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Estimation of the Velocity of the Salivary Film at the Different Regions in the Mouth – Measurement of Potassium Chloride in the Agar Using Atomic Absorption Spectrophotometry

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1. Introduction

Saliva is secreted into the mouth at a rate of 0.3 to 0.4 ml per minute. Retained saliva in the mouth physiologically triggers swallowing to carry the saliva out of the mouth. Dawes (1983) have reported the volume of saliva in the mouth just before swallowing, the rate of swallowing, the volume swallowed per swallow, and the volume of saliva in the mouth just after swallowing. Clearance of materials from the mouth is facilitated by alternately-performed saliva secretion and swallowing, and thereby the oral environment is maintained relatively constant (Fig.1). The unstimulated salivary flow rate and saliva volume in a single swallowing have the most influence on the efficiency of clearance.
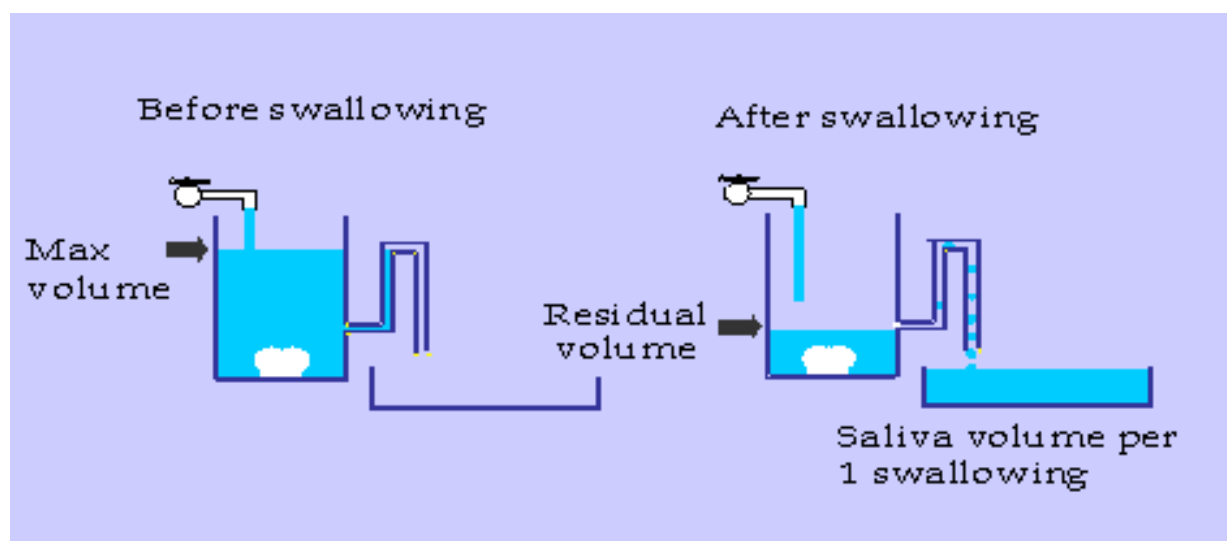

Fig. 1. Saliva volume in the mouth

Saliva is a crucial factor for protection of the oral environment. The rate of oral clearance of sugar and acid is inversely related to the onset and progression of dental caries, as shown particularly in persons with severe hyposalivation.

Saliva secreted into the mouth flows slowly as a thin film, over the tooth surfaces and mucosa and is cleared from the mouth by swallowing (Fig. 2). However, saliva does not flow equally throughout the mouth, and there are differences in the different areas. Measurement of the volume of saliva and velocity of the salivary film at different locations in the mouth are important for understanding the site-specificity of dental caries and periodontal disease.

Using agar as an artificial-plaque, we have conducted studies on the five following items by measuring the clearance of potassium chloride from the agar using an atomic absorption spectrophotometer.

1) Salivary clearance from different regions of the mouth. 2) Salivary clearance in children with complete primary dentitions. 3) Influence of the location of the parotid duct orifice on oral clearance. 4) Effect of salivary flow rate on fluoride retention in the mouth. 5) Estimation of the velocity of the salivary film at different locations in the mouth.
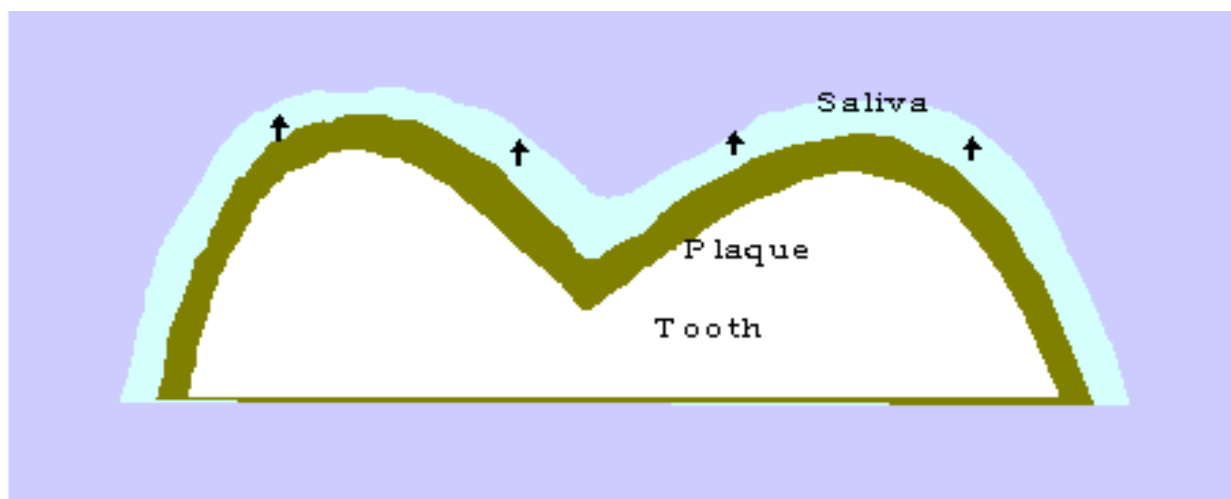

Fig. 2. Salivary film and plaque

2. Study

2.1 Salivary clearance from different regions of the mouth

2.1.1 Aim

Very little research has been carried out on the rates of diffusion of substances into or from dental plaque in vivo. Primosch et al. (1986) studied topical fluoride distribution in the oral cavity and rates of clearance following different methods of dissolution of fluoride tablets. They found that after the chewing, sucking, or passive dissolution of the tablets, fluoride was not evenly distributed in the mouth, and that retention of fluoride was reduced by increased salivary flow rate. Thus, it would seem likely that the rate of renewal of the film of saliva over plaque must influence diffusion rates into and from plaque.

The aim of this study was to determine the velocity of the salivary film by determining the rate of diffusion of potassium chloride from an artificial plaque at different sites in the mouth.

2.1.2 Materials and methods

- Determination of the rate of potassium chloride clearance:

A 1-mol/L solution of potassium chloride was mixed with sufficient agarose (Electrophoresis Purity Reagent; BioRad Laboratories, Richmond, CA) to give a 1.0% solution which was heated until the agarose dissolved. The acrylic chambers（Fig. 3） to hold the gel were rectangular (16 mm in length, 8 mm wide, and 1.5 mm thick) with a cylindrical central depression (6 mm diameter and 1.5 mm depth).
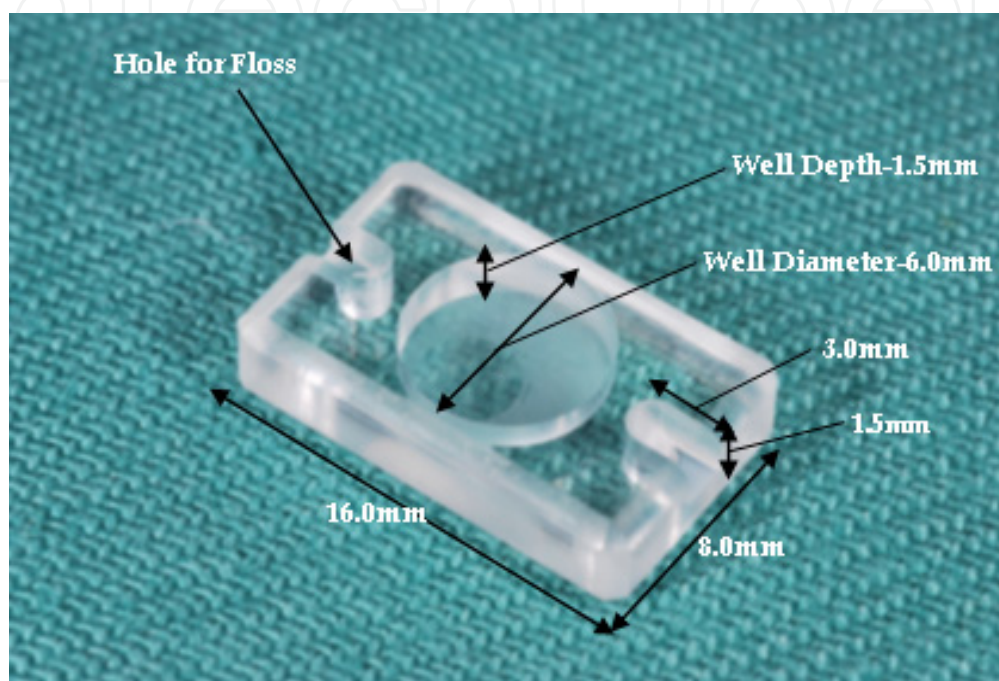

Fig. 3. Diagram of design of the diffusion chambers

The weight of the agarose held in the center well of each chamber was measured six times using an electronic balance (FX-3200; A&D, Tokyo, Japan), and chambers in which the mean weight of agarose was more than 2 SD from the mean were excluded.

Two chambers were initially covered with a layer of Parafilm (American Can, Greenwich, Conn., USA), were attached bilaterally by floss to the teeth, with the gel surface away from the teeth. The chambers were attached to the upper first molars for measurement of the posterior sites (UPB) and to both upper incisors for measurement of the anterior site (UAB) (Fig. 4).

After temperature and salivary flow equilibration, the Parafilm was removed at time 0. The first diffusion chamber was removed from the mouth after being exposed to saliva for a selected period of time and the gel was transferred to flasks containing 400 ml of (100 ppm) sodium chloride. Subsequently the second chamber was removed and the potassium chloride extracted by the same procedure. The fluid was agitated intermittently for 90 min, and the potassium concentration was assayed by atomic absorption spectrophotometry (Shimadzu AA-6105, Kyoto, Japan). The times were chosen so that between about 30 and 60% of the potassium chloride would have diffused from the agarose discs. The initial KCl concentration in the agarose discs, which had not been placed into the mouth, was also measured.

Fig. 4. Acrylic chamber attached to the upper central incised

- Calculation of the half-time (the time for half the KCl to diffuse from the gel) (Lecomte & Dawes, 1987) for clearance.

The rate of potassium chloride clearance from the gels into a large, stirred volume was determined. One involved suspending the filled chambers in one liter of 100 ppm NaCl, stirred by a magnetic stirrer, either at room temperature or at 37℃. The diffusion chambers were taken from the fluid at selected time intervals and the gels transferred quantitatively with a sewing needle to flasks containing 500 ml of 100 ppm sodium chloride. The fluid was agitated intermittently for 90 min, since preliminary studies showed that the remaining potassium chloride was extracted from the gel in this time interval. The potassium concentration was also measured in identically prepared agarose discs which had not been put into the 100 ppm NaCl, to give the initial concentration.

A least-squares straight line was fitted, by computer, to the potassium concentration plotted against the square root of time. This gives a very good approximation of the theoretical clearance curve until about 65% of the diffusant has been lost from the gel (see 2-1-5). From the results, the half-time was calculated.

2.1.3 Subjects and locations

The subjects were 6 adults with a mean age of 26 years. They had a complete dentition up to the second molar and no malocclusion.

Seven different sites in the mouth were chosen for measurements. These were the Lower anterior lingual (LALi) and buccal (LAB), lower posterior buccal (LPB) and lingual (LPLi), upper posterior lingual (UPLi) and buccal (UPB), and upper anterior buccal (UAB). The flow rate of unstimulated whole saliva was measured on each occasion for 5 min by being allowed to drip off the lower lip into a weighed container.

2.1.4 Result

The half-times in the mouth varied with locations and with salivary flow rate, as shown in Fig. 5 When the flow rate was unstimulated, the shortest halftimes occurred in the LALi site and the longest in the UAB site. In both groups, the difference was significant at $p<0.001$.
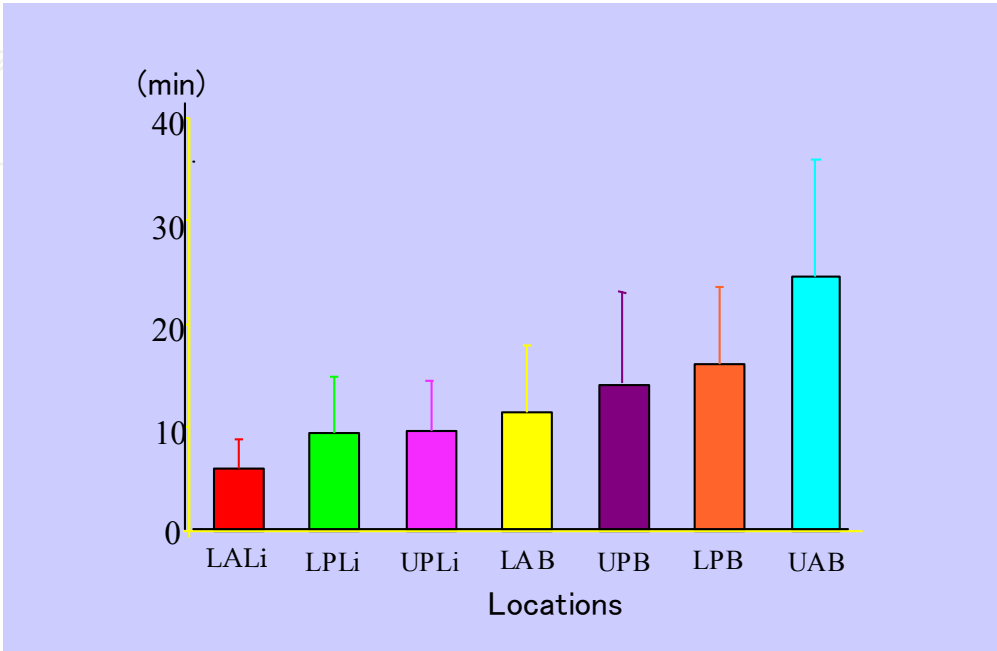

Fig. 5. Half-time when salivary flow rate is unstimulated

2.1.5 Discussion

In this study, we measured the concentration of residual potassium in agarose gel to determine the velocity of salivary flow for the 7 different sites. The reason why potassium chloride was used as the target substance (with an agarose gel used as artificial plaque) is that it is readily soluble in water, harmless, has a low molecular weight enabling it to diffuse easily, is present at a low concentration (around 20 mM) in saliva and can be measured relatively easily. Because the potassium concentration in the agarose gel used in this study was much higher than that in the saliva of the subjects, it was unlikely that the potassium concentration in the saliva affected that in the gel.

The relationship between time and the quantity of potassium diffused from the gel into the saliva was pre-determined in a pilot study. Clearance was evaluated by determining the half-time, the time at which the concentration at time 0 is reduced to half, from the relationship between 3 time points, including time 0, and potassium concentration, as well as by comparing the mean half-time between different sites. Since the correlation between time and the quantity of potassium eluted from the agarose gel was found to decrease in the early and late phases of the test (Lecomte P, Dawes C, 1987), the time to hold the holder in the mouth was determined so that the half-time would be almost at the mid-point of the test. For the measurement of potassium concentration, sodium chloride solution was used as the solvent to avoid errors in measurements due to ionization of potassium.

2.2 Salivary clearance in children with complete primary dentitions

2.2.1 Aim

Nursing bottle caries (Fig.6) is a specific form of rampant decay on the buccal surface of the upper anterior primary teeth. Some etiological factors, such as the types of microorganisms, tooth structure, and diet, have been reported, but there is little information about the influence of the salivary flow rate.

Fig. 6. Nursing bottle caries

Very little research has been carried out on the salivary flow rate or salivary clearance in children. Although the average thickness of the salivary film covering teeth and oral mucosa in children is essentially identical with values reported for adults, marked differences were found between children and adults for such parameters as unstimulated and stimulated whole-salivary flow rates, the volume of saliva in the mouth before and after swallowing, and the surface area of the mouth.

The aim of this study was to evaluate the rates of salivary clearance at different locations in the mouths of children and the effect of the spaces in the primary dentitions to determine whether prolonged clearance would occur in sites particularly susceptible to nursing bottle caries.

2.2.2 Materials and methods

The determination of the rate of potassium chloride clearance was done by the same methods as in study 1. A 1-mol/L solution of potassium chloride was mixed with sufficient agarose (Electrophoresis Purity Reagent; BioRad Laboratories, Richmond, CA) to give a 1.0% solution which was heated until the agarose dissolved. The acrylic chambers（Fig. 3）to hold the gel were rectangular (16 mm in length, 8 mm wide, and 1.5 mm thick) with a cylindrical central depression (6 mm diameter and 1.5 mm depth). The potassium concentration in agarose was analyzed by absorption spectrophotometry.

The subjects were 4 boys and 8 girls, 5 years of age, who were all in good health and with complete primary dentitions. Six subjects had primary spaces (located mesial to the maxillary and distal to the mandibular canines) and developmental spaces (present between the remaining teeth) (Fig. 7), and the other 6 subjects had no spaces in their arches (Fig. 8). The mean values of right and left primary spaces and total developmental spaces for 6 subjects who have spacing arch were 1.1 ± 0.4 mm, 1.2 ± 0.4, and 4.2 ± 1.9 mm for the upper dentition and 0.8 ± 0.3 mm, 0.7 ± 0.3, and 3.8 ± 1.4 mm for the lower dentition, respectively.

Fig. 7. Spacing arch at 5 years old.

Fig. 8. No spacing arch at 5 years old.

Seven different sites in the mouth were chosen for measurements. These were the lower anterior lingual (LALi) and buccal (LAB), lower posterior buccal (LPB) and lingual (LPLi), upper posterior lingual (UPLi) and buccal (UPB), and upper anterior buccal (UAB). The flow rate of unstimulated whole saliva was also measured on each occasion for 5 min by being allowed to drip off the lower lip into a weighed container.

The rate of potassium chloride clearance from the gels into a large volume (1 liter) of 100 ppm NaCl fluid at 37℃ stirred by a magnetic stirrer was determined for the estimation of the half-time.

2.2.3 Result

The mean half-times for in vitro clearance into the large volume of stirred 100 ppm NaCl at 37°C was 3.9 ± 0.5 min.

The half-times in the mouth varied with location as shown in Table 1. The half-times of all sites for the spacing arch and of the LALi site for the no-spacing arch were reduced (p<0.05) as compared with the values when the flow rate was unstimulated.

	Location						( )SD
	LALi	UPLi	LPLi	UPB	LAB	LPB	UAB
Spacing arch	5.9***	9.6*	9.4*	14.2	11.5	16.1	24.6
	(±2.8)	(±4.8)	(±5.4)	(±8.8)	(±6.4)	(±7.4)	(±11.4)
No-spacing	5.3 ***	9.1 **	10.1*	13.8	13.8	17.4	25.9
arch	(±2.1)	(±4.4)	(±5.3)	(±6.8)	(±7.0)	(±8.1)	(±9.4)

Statistical analyses werw carried out between UAB site and the other sites in each group: *p<0.05, **p<0.01, ***p<0.001.
Mean unstimulated salivary flow rates were 0.47±0.2 ml/min.

Table 1. Half-times (mean ± SD) and salivary flow rates when salivary flow was unstimulated

When the saliva flow rate was stimulated (Table 2), the shortest halftimes occurred in the LALi site and the longest in the UAB site. The clearance from the LAB site in the spacing arch showed almost the same value as those from the LALi sites in both groups.

Halftimes(mean±SD)and salivary flow rates when salivary flow was stimulated

	Location		
	LALi	LAB	UAB
Spacing arch(n=6)			
Halftime, min	4.0±0.3**	4.5±0.8**	11.3±4.5
Flow rate, ml/min	3.4±1.5	4.1±2.1	3.4±1.7
No-Spacing arch(n=6)			
Halftime, min	4.0±0.2**	9.4±2.8*	15.4±4.2
Flow rate, ml/min	3.7±1.2	3.9±1.7	4.8±1.7

Halftime in large volume at 37°C=3.9 ± 0.5min.Statistical analyses were carried out detween UAB site and the other sites in each group :*p＜0.05 **p＜0.01

Table 2. Halftimes (mean ± SD) and salivary flow rates when salivary flow was stimulated

2.2.4 Discussion

The present study showed that the rate of clearance of substances from agarose gels into saliva varies markedly in different regions of the primary dentition. The location closest to the submandibular and sublingual ducts (LALi) showed the lowest half-time, whereas the UAB site had a clearance half-time 6.5 times longer than that for clearance into a large volume in vitro. As the opening of the parotid duct is situated on the rearward of the upper second primary molar in the children's mouth, there was a relatively long half-time in the UPB site. Since the mean salivary film thickness in 5-year-old children has been estimated to have almost the same value (0.06-0.09 mm) (Watanabe and Dawes, 1990) as in adults (Collins and Dawes, 1987), these results suggest that the velocity of the salivary film varies in different regions.

The relative order of the half-times at the different sites in the no-spacing arches was identical with that found in a study on adult subjects when saliva flow was unstimulated. Although in the spacing arch, the LAB site had a shorter clearance half-time than the UPB site. These results may be due to the fact that in the spacing arches, the tongue pushes out a portion of saliva from the lingual to the buccal side during swallowing, and this is in accordance with clinical findings that these sites are not susceptible to caries. The ideal arch in the primary dentition has spacing between the teeth (Pinkham et al, 1988), but Foster and Hamilton (1969) reported that only 33% had spacing between all the incisors in the upper and lower arches and that only 12% had spacing between all teeth in both arches in 100 British children aged 30-36 months.

Although it is known that nursing bottle caries depends on the feeding pattern in infancy, the present results suggest that the upper anterior buccal site in a no-spacing arch will be the most cariogenic site in a child's mouth because it has the lowest rate of salivary clearance.

2.3 Influence of the location of the parotid duct orifice on oral clearance

2.3.1 Aim

The rate of oral clearance was shown to vary markedly at different locations in the mouth. Oral clearance is slower for teeth in the maxilla than for those in the mandible and slower for the buccal surfaces of the teeth than for the lingual. Oral clearance on the labial surface of the upper anterior region is the slowest, while that for the lingual surface of the lower anterior region is the fastest. The lingual surface of the lower anterior region is near the openings of the ducts of the submandibular and sublingual glands, which probably accounts for the fastest rate of oral clearance being there. The effect of unstimulated parotid saliva on clearance around the maxillary first molar is not very striking, perhaps because the volume ratio of parotid saliva to total saliva is only about 15% at rest for each side. However, with stimulation, the proportion of parotid saliva increases, increasing clearance over the maxillary first molar, which is closest to the parotid duct. Few studies have examined positional relationships between the parotid duct orifice and the maxillary molars or individual differences in this positional relationship (Suzuki et al. 2009). The present study sought to ascertain the location of the parotid duct orifice in relation to the maxillary molars and whether oral clearance at locations 1 cm mesial and distal to the duct opening would be as rapid as that directly opposite the opening of the duct.

2.3.2 Materials and methods

2.3.2.1 Location of the parotid duct orifice

- Subjects

These were 35 consenting adults (20 men, 15 women) with a mean age of 27.1 years (range, 23-35 years). They had a complete dentition up to the second molar and no malocclusion. In each subject, plaster models were made after taking impressions of the upper and lower dentitions.

- Impressions of the right and left parotid duct orifice

Before taking an impression of the parotid duct orifice, a 2-mm hole was made at the centre of an adhesive therapeutic agent for aphthous stomatitis (Aftach; Teijin, Tokyo) and the agent was placed on the mucosa so that the hole matched the parotid duct orifice. Next, using a vinyl siloxane impression material (Stat BR; Car Japan, Tokyo), an impression of the buccal tooth surfaces and mucosa around the Aftach was taken with the teeth in centric occlusion to localize the duct opening in relation to the teeth.

- Reference plane setting

To take standard photos, a horizontal reference plane was set for each maxillary plaster model. This was a triangular plane defined by the occlusal plane at the maxillary midline and the distobuccal cusp of the left and right maxillary first molars.

- Taking standard photos

The standard plane was set horizontally and the plaster model was matched with the impression of the parotid duct orifice. In order to take standard photos from the same angle, the line connecting the disto- and mesio-buccal interdental papillae of the maxillary first molar was set orthogonal to the imaging direction.

- Location of the parotid duct orifice

After defining the reference plane on standard photos as the X axis and the line perpendicular to the X axis passing through the distal plane of the first molar as the Y axis, the location of the parotid duct orifice was measured in relation to the reference point.

In one subject the location of one parotid duct was determined six times in order to assess the reliability of the method.

2.3.2.2 Oral clearance on the buccal surface of the upper molar region

- Subjects

Subjects comprised 12 (8 men, 4 women mean age 28.3 years) of the original 35 subjects whose parotid duct orifice fell within 1 SD of the mean values for the X and Y coordinates obtained in Study 2-3-2-1.

- The rate of secretion by the parotid gland

The subjects had not eaten for at least one hour prior to the study and the studies were done in either the mid-morning or mid-afternoon. In the 12 subjects for whom oral clearance was measured, Lashley cups were attached over the left and right parotid duct orifices and with

the agar holders in position, parotid saliva was collected on 5 separate occasions for a 5-min period without stimulant.

- Diffusing substance and agar holder

Oral clearance was assessed using the same methods of the study 2-1. 1% agar containing 1 mol/l potassium chloride was placed into cylinders (diameter, 4 mm; depth, 1 mm) held by an acrylic holder (width, 30 mm; height, 10 mm; thickness, 2 mm). The open surfaces of the cylinders were initially covered with microscope slides to allow the agar to set. In each agar holder, 3 cylinders were placed horizontally at 6-mm intervals (Fig. 9).
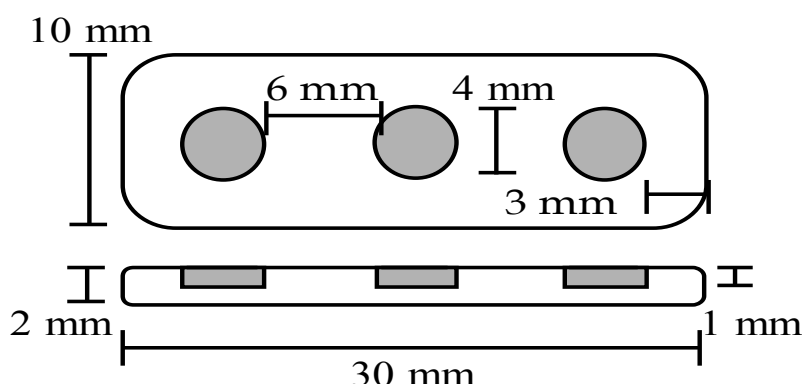

Fig. 9. Agar holder

The cylinders were attached to the teeth using Hydroplastic (TAK, Tokyo) so that the central cylinder would be on the buccal surface of the first molar, at the coordinates of the mean X and Y values obtained in Study 2-4-2-1. After salivary secretion stabilized, which took about 1 minute when parotid flow was measured with a Lashley cannula, the Parafilm was removed to initiate the experiment. On separate occasions, the holder was retained for 5, 10, 20 or 40 min without stimulant. At each time point, the concentration of residual potassium in the agar was measured for calculation of the half- time (the time for half of the potassium chloride to diffuse from the gel), as described by the study 1. Concentrations of potassium were measured by removing the agar cylinders from the holder, soaking each in 300 ml of 100 ppm sodium chloride solution for 90 min, and measuring the levels of eluted potassium by atomic absorption spectroscopy using an ANA-182 spectroscope (Tokyo Koden, Tokyo). The experiment was performed 3 times on both sides of each subject, and mean values were calculated. During the experiment, subjects were asked to refrain from touching the agar holder with their tongue or talking.

2.3.3 Result

Along the X axis, the location of the left and right parotid duct orifices varied within a range of -7.5 to $+6.1$ mm (Mean ± S.D.) from the reference point. Mean location (-0.36 ± 3.76) was just mesial to the reference point. Along the Y axis, the orifice was always located on the positive side of the reference point, ranging from +3.8 to +10.4 mm (mean value: 7.21 ± 2.15) (Fig. 10). This suggests that the parotid duct orifice is located above the reference plane near the contact surface between the maxillary first and second molars. Also, ranges of 13 mm in the mesiodistal direction and 6 mm in the perpendicular direction were noted, showing that

there was a high degree of inter-individual variation. No significant left-right differences were identified. The intra-individual right-left differences were significantly less (P< 0.001) than the overall variability among subjects.

The unstimulated parotid saliva flow rates for left and right sides were 0.02 ± 0.02 and 0.02 ± 0.02 ml/min, respectively and no significant difference was found between results for the two sides. No significant differences in half-time could be detected between comparable left and right regions. Fig.11 shows the half-times for the right and left sides without stimulant. The half-time of the central cylinder was the shortest, followed by the mesial and then the distal cylinders, in that order, for both left and right sides. The half-time values among the 3 cylinders were all significantly different.
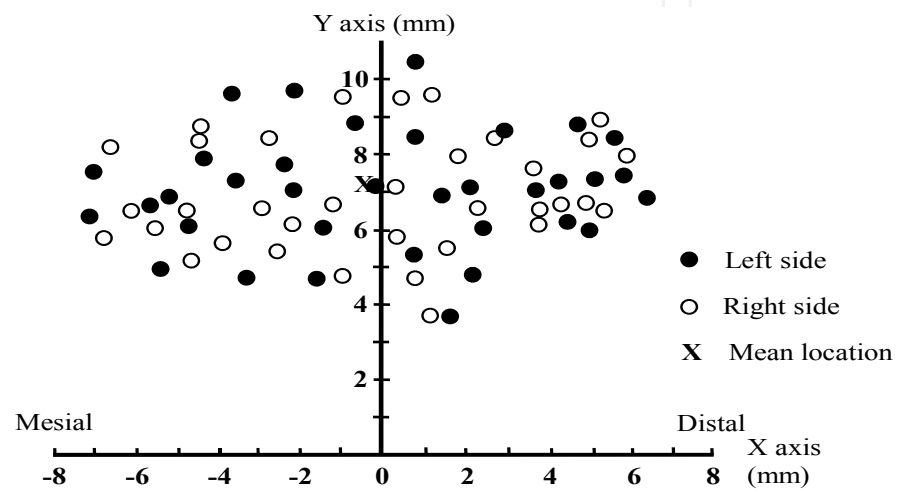

Fig. 10. The location of the parotid duct orifice. The symbols indicate the individual results for the 35 subjects. The x indicates the mean position of the duct orifice.
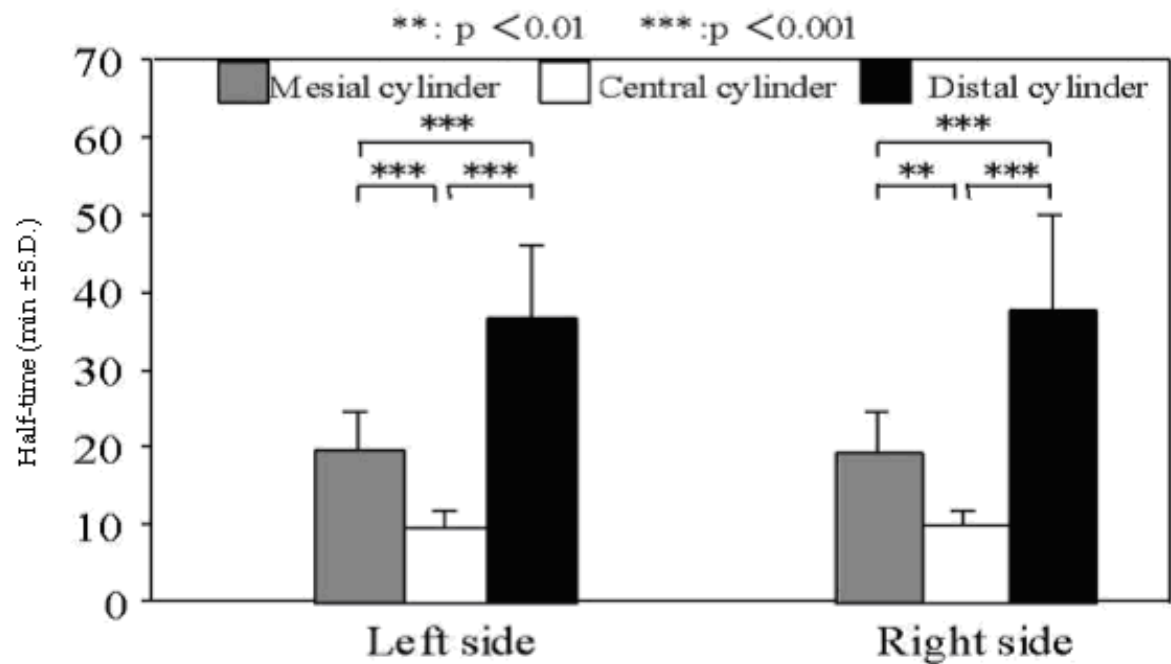

Fig. 11. Half-times when saliva flow was unstimulated

2.3.4 Discussion

The main finding from the second study was that clearance from a site directly opposite the opening of the parotid duct was significantly faster than from sites only one cm either mesially or distally. When salivary flow was unstimulated or stimulated, the clearance half-times mesial or distal to the duct opening were two or more times longer than those opposite the duct opening.

The present results are in conformity with those of Weatherell et al. (1986) who found that when a fluoride tablet was placed in the buccal vestibule, the fluoride concentration peaked in the fluid adjacent to the tablet but was much lower both mesially and distally. The previous reports and our results suggest that when parotid saliva exits the parotid duct, it primarily flows downwards and then, from the results of Weatherell et al. (1986), probably lingually over the occlusal surface of the teeth, rather than flowing mesially or distally in the buccal sulcus. If it flowed primarily in either of these two directions, one would have expected very little difference between the clearance rates from the mesial or distal agar cylinder and that from the cylinder positioned over the parotid duct opening. Sass and Dawes (1977) also reported that very little parotid saliva appeared to flow mesially when flow was either unstimulated or stimulated by the use of chewing gum.

In conclusion, the degree of individual variation in the location of the parotid duct orifice is great and its exact location will markedly affect oral clearance at different positions on the buccal surfaces of the upper molars.

2.4 Effect of salivary flow rate on fluoride retention in the mouth

2.4.1 Aim

Salivary clearance rates in different parts of the mouth are known to vary. The clearance half-times on the buccal surfaces of the upper anterior teeth were the longest of any site in the mouth. These show that the saliva secreted into the oral cavity is not perfectly mixed. Weatherell et al (1986) reports the difference by the fluoride distribution in the mouth after fluoride rinsing. Duckworth and Morgan (1991) and Heath et al. (2001) have also reported oral fluoride retention after use of fluoride rinse. These researches demonstrate the mechanism of the salivary clearance reported by Dawes (1983). According to Lear et al (1965), the salivary flow rate in the sleep is almost similar to the zero, but there are few reports the clearance of the fluoride in the sleep.

The purpose of this research was to measure the site-specificity of fluoride clearance when the subjects were awake and when they had been sleeping.

2.4.2 Materials and methods

40 mg of NaF and 5 ml distilled water were mixed with 0.15 g agarose which was heated until the agarose dissolved. Aliquots were pipetted into holders (diameter 4 mm, depth 1 mm) and these were bonded onto mouthguards produced from plaster casts of each subject (Fig.12).

The bonding sites were on the labial of maxillary incisors (UAB), the buccal of left maxillary molars (UPB) and the lingual of lower incisors (LAL). When the subjects were awake, the

upper and lower mouthguards were fixed in the mouth and exposed to saliva for 15, 45 minutes. The agarose was taken out of the holder and put into 2 ml of distilled water mixed with 0.1 ml of the total ion strength adjustment buffer (TISABⅢ, Thermo Orion, IL, USA) for 90 minutes and the fluoride concentration was measured by atomic absorption spectrophotometry (Shimadzu AA-6105, Kyoto, Japan) as described in study 1. The fluoride concentration of the agarose held in the holder of each mouthguard was measured six times, and holders in which the mean concentration of agarose was more than 2 SD from the mean were excluded. To examine the retention of fluoride in the mouth during sleep, the mouthguards were placed before going to bed (0:00 a.m.) and removed at 6:30 a.m. and the fluoride concentration measured by a fluoride electrode (Thermo Fisher Scientific , MA, USA). The subjects, 6 adults who were all in good health and whose salivary flow rates exceed 0.3 ml/min were selected. Before the experiment, the subjects were explained the purpose and got their cooperation. In order to determine the effects of site specificity of salivary clearance, the data were analyzed by analysis of variance in randomized blocks and by Duncan`s New Multiple Range Test.
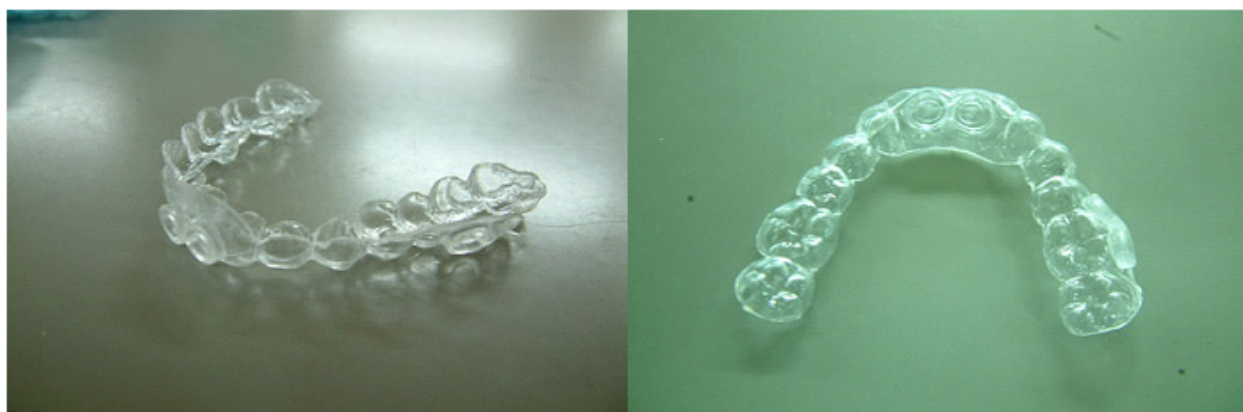

Fig. 12. Mouthguard with agarose holders. (Left: Upper, Right: Lower)

2.4.3 Results

Fig.13 showed the comparison of the mean half-times of each place, expressed as a standard in the value at LAL. The half-times were lowest in LAL and were highest in UAB. There were significant differences between the LAL and UAB (p<0.01), and between the LAL and UPB (p<0.05).

Table 3 showed the comparison of the mean volume of fluoride retention at 6:30 am. when the subjects had been sleeping. The fluoride concentrations were expressed as a percentage of that of the initial agarose which did not expose to saliva in the mouth. The values in LAL were also lowest, and UAB were highest. There were significant differences between the LAL and UAB (p<0.05) and between the LAL and UPB (p<0.05).

Most studies on fluoride clearance in the mouth have been carried out when the subjects were awake, and there is little information when they were sleeping. Ekstrand et al. (1986) and Featherstone et al.(1986) have suggested that fluoride, even at low concentrations, is necessary in the oral fluids to obtain maximum caries inhibition and have concluded that continuous or frequent elevation of the fluoride concentration in the oral fluids would be advantageous.
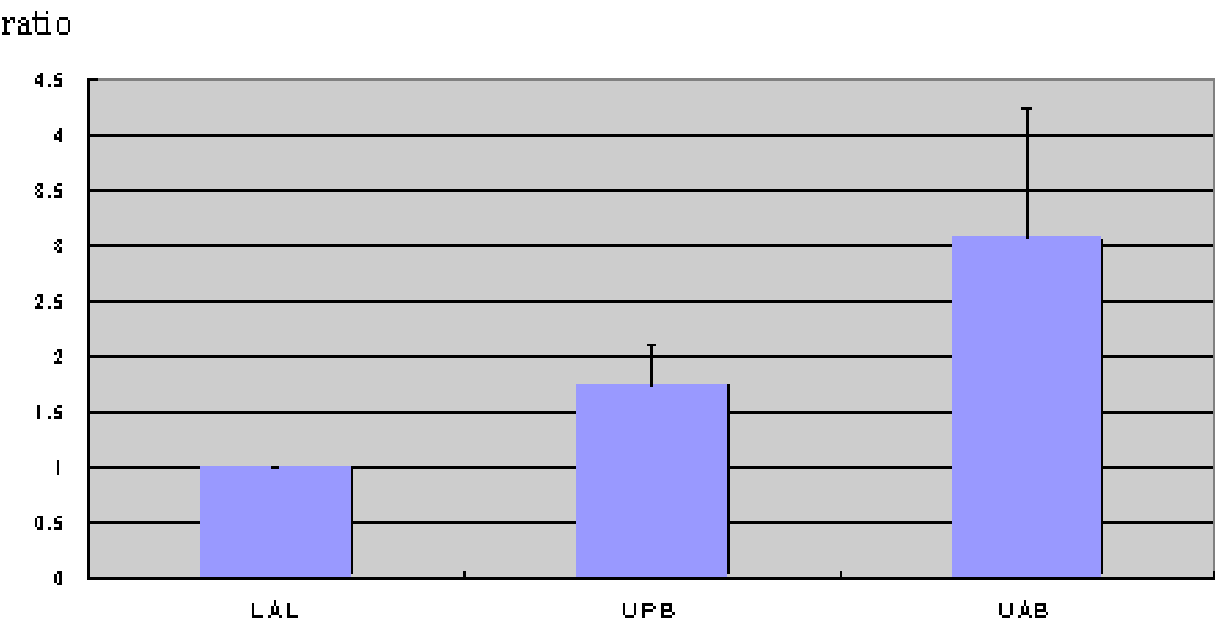
ratio

Fig. 13. The comparison of the mean half-times of each place, expressed in relation to the value for LAL. (p<0.01: LAL vs. UAB, p<0.05: LAL vs. UPB)

	LAL	UPB	UAB
	Mean S.D.	Mean S.D.	Mean S.D.
Subject A	1.2 ±0.3	1.5 ±0.2	3.4 ±2.2
Subject B	1.6 ±0.6	2.6 ±1.7	4.0 ±1.7
Subject C	0.9 ±0.2	0.6 ±0.2	2.6 ±1.8
Subject D	1.8 ±0.6	1.8 ±0.7	3.0 ±2.4
Subject E	1.7 ±0.8	4.1 ±1.2	3.3 ±2.6
Subject F	2.1 ±0.8	2.3 ±1.0	3.5 ±1.4
Mean	1.6 ±0.6*	2.2 ±1.2*	3.3 ±1.8

(*p<0.5: Significantly different from the mean volume of UAB)

Table 3. The mean volume (%) of fluoride retention at 6:30 a.m. when the subject had been sleeping

In this study it was shown that the fluoride concentration in the saliva was kept at high level for a long time during sleeping. In order to prevent dental caries at the buccal surfaces of the upper anterior teeth, it seems to be good to use a fluoride rinse before going to bed.

2.5 Estimation of the veloci1ty of the salivary film at different locations in the mouth

2.5.1 Aim

Although a great deal of information is available about the overall flow rate of whole saliva in man, there is no quantitative information on the velocity of flow of the salivary film in different regions of the mouth. Once secreted into the oral cavity, saliva forms a thin film, approximately 0.1 mm thick, which moves around inside the mouth until it is eventually swallowed. The higher the saliva secretion rate, the more frequently swallowing occurs, and the cleaner the mouth will be remain, However, this salivary film does not distribute evenly or reach all parts of the mouth.

The aim of this study was to estimate of the velocity of the salivary film at different locations in the mouth.

2.5.2 Materials and methods

- The equipment used in the salivary film velocity studies.

An extraoral device was used to adjust the flow rate of a 0.1-mm-thick film of artificial saliva over an agarose disk to determine the clearance half-time in the same manner as that performed intraorally (Dawes et al, 1989). Then, from the relationship between the intraoral and extraoral half-times, the salivary film velocities of the UAB and UPB sites were estimated. The half-time at UAB and UPB were evaluated by the method of study 2.1.

Fig. 14 shows the equipment used. The diameter of the well in the lower part of the device was 6 mm, the same as the width of the 0.1-mm-deep slot in the upper part. Thus, the fluid was directed over the surface of the gel. The well was 4 mm from the end of the device.

The well in the lower part of the device was filled with 1 mol/L KCl in 1 % agarose, as described for study 2.1 (Fig.3) and the upper and lower parts of the device were held together with three spring clamps.
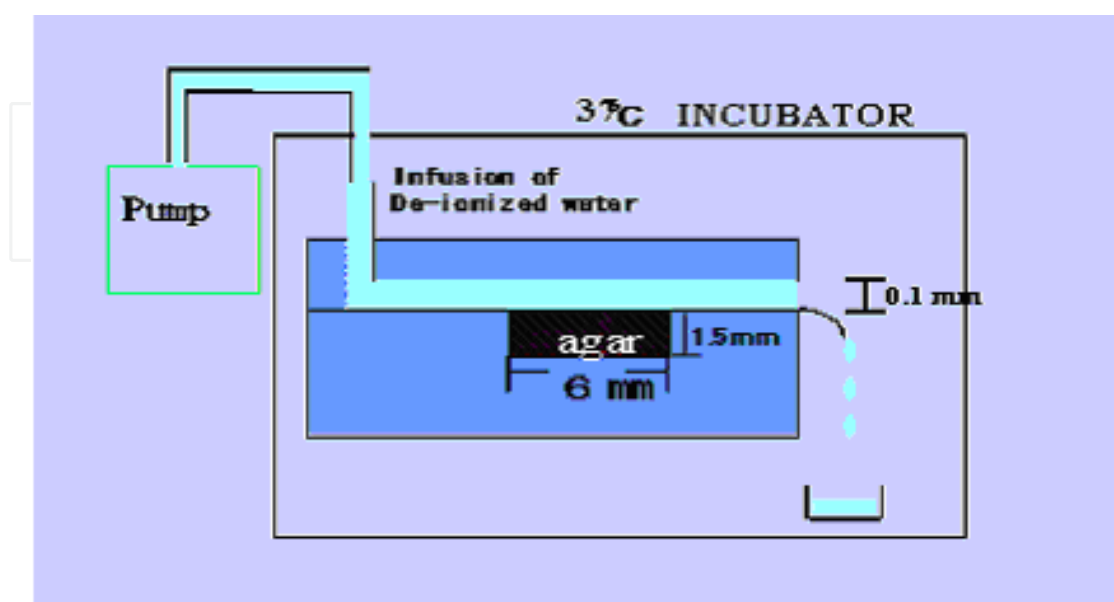

Fig. 14. An extraoral device for salivary flow rate study.

The device was maintained at 37 °C, and de-ionized water at the same temperature was infused with an infusion pump (Model 2000 IW, Harvard Apparatus Co., USA), the flow rate of which was adjustable over a wide range. The pump activated a 5-mL syringe connected to the device via polyethylene tubing.

For an experiment to be initiated, the flow rate of the pump was set to 1.07 ml/min. As soon as the water filled the tubing and completely covered the gel, a stop watch was started, and the flow rate of the pump was set to the desired value. After a pre-determined time, the pump was stopped, the two halves of the acrylic device were separated, and the agarose gel was removed with a needle and transferred to an appropriate volume of 100-ppm NaCl. The potassium concentration was determined by atomic absorption spectrophotometry.

For each flow rate, the experiment was repeated using five different gels for different durations to enable up to 70% of the KCl to be cleared from the gel. For each flow rate, a control gel that had not been exposed to water was used to determine the initial potassium concentration. The experiment was repeated three times for each flow rate. A least-squares straight line was fitted by computer to the potassium concentration plotted against the square root of time, and the half-time was calculated.

2.5.3 Results

There was a significant difference in the mean clearance half-time for the UAB between unstimulated (51.2 ± 19.3 min) and stimulated (40.1 ± 15.1 min) ($P < 0.05$) salivary flow rates. A significant difference was also found in the mean half-time for the UPB between unstimulated (19.2 ± 6.9 min) and stimulated (12.1 ± 5.2 min) ($P < 0.01$) salivary flow rates.

The results on the effect of velocity on the clearance half-time are shown in Table 4. With the flow rates set, the film velocity varied from 0.67 to 100 mm/min. The clearance half-times were inversely related to the velocity of fluid flow, and varied from 2.2 min to 58.3 min.

Fluid Flow Rate (ml/min)	Velocity of Fluid flow (mm/min)	Half-time (min) 6 mm in diam
0.06	100	2.2 ± 1.9
0.005	8.33	8.3 ± 1.2
0.003	5.00	15.1 ± 1.9
0.001	1.66	21.5 ± 2.8
0.0005	0.83	39.4 ± 4.2
0.0004	0.67	58.3 ± 6.1

Table 4. Effect of velocity of fluid flow on the mean half-time ± S.D. for clearance of KCL from an agarose gel, 1.5 mm in depth

Table 5 shows the in vivo clearance half-times for the UAB and the UPB as well as the estimated velocities of flow of the salivary film, as determined from the data in Table 1.

When salivary flow was unstimulated, the velocity of the salivary film of the UAB was estimated as 0.8 mm/min, whereas for the UPB it was estimated as 40.1 mm/min. When

salivary flow was stimulated, the velocity of flow for the UAB was estimated as 2.3 mm/min, whereas for the UPB it was estimated as 12.1 mm/min.

Site	Salivary Flow	Clearance Half-time* (min)	Estimated Velocity of Salivary Film** (mm/min)
UAB	U	51.2 ± 19.3	0.8
	S	40.1 ± 15.1	2.3
UPB	U	19.2 ± 6.9	40.1
	S	12.1 ± 5.2	12.1

U = unstimulated (mean S.D = 0.42 ± 0.21 ml/min).

S = stimulated (mean S.D. = 4.5 ± 2.3 ml/min).

* = in vivo data

** = from the data for the half-time in Table 1 of this study.

Table 5. Estimated velocity of the salivary film at UAB and UPB sites in the mouth

2.5.4 Discussion

Extraoral device for estimating salivary velocity at both sites:

Lagerlöf and Dawes (1984) measured oral salivary volume immediately before and after the onset of swallowing and reported the mean volumes to be 1.07 and 0.77 ml, respectively. Collins and Dawes (1987) and Watanabe and Dawes (1990) measured the surface area of the mouth, and based on the oral salivary volumes reported by Lagerlöf and Dawes (1984), they estimated the mean thickness of the salivary film in the mouth to be 0.1 mm. The extraoral device on which a 0.1-mm-thick salivary film flows on an agarose gel was designed on the basis of these reports to reproduce the situation in the mouth extraorally. Artificial saliva was allowed to flow onto an agarose gel in the same holder as that used in the mouth at different flow rates to determine the relationship between flow rate and clearance half-time, based on which salivary velocity at the two sites in the mouth was calculated. The velocity estimated with this device appears to be more useful for comparing salivary velocity between the different sites in the mouth than in determining actual salivary velocity. The mean half-time at stimulated salivary flow was 12.1 ± 5.2 min in the present study, which is substantially different from that obtained at unstimulated salivary flow. This may be attributable to the substantial difference in the secretion rate of saliva between when saliva is unstimulated and stimulated condition.

3. Conclusion

In clinical dentistry, it is generally accepted that the mandibular front teeth has low caries sensitivity, and the maxillary front teeth has high (e.g. nursing bottle caries) and the caries incidence of the buccal side of teeth in old person is higher than the lingual side. The results of this study have confirmed these clinical situations.

The author has also concluded that the velocity of salivary film is different by each site in the oral cavity and the slow velocity of the salivary film over the different surfaces of the teeth will retard clearance from diffusants of plaque such as acid. This suggests that the pH of each site in the oral cavity is slso different (Watanabe 2008),

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