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Estimation of the Velocity of the Salivary Film at Some Different Locations in the Mouth

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Previously, we studied the clearance rates of KCl from agarose gels positioned at different locations in the mouth, and showed that the rates were much slower than when clearance was into a well-stirred solution. We designed the present *in vitro* study to test the effect on KCl clearance of the velocity of a 0.1-mm-thick film of water flowing over an agarose gel of the same diameter and composition as those used *in vivo*. The thickness of the salivary film overlying dental plaque has been estimated to be about 0.1 mm, and we assumed that when clearance rates *in vitro* matched those found *in vivo*, velocities of the fluid film (*in vitro*) and the salivary film (*in vivo*) must be equal. On this basis, it was calculated in the present experiments that when salivary flow was unstimulated, the velocity of the salivary film at the level of the teeth varied between about 0.8 mm/min (upper-anterior buccal region) and 8.0 mm/min (lower-anterior lingual region). When salivary flow was stimulated, this was estimated to increase the velocity of the salivary film from 2 to 40 times, depending on the location in the mouth. It is postulated that the slow movement of the salivary film when flow is unstimulated allows for accumulation of diffusants from dental plaque, which reduces the concentration gradient for diffusion from plaque and prolongs the clearance time of such metabolic products as acid.

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Introduction.

Although a great deal of information is available about the overall flow rate (mL/min) of whole saliva in man (Dawes, 1987), there is no quantitative information on the velocity of flow (mm/min) of the salivary film in different regions of the mouth, although clearance rates in different parts of the mouth are known to vary (Weatherell *et al.*, 1984; Primosch *et al.*, 1986). Collins and Dawes (1987), from measurement of the surface area of the mouth, and from a knowledge of the volume of saliva in the mouth (Lagerlöf and Dawes, 1984), have calculated that the salivary film averages only 0.1 mm or less in thickness.

Lecomte and Dawes (1987) studied the rates of clearance of potassium chloride from agarose gels (artificial plaque) positioned at different sites in the mouth. On the lingual surfaces of the teeth, the half-times for clearance were about double that for clearance into a well-stirred solution. However, on some buccal tooth surfaces, clearance half-times were increased up to 14 times. The differences in half-times were attributed to differences in the velocity of flow of the salivary film at different sites in the mouth. The slower the velocity, the greater would be the opportunity for accumulation of diffusant from the plaque, with a lessening of the concentration gradient, thus causing an increase in the half-time for clearance.

The objective of the present study was to estimate the ve-

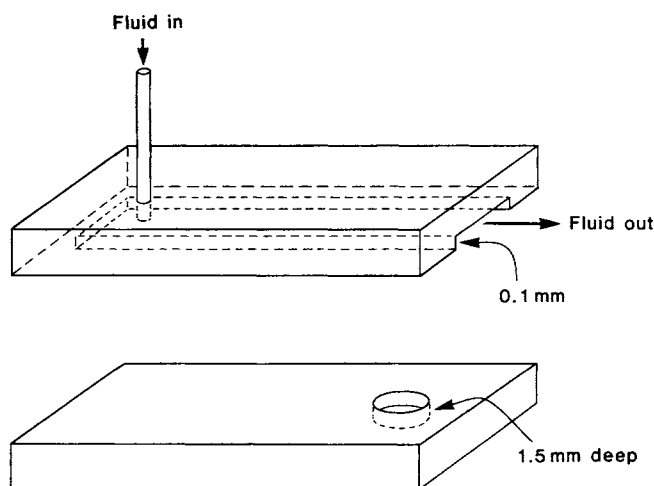


Fig. 1—An exploded view of the design of the equipment used in the salivary film velocity/clearance studies. The 1.5-mm-deep well was filled with 1% agarose containing 1 mol/L KCl, which diffused into the 0.1-mm-thick fluid film moving at known velocity.

locity of the salivary film at different locations *in vivo*. To do this, we designed an *in vitro* system similar to that used by Lecomte and Dawes (1987) *in vivo*, but in which the velocity of the fluid film flowing over the agarose gel could be varied. If, by varying the velocity of the fluid film *in vitro*, the half-times for potassium chloride clearance from agarose gels *in vitro* could be made to correspond with those *in vivo*, presumably the velocities *in vivo* and *in vitro* would be equal.

Materials and methods.

Fig. 1 illustrates a device that was specially machined from clear polymethacrylate by the University of Manitoba Machine Shop. 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. Prior to use, the upper and lower parts were dipped in a 1:200 dilution of Photo-flo 200 (Kodak Canada Ltd., Toronto) and allowed to dry. This was found necessary to ensure that fluid would flow uniformly across the acrylic surfaces. When a red dye was infused into the system, the dye was seen to move with a linear front over the gel.

The well in the lower part of the device was filled with 1 mol/L KCl in 1% agarose, as described previously for the *in vivo* study (Lecomte and Dawes, 1987), and the upper and lower parts of the device were held together with three spring clamps.

The device was maintained at 37°C, and de-ionized water at the same temperature was infused with an infusion pump (Model 975, Harvard Apparatus Co., Inc., Dover, MA), the

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TABLE 1

EFFECT OF VELOCITY OF FLUID FLOW ON THE MEAN HALF-TIME \pm S.D. FOR CLEARANCE OF KCl FROM AN AGAROSE GEL, 1.5 mm IN DEPTH

| Fluid Flow Rate (mL/min) | Velocity of Fluid Flow (mm/min) | Flow Time* (min) | Half-time (min) | |
|--------------------------|---------------------------------|------------------|----------------------------------|---------------------------------|
| | | | Cylindrical Gel* 6 mm in diam | Square Gel** 6 \times 6 mm |
| 0.545 | 908 | 0.003 | 3.65 \pm 0.43 | 4.05 \pm 0.15 |
| 0.0517 | 86.2 | 0.035 | 4.38 \pm 0.46 | 4.76 \pm 0.26 |
| 0.0049 | 8.17 | 0.367 | 8.26 \pm 0.62 | 8.85 \pm 0.26 |
| 0.0025 | 4.17 | 0.719 | 14.83 \pm 4.96 | not tested |
| 0.00128 | 2.13 | 1.41 | 20.65 \pm 1.30 | 23.08 \pm 3.45 |
| 0.000912 | 1.52 | 1.97 | 29.92 \pm 2.25 | 38.35 \pm 2.61 |
| 0.000651 | 1.08 | 2.78 | 38.28 \pm 5.39 | 44.67 \pm 2.94 |
| 0.000465 | 0.78 | 3.85 | 61.22 \pm 8.92 | 64.77 \pm 7.76 |

* $n = 3$, ** $n = 4$, for each individual flow rate.

+ Flow time = time for the fluid film to flow 3 mm (*i.e.*, replace half the film over the 6-mm-wide gel).

flow rate of which was adjustable over a wide range. The pump activated a 5- or 10-mL syringe that was connected to the device with 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 (which took about 3 s, a very small fraction of the experimental time), and the agarose gel was removed with a needle and transferred to an appropriate volume of 100-ppm NaCl. This was agitated for 1.5 h so that the remaining potassium chloride could be extracted from the gel, and then the potassium concentration was determined by atomic absorption spectrophotometry (Lecomte and Dawes, 1987). The 100-ppm NaCl acts to reduce potassium ionization. For each flow rate, the experiment was repeated with from three to five different gels, for different periods of time that would span the time for 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 for determination of the initial potassium concentration. A least-squares straight line was fitted by computer to the potassium concentration plotted against the square root of time (Lecomte and Dawes, 1987), and the half-time (the time for half the KCl to diffuse from the gel) was calculated. The experiment was repeated three times for each flow rate. Similar studies were also carried out with wells that were 6 mm square and 1.5 mm deep. The data from the latter studies were subsequently used so that a computer model of the system could be tested (Dawes, 1989). Only cylindrical gels were tested *in vivo* by Lecomte and Dawes (1987).

Results.

The results on the effect of fluid velocity on the clearance half-time are shown in Table 1. With the flow rates chosen, the film velocity varied over the range of 0.8 to 908 mm/min. As expected, the clearance half-times were inversely related to the velocity of fluid flow, and they varied from less than 4 min to over 60 min. As was also expected, for any given fluid velocity, the half-times for the 6-mm-square gels were somewhat larger than those for the cylindrical gels of 6-mm diameter, because of the larger surface area of the former.

There was only a relatively small effect on clearance half-time as the fluid velocity fell from 908 to less than 8.2 mm/

TABLE 2

ESTIMATED VELOCITY OF THE SALIVARY FILM AT DIFFERENT SITES IN THE MOUTH

| Site | Salivary Flow | Clearance Half-time* (min) | | Estimated Velocity of Salivary Film** (mm/min) |
|-------------------------|---------------|----------------------------|---|--|
| | | U | S | |
| Lower-anterior Lingual | U | 9.7 \pm 5.5 | | 7.6 |
| | S | 4.5 \pm 1.4 | | ca. 350 |
| Upper-posterior Lingual | U | 11.1 \pm 3.3 | | 6.8 |
| Lower-anterior Buccal | U | 42.2 \pm 30.6 | | 1.0 |
| | S | 20.6 \pm 10.0 | | 2.4 |
| Upper-anterior Buccal | U | 56.1 \pm 30.6 | | 0.8 |
| | S | 34.1 \pm 27.1 | | 1.3 |

U = unstimulated (mean \pm S.D. = 0.46 \pm 0.29 mL/min*).

S = stimulated (mean \pm S.D. = 4.1 \pm 2.0 mL/min*).

* = *in vivo* data from Lecomte and Dawes (1987).

** = from the data for the cylindrical gels in Table 1 of this study.

min. However, when the fluid velocity was less than 2 mm/min, slight reductions led to very large increases in clearance half-times. That is, the relationship between the velocity of fluid flow and the clearance half-time had the form of a hyperbola.

In Fig. 2, the clearance half-time for the square gels is plotted against flow time, which is the time for half the fluid film over the gel to be replaced with fresh fluid. The relationship was linear ($r = 0.996$), with a slope of 15.68.

Table 2 gives the *in vivo* clearance half-times reported by Lecomte and Dawes (1987) for different sites in the mouth, with salivary flow either unstimulated, or stimulated by having the subject suck on sour lemon drops. This Table also gives the estimated velocities of flow of the salivary film, as determined from the data in Table 1 for the 6-mm-diameter cylindrical gels.

When salivary flow was unstimulated, the highest velocity of the salivary film was estimated to be 7.6 mm/min in the lower-anterior lingual region of the teeth, whereas the lowest velocity was estimated to be 0.8 mm/min in the upper-anterior buccal region of the teeth.

When salivary flow was stimulated, the velocity of flow in the lower-anterior lingual region of the mouth was estimated to be about 350 mm/min, but it was not possible for a very reliable estimate to be provided. This is because, as film velocity increases, the clearance half-time approaches asymptotically the value for clearance into a well-stirred solution. The clearance half-time of 4.5 min for the lower-anterior lingual region when salivary flow was stimulated is fairly close to the minimum value. In the other two sites tested, when salivary flow was stimulated, the estimated velocities were 2.4 and 1.3 mm/min in the lower- and upper-anterior buccal regions, respectively. These values were about double those when flow rate was unstimulated.

Discussion.

Our study is based on the assumption that the thickness of the salivary film at different locations in the mouth is uniform and about 0.1 mm (Collins and Dawes, 1987). If the salivary film between adjacent layers of oral mucosa or between the teeth and mucosa were not of uniform thickness—for instance, on the buccal and lingual aspects of the teeth—this would presumably be caused by unequal pressure on the teeth from the tongue and from the cheeks. Since the position of the teeth tends to be stable, presumably the buccal and lingual forces

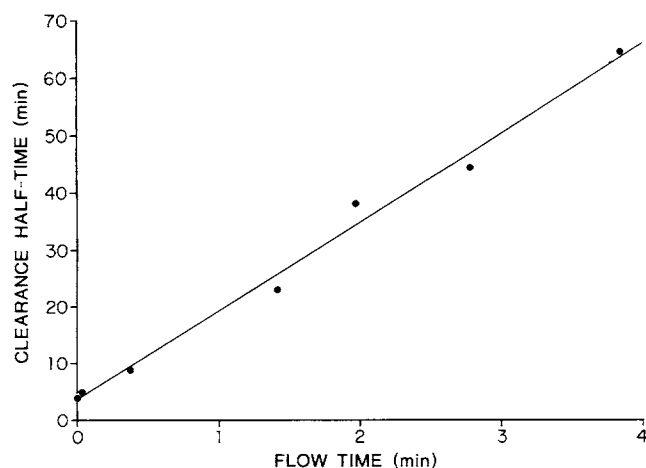


Fig. 2—The clearance half-times for the 6-mm-square gels plotted against Flow Time (the time for the fluid film to move 3 mm, *i.e.*, half the length of the gel). The line is the regression line ($r = 0.996$) with intercept 3.62 and slope 15.68.

are approximately in balance and thus, the thickness of the salivary film is likely to be similar on each surface.

Another assumption of the technique is that the rate of diffusion into water is about the same as that into a film of saliva. Since saliva is 99.5% water (Jenkins, 1978), this assumption seems reasonable and has received some experimental confirmation (Dibdin, 1984). Furthermore, the linear relationship seen in Fig. 2 between the clearance half-time and the flow time (time for half the fluid film over the agarose gel to be replaced by fresh fluid) is of interest, since it implies that clearance of diffusant from the gel was controlled largely by the bulk flow over the surface, rather than by diffusion processes alone. Thus, a slight difference between the rates of diffusion in water and in saliva would be of little importance. This relative lack of importance of the diffusion coefficient has also been confirmed in a computer model of this system (Dawes, 1989). The intercept on the y axis (3.62 min) of Fig. 2 is very close to the half-time for diffusion into a well-stirred solution (3.9 min), and the slope (15.68) is very close to 16, the ratio of the thickness of the gel plus fluid film (1.6 mm) to the thickness of the fluid film (0.1 mm).

The surfaces of the oral mucosa and teeth are not uniformly smooth, but in certain areas, such as the occlusal fissures of the teeth and dorsum of the tongue, are covered by micro-invaginations. It seems likely that exchange of fluid between the moving salivary film and that in the micro-invaginations will be relatively slow, because of the forces of capillary attraction. The studies of Lecomte and Dawes (1987) were carried out mostly in areas of the mouth where there are no micro-invaginations, except for the palatal aspects of the upper teeth that would be in contact with the dorsum of the tongue. Thus, we do not believe that this factor would have significantly affected the experimental results of Lecomte and Dawes (1987).

The present studies give no information about salivary film velocities over the approximal surfaces of teeth, or over plaque present in interproximal spaces. It seems likely, however, that the flow patterns will be disturbed in such locations because of individual anatomical factors. Salivary film thickness will also not be uniform over interproximal plaque.

This study is the first to give estimates of the velocity of flow of the salivary film in different locations in the mouth. When salivary flow was unstimulated, the results suggest that the velocity of flow at the level of the teeth in all sites tested

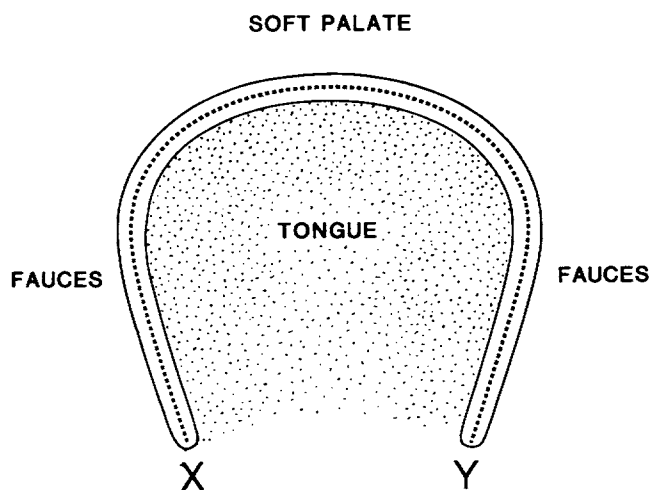


Fig. 3—Section of the tongue in the coronal plane posterior to the teeth, to illustrate the linear distance (dotted line from X to Y) across which the salivary film leaves the oral cavity.

was less than 8 mm/min. Values ten times less than this were estimated for the upper-anterior buccal region of the teeth, where minor mucous gland secretions will probably be by far the main type of saliva present. Minor mucous gland secretions are much more viscous than those of the secretions from the major salivary glands. This factor, together with the slow rate of secretion by the minor salivary glands, probably accounts for the slow film velocity on the anterior buccal aspect of the teeth.

The locations in the mouth for which clearance times were determined by Lecomte and Dawes (1987) were all on the buccal or lingual aspects of the teeth, regions where plaque commonly accumulates. If we consider the uppermost part of, for instance, the anterior buccal sulcus, it may well be that here, the salivary film is actually stationary, as indicated in Fig. 3 of Lecomte and Dawes (1987).

When salivary flow was stimulated, the velocity of flow in the upper- and lower-anterior buccal regions approximately doubled, but in the lower-anterior lingual region, the increase was much more dramatic (over 40-fold).

From the study by Collins and Dawes (1987), the linear distance from the depth of one lingual sulcus to the depth of the other across the posterior part of the tongue is about 10 cm (from X to Y in Fig. 3). This is the linear distance across the posterior part of the mouth, which is available for saliva to cross as it exits the oral cavity. The average unstimulated salivary flow rate, determined in a number of large studies, is about 0.3 mL/min (Dawes, 1987). Thus, if the salivary film were uniformly 0.1 mm in thickness over the 10-cm linear distance as it exits the oral cavity, the average velocity of flow over all surfaces of the posterior region of the tongue would be 30 mm/min. This is over four times higher than the 6.8 mm/min calculated for the velocity over the lingual surfaces of the upper posterior teeth (Table 2), or what may be calculated from Table 1 of Lecomte and Dawes (1987) for the lingual surfaces of the lower posterior teeth of two subjects.

This discrepancy may be because of the fact that the velocity of flow in the posterior region of the tongue may be greatly increased for a very short time during each swallow. Thus, the velocity of the salivary film could be about 7 mm/min for the time between swallows, but be much greater during a swallow. Another possibility is that saliva may pool in the depths of the two lingual sulci, below the level of the teeth—where the ve-

locity of flow could be higher than that over the dorsum of the tongue—and the thickness of the salivary film in the lingual sulci could be greater than 0.1 mm. Pooling in the depths of the lingual sulci is to be expected, because of the effects of gravity, but also because the ducts of both the submandibular and sublingual glands open into the floor of the mouth. These glands produce about 70% of the unstimulated saliva (Schneyer and Levin, 1955). The flow patterns of the salivary film are also likely to be disturbed during chewing and sucking.

We have suggested (Lecomte and Dawes, 1987) that the slow velocity of flow of the salivary film over the different surfaces of the teeth will retard clearance from plaque of diffusants such as acid (and thus prolong the Stephan curve), and that the slower the velocity, the more pronounced this effect will be.

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