes acids in the mouth. The buffer capacity of human saliva is regulated by 3 buffer systems: the carbonic acid/bicarbonate system, the phosphate system, and the proteins. In non-stimulated saliva, the concentration of inorganic phosphate is rather high, while the concentration of carbonic acid/bicarbonate system is low. Carbonic acid/bicarbonate system is the most important buffer in stimulated saliva due to its higher concentration. The aim of this study was to determine salivary bicarbonates levels in patients with different degree of caries activity. We examined 60 children of both sexes, 16 years of age, which were divided in 2 groups according to the condition of the teeth, i.e. the DMFT - index: the first group consisted of 30 examinees with very low caries index (0-3), and the second group consisted of 30 examinees with high value degree of caries (>10). Concentration of salivary bicarbonates was determined with the enzyme method of continuous measuring ("Cobas Mira" - Roche Diagnostic Systems), within different periods: 5, 30, 60 and 120 min. after consuming the meal, as well as before consuming the meals - basic values.

The results refer to close connection between the concentrations of the salivary bicarbonates with the occurrence of dental caries. The concentration of the salivary bicarbonates were remarkably higher ( $p < 0.01$ ) in examinees with lower DMFT- index, compared with the examinees with higher values of DMFT. This refers to the basic values as well as to the values of the bicarbonates in stimulated saliva. The obtained results confirm the importance of the buffer capacity role of the salivary bicarbonates within the oral media and may serve as parameters for determining the caries risk; according to that, we can plan and take appropriate caries-preventive measures.

**Keywords:** Saliva; Salivary Bicarbonates; Dental Caries

**E. Zabokova-Bilbilova, B. Bajraktarova,  
A. Sotirovska-Ivkovska, A. Fildisevski**

Department of Paediatric Dentistry  
School of Dentistry, Skopje, FYROM

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## Introduction

General term "saliva" refers to the fluid that surrounds all oral hard and soft tissues. This oral fluid (that is, whole saliva) represents a mixture of individual fluids and components derived from several sources. Major and minor salivary glands make the bulk contribution to whole saliva, with minor contributions from non-glandular sources, such as crevicular fluid, oral microorganisms, host-derived cell, and cellular constituents, as well as diet-related components<sup>1,2,11</sup>.

The fluids secreted by the parotid, submandibular, sublingual, and minor salivary glands have been shown to differ considerably from each other, to be complex in composition, and to be affected by (1) type, intensity, and

duration of stimulation, (2) time of day, (3) diet, (4) age, (5) a variety of diseases, and many pharmacologic agents.

During the day 0.5 - 1.0 litre per day saliva is produced. Whole saliva is about 99% water and contains a mixture of inorganic ions, including calcium, phosphate, sodium, potassium, chlorine, bicarbonate and magnesium, together with some minor ionic components, including fluoride. Apart from these inorganic components, pooled saliva also contains very wide range of organic molecules. Some of these are simple proteins, such as the enzyme albumin, together with free amino acids. However, the bulk of the organic component is made up of a group of complex glycoprotein, the mucins<sup>13,15,16</sup>.

Salivary secretion is an important factor for oral health, accomplishing mechanical cleansing and protective

functions through various physiological and biochemical mechanisms<sup>3,9,18</sup>.

Theoretically, saliva can affect caries in four general ways:

- mechanical cleansing, resulting in less accumulation of plaque;
- reducing enamel solubility by means of calcium, phosphate and fluoride;
- buffering and neutralizing the acid produced by cariogenic organisms or introduced directly through diet;
- by anti-bacterial activity.

### Buffer Systems

Solutions containing both weak acids and their salts are referred to as buffer solutions. These solutions have the capacity of resisting changes of pH when either acids or alkalis are added to them.

Maintaining of buffer capacity of the acid - base balance is one of the most important protective functions of the saliva. The buffer capacity of human saliva is regulated by 3 buffer systems - the carbonic acid/bicarbonate system, the phosphate system, and the proteins. The carbonic acid/bicarbonate system is the most important one in saliva, but only at high flow rates. Its concentration varies from less than 1 mmol/l in non-stimulated parotid saliva to almost 60 mmol/l at very high flow rates. Thus, in non-stimulated saliva, the level of bicarbonate ions is too low to be an effective buffer<sup>7,8</sup>.

Several studies have show that bicarbonate is one of the salivary components that potentially modifies the formation of caries by changing the environmental pH and possibly the virulence of bacteria that cause decay. Tanzer et al<sup>20,21</sup> tasted the efficacy of a sodium bicarbonate based dental power and paste with the addition of fluoride on dental caries and on *Streptococcus sobrinus* or *Streptococcus mutans* recoveries in rats. These authors observed that the caries reductions in these studies ranged from 42 to 50% in the rats treated with bicarbonate dentifrices when compared with rats treated with water<sup>4,10,12,14,20,21</sup>.

The **aim** of this study was to determine salivary bicarbonates and urea levels in the patients with different degree of caries activity.

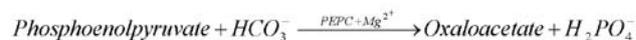
## Material and Method

60 children (30 males and 30 females), 16 years old, with same diet habits, in good health except dental caries, took place in our examination. According to their DMFT-index, they were divided in 2 groups: first group consisted of 30 examinees with very low caries index (0-3), and second group consisted of 30 examinees with higher value degree of caries (>10).

The concentration of salivary bicarbonates wa determined within different periods: 5, 30, 60 and 120 min.

after consuming the meal, as well as before consuming the meals - basic values.

For the collection of non-stimulated saliva, the patient was seated comfortably, with their eyes open, in a standard dental chair. The child sat with their head bent forward and after an initial swallow spat out into a graduated tube approximately every 30s for 5 min. The samples were taken in sterile calibrated bottles (specially intended for this purpose). The collection volume was about 5 ml. The saliva was kept at 4<sup>0</sup>C and transported to the laboratory within 30 minute, centrifuged for 30 minute and the supernatant part was analyzed. HCO<sup>3-</sup> concentration was determined by enzymatic colorimetric method using a commercial kit from GmbH Diagnostic. For enzymatic test phosphoenolpyruvate carboxylase (PEPC) and a stable NADH analogue were used<sup>17</sup>, utilizing the principle:



The decrease of reduced cofactor concentration was measured at 405 or 415 nm and it was proportional to the concentration of total carbon dioxide in the sample.

For statistical evaluation, a 1-way analysis of variance (ANOVA) was initially used to see if there was a significant difference between 2 groups; the Student "t" test was used to compare the DMFT and concentration of HCO<sup>3-</sup> between 2 groups.

## Results

Table 1 shows the basic values of concentration of salivary bicarbonates in both groups. There was a significant difference (p < 0.01) in bicarbonate concentration between first and second group.

Table 1. Basic values of concentration of salivary bicarbonates (mmol/l)

Group	$\bar{x}$	SD	SE	min	max
I	7.94	0.8700	0.1588	6.70	9.80
II	2.48	0.7993	0.1459	1.00	3.90

t = 25.298; df = 58; p < 0.01

Values of the salivary bicarbonate in 5 min period after consuming the meal are illustrated in table 2. The concentration of the salivary bicarbonate in first group was 6.76 ± 1.3402 (SE 0.2447), and 4.66 ± 0.9409 (SE 0.1718)

in the second group. The results display high statistically significant difference ( $p < 0.01$ ) between both groups.

Table 2. Values of salivary bicarbonates in 5 min period after consuming the meal (mmol/l)

Group	$\bar{X}$	SD	SE	min	max
I	6.76	1.3402	0.2447	3.90	9.10
II	4.66	0.9409	0.1718	2.70	5.90

$t = 7.046$ ;  $df = 58$ ;  $p < 0.01$

After 30 min of consuming the meal, concentration of the salivary bicarbonate in first group was  $5.94 \pm 1.996$  (SE 0.2190), and  $3.74 \pm 1.0539$  (SE 0.1924) in the second group (Tab. 3). The results display statistically significant difference ( $p < 0.01$ ) in bicarbonate concentration between first and second group.

In table 4 the values of the salivary bicarbonate concentration after 60 min and 120 min of consuming the meal are illustrated, and it's very clearly that the values are lower than the values after 5 min of consuming the meal; however, there is still some difference between both groups.

Table 3. Values of salivary bicarbonates in 30 min period after consuming the meal (mmol/l)

Group	$\bar{X}$	SD	SE	min	max
I	5.94	1.1996	0.2190	3.00	7.90
II	3.74	1.0539	0.1924	1.50	5.30

$t = 7.546$ ;  $df = 58$ ;  $p < 0.01$

Table 4. Values of salivary bicarbonates in 60 min and 120 min period after consuming the meal (mmol/l)

Time	Group	$\bar{X} \pm SD$	t	df	p
Bicarbonates - 60 min	I	$4.85 \pm 0.9580$	6.926	58	$p > 0.01$
	II	$3.08 \pm 1.0206$			
Bicarbonates - 120 min	I	$4.51 \pm 0.9011$	6.439	58	$p > 0.01$
	II	$2.94 \pm 0.9856$			

## Discussion

Dental caries is a multifactorial disease, which has been afflicting people throughout ages. An important factor which influences the development of dental caries is

saliva. There are also studies showing the effect of diet on saliva secretion and caries development. Saliva provides one of the principal defence mechanisms in the mouth and is know to be important in the pathogenesis of dental caries. Saliva also helps acids quickly to clear away food debris from the mouth and to buffer the organic acids that are produced by the bacteria.

Saliva's protective role is very important to maintain a neutral pH in plaque and in the oral cavity. Its ability to perform this function can largely be attributed to bicarbonates and to a lesser extent to phosphate, as well as other factors. The chief salivary buffer is the carbonic acid/bicarbonate system, while phosphates and proteins play a minor role. The bicarbonate ions, possibly other salivary components, are important in the buffering capacity of this oral fluid and their neutralization of dietary acids will help to determine the pH at the tooth surface after eating.

When saliva secretion is stimulated, the increased rate of flow through the ducts means that there is little time for the ducts to re-absorb sodium and chloride, and the fluid resembles the isotonic primary secretion. Another changer the secretion of bicarbonate ions, which means the composition of saliva, now, is very different from the resting secretion. This bicarbonate raises the pH of the saliva, and greatly enhances its buffering power. The saliva is now more effective in neutralizing and buffering foods, and acids arising in plaque from the fermentation of carbohydrate<sup>5,6,19</sup>.

The results obtained in this study refer to the close connection between the concentrations of the salivary bicarbonates with the occurrence of dental caries. The concentration of the bicarbonates were remarkably higher ( $p < 0.01$ ) in examinees with lower DMFT index, compared to the examinees with higher values of DMFT. This refers to the basic values as well as to the values of the bicarbonates in saliva within different periods from the moment/time of mechanical stimulation (having a meal).

The obtained results confirmed the importance of the buffer capacity role of salivary bicarbonates within the oral media and its responsibility for rapid neutralization of the acid.

## Conclusions

Saliva has an important role in maintaining oral health. Saliva accomplishes its mechanical cleaning and protective functions through various physical and biochemical mechanisms. Saliva has a buffer capacity which neutralizes acids in the mouth.

The carbonic acid/bicarbonate system is the most important buffer in stimulated saliva due to its higher concentration. The values of the bicarbonates in saliva may serve as parameters for determining the caries risk patients and, according to that, we can plan and take appropriate caries-preventive measures.

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Correspondence and requests for offprints to:

Efka Zabokova-Bilbilova  
 Department of Paediatric Dentistry  
 School of Dentistry  
 Vodnjanska 17  
 1000 Skopje, Macedonia  
 e-mail: efka\_zabokova@hotmail.com