The Conditioning Role of Saliva in Streptococcal Attachment to Hydroxyapatite Surfaces

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The solution properties of saliva and its role in conditioning both the substrate and the bacterial surface have been investigated with regard to the attachment of oral streptococci to hydroxyapatite surfaces. Saliva from eight subjects was used and the attachment of three organisms, *Streptococcus mutans* strains FA-1 (serotype b) and KPSK2 (serotype c) and *S. sanguis* T175-1, was studied. An adsorbed salivary layer on a hydroxyapatite surface substantially reduced the affinity of the organisms for the surface. Adsorbed saliva on the bacterial surfaces, however, tended to increase the organisms' affinity for saliva-coated apatite. The source of saliva was important in determining the extent of inhibition of attachment. The data indicated that the negatively charged and hydrophilic nature of salivary conditioning films was important in controlling bacterial adsorption to hydroxyapatite. The results also suggested that hydrophobic salivas could promote binding of the more hydrophobic bacteria known to be early colonizers of the teeth.

INTRODUCTION

The attachment of microbes to surfaces and their subsequent colonization is common in aqueous environments (Berkeley *et al.*, 1980). In natural environments the substrate is usually covered by a pre-adsorbed film of biological macromolecules and the oral environment is no exception. A freshly cleaned tooth surface is quickly coated with an adsorbed layer of proteins and glycoproteins derived from saliva. This is called the acquired pellicle and must influence the later adsorption of bacteria (Moreno *et al.*, 1976; Gibbons, 1980). Saliva can also modify the surface properties of bacteria (Orstavik, 1980). The aim of this work was to investigate the relative effects of conditioning by saliva of the substrate and bacterial surface on streptococcal attachment to hydroxyapatite surfaces.

Hydroxyapatite crystals and beads have been used as a substitute for the enamel surface (Slade, 1976) and are often treated with saliva to simulate the acquired pellicle (Gibbons, 1980). Commercially available beads have a relatively small surface area and provide only a limited area for binding (Appelbaum *et al.*, 1979; Gibbons *et al.*, 1983). We therefore used a hydroxyapatite powder with a large surface area. The powder was treated with saliva to provide an experimental pellicle very similar to the natural material (Douglas, 1983).

We have attempted to measure the initial interaction of the organism with the surface following previous deposition studies (Rutter & Abbott, 1978; Abbott, *et al.*, 1980, 1983) and have used radiolabelled cultures of three oral streptococci, *Streptococcus mutans* strains FA-1 (serotype b) and KPSK2 (serotype c) and *S. sanguis* T175-1. Saliva samples from eight subjects were obtained and their effects on (i) the apatite surface, (ii) the cell surface and (iii) cell-surface affinities were investigated.

METHODS

Organisms. The strains used were *S. mutans* FA-1 (serotype b) and KPSK2 (serotype c) (kindly supplied by J. R. Hunter, CAMR, Porton, UK) and *S. sanguis* T175-1 (from J. D. de Stoppelaar, University of Utrecht, The Netherlands). Stock cultures were maintained by fortnightly subculture on blood agar plates. Strain characteristics were checked using API 20E and 50E identification systems (Buisserie, 1972). These proved suitable for the identification of these strains and results were confirmed using tests outlined by Hardie & Bowden (1976).

Saliva. Fresh, whole, unstimulated saliva was collected over ice from eight individuals and clarified by centrifugation (30 min, 4 °C, 18000 g). It was pasteurized before being added to growth medium.

Radiolabelled bacteria. Bacteria were grown in 10 ml brain heart infusion broth (Difco), containing 0.001 mg thymidine, for 18 h at 37 °C; this was used to inoculate 50–100 ml of a complex medium (Abbott *et al.*, 1983) containing 10 µCi (370 kBq) [methyl-³H]thymidine ml⁻¹ (Amersham; specific activity 44 Ci mmol⁻¹; 1.628 TBq mmol⁻¹). All incubations were in an atmosphere of H₂/CO₂ provided by a Gaspak generator (BBL) in an anaerobic jar for 18 h at 37 °C.

Treatment of bacteria. Radiolabelled cultures were sonicated for 1.5 min at 180 W using a 9 cm probe (Ultrasonic Rapidis 180 Ultrasonic Disintegrator, Shipley, UK) and the cells harvested by centrifugation (15 min, 4 °C, 7000 g). This produced mainly individual cells with no loss of viability nor leakage of intracellular material (Abbott, 1981). (i) *Untreated cells.* The cells were washed and resuspended in KCl solutions (Aristar grade, prepared in double glass-distilled water) of different ionic strengths between 0.001 M and 0.1 M and the pH value adjusted to 6.5 with KOH. (ii) *Saliva-treated cells.* Washed cells were also resuspended in clarified saliva and shaken for 1 h before harvesting, washing and resuspending in 0.05 M-KCl. (iii) *Saliva-grown cells.* Other radiolabelled cells were grown in equal volumes of medium plus whole clarified, pasteurized saliva, also followed by harvesting and washing. The single cell concentration in suspension was determined in a Hawksley-Helber chamber (0.0025 mm² × 0.02 mm) and related to the radioactive counts (see below) to obtain the specific activity of the bacteria.

Hydroxyapatite. The powder used in the adsorption studies was prepared by the method of Langdon *et al.* (1980) under an atmosphere of nitrogen. The powder had a Ca/P molar ratio of 1.76 on analysis and X-ray diffraction and IR spectra indicated pure apatite with very little carbonate contamination (D. J. Langdon, personal communication). The surface area of the experimental powder (after finely grinding in a glass pestle and mortar and determination by N₂ adsorption using an Area Meter) was 3.4 (±0.17) × 10⁶ mm² g⁻¹. The powder was washed twice and suspended in the KCl solution used in a particular experiment. This allowed the powder to equilibrate in the suspending fluid and also removed the 'fines'.

Treatment of hydroxyapatite. Hydroxyapatite was coated with saliva by end-over-end rotation of 10 mg powder with 3 ml saliva supernatant for 3 h at ambient temperature. Excess saliva was removed by washing by rotation in 0.05 M-KCl, pH 6.5 for 30 min and the saliva-coated powder samples were stored in the appropriate KCl solutions overnight at 4 °C before use. In some experiments, saliva diluted 1:1 with H₂O was used and washed as described. Microscopic examination of the powders indicated little or no aggregation after treatment.

Estimation of bacterial adsorption. This was as described by Gibbons *et al.* (1976) but with an excess surface area available for adsorption. Radiolabelled cell suspensions (2 ml) were shaken by end-over-end rotation for 3 h at ambient temperature with 10 mg saliva-coated or uncoated hydroxyapatite powder in plastic scintillation vials (Koch-Light). The powder was then separated from the supernatant by settling and washed once in 3 ml suspending fluid to remove loosely attached organisms. The radioactivity associated with: 0.1 ml samples of the suspensions; 0.5 ml samples of the supernatants and washes; and the powder with adsorbed bacteria, was determined in 3.5 ml Beckman Ready-Solv HP scintillation cocktail using an LKB 1217 Rackbeta liquid scintillation counter. Controls indicated that no significant quenching of counts could be attributed to either the aqueous samples or the powders. Triplicate samples were used in each experiment which was performed at least twice.

Zeta-potential determinations. Electrophoretic mobilities of the bacterial suspensions and the powders in KCl solutions over a range of ionic strengths (0.001–0.1 M) pH 6.5, were determined using a Rank Brothers micro-electrophoresis apparatus in a thin-walled cylindrical cell with platinum electrodes at ambient temperature under dark ground illumination. Zeta potentials were derived from the mobility data using the tables of Ottowill & Shaw (1972) based on computations of Wiersema *et al.* (1966).

Aggregation of saliva-treated bacteria. Organisms were sonicated (as above) and suspended in 0.05 M-KCl, pH 6.5 at an *A*₅₄₀ of 0.5 as measured in a 1 cm cell using a Pye-Unicam SP600 series II spectrophotometer. Changes in *A*₅₄₀ over a 60 min period were recorded and results expressed as Δ*A*₅₄₀ min⁻¹. A marked decrease in absorbance compared to a control indicated gross aggregation.

Relative wettability of saliva films on hydroxyapatite. Discs of hydroxyapatite were prepared by pressing 150 mg of powder under 5 tons (5.08 tonnes) pressure. These were coated with saliva and air-dried. The base diameter of a 5 µl drop of water placed on the saliva-coated discs was measured after 10 s using a binocular microscope with an eyepiece graticule to assess the relative wettabilities of the films. The greater the diameter of the spread water droplet, the more wettable was the saliva film. Parafilm was used as a hydrophobic control.

Relative hydrophobicities of the organisms. Two methods were used. The first depended on the partition of sonicated ^3H -labelled organisms, suspended in 0.05 M-KCl, between a 3 ml water layer and a 0.1 ml n-hexadecane layer, after vigorous mixing. The number of organisms in the hexadecane phase was determined by chilling the phase mixtures after separation and removing all the solidified top phase (hexadecane freezes at 18 °C) into scintillation fluid for radioactive counting as previously described. Hydrophobicity was expressed as the percentage of organisms in the hexadecane phase.

The second method, hydrophobic interaction chromatography, was as described by Ollson & Westergen (1982) except that organisms were grown in the medium described above and were sonicated before suspending in 0.05 M-KCl, pH 6.5. Octyl-Sepharose CL4B and phenyl-Sepharose CL4B (Pharmacia) were used in the columns and control columns of Sepharose 4B were also run.

Chemicals. All reagents were from BDH and were made up in deionized, distilled water unless described otherwise.

Statistical analyses. Analyses of means were carried out using Student's *t*-test and comparison of slopes from regression lines by established analysis of variance techniques.

RESULTS

Affinity of streptococci for saliva-treated and untreated hydroxyapatite

Adsorption of all organisms to all surfaces showed a linear relationship with suspension concentration in the range 0.5×10^8 – 50×10^8 cells ml^{-1} . Regression coefficients were always >0.9 and the intercepts with the y-axis all approached zero. For each experiment, data are presented as the slope (*b*) of the plot of adsorbed cells against total cells in suspension. These slopes indicate the affinity of the organisms for the surfaces. The 95% confidence limits on *b* were also calculated.

Table 1 shows the affinity of *S. mutans* FA-1 and *S. sanguis* T175-1 for hydroxyapatite powders treated with different salivas. Group means ($\bar{b}$) were calculated assuming that the individual mean was the best estimate for each saliva sample; differences in sample numbers were ignored. Adsorption onto untreated hydroxyapatite is given for comparison. *Streptococcus sanguis* showed a greater affinity for all saliva-treated surfaces than did *S. mutans* FA-1. Individual mean slopes and group means were all significantly different at $P < 0.001$. The adsorption of both organisms to untreated hydroxyapatite was similar and significantly greater ($P < 0.001$) than to saliva-treated powders. The adsorption affinity of both organisms for saliva-treated surfaces varied widely depending on the source of the saliva.

Table 1. *Affinity of streptococci for hydroxyapatite powder*

Affinity is represented by slopes (*b*) of the regression plot of numbers of bacteria adsorbing in 3 h to 10 mg of powder versus total number of cells in suspension (in the concentration range 0.5×10^8 – 50×10^8 cells); 95% confidence limits on *b* are given. The different salivas used to treat the hydroxyapatite are identified by the initials of the eight subjects.

Saliva	<i>S. mutans</i> FA-1		<i>S. sanguis</i> T175-1	
	<i>b</i>	<i>n</i> *	<i>b</i>	<i>n</i> *
None	0.944 ± 0.014	26	0.883 ± 0.051	22
A. A.	0.036 ± 0.006	26	0.076 ± 0.011	22
B. L. S.	0.049 ± 0.020	15	0.210 ± 0.025	15
C. M. W.	0.032 ± 0.004	15	0.267 ± 0.030	18
R. P. S.	0.036 ± 0.006	15	0.175 ± 0.012	18
D. J.	0.011 ± 0.005	18	0.100 ± 0.025	18
J. R.	0.011 ± 0.002	18	0.138 ± 0.016	18
J. E. T.	0.005 ± 0.001	18	0.245 ± 0.028	15
M. K. C.	0.010 ± 0.005	18	ND	
	$\bar{b} = 0.024$	$n\ddagger = 8$	$\bar{b} = 0.173$	$n\ddagger = 7$

ND, Not determined.

* No. of samples.

† No. of subjects.

Table 2. *Influence of electrolyte concentration on the adsorption of streptococci to hydroxyapatite*

Adsorption of radiolabelled bacteria in 3 h to 10 mg untreated hydroxyapatite and saliva-treated hydroxyapatite from suspensions of *S. mutans* FA-1 (2.04×10^9 cells ml⁻¹), *S. mutans* KPSK2 (2.34×10^9 cells ml⁻¹) and *S. sanguis* T175-1 (2.25×10^9 cells ml⁻¹) was measured. The numbers of adsorbed cells are given as means ± 2 standard deviations of two experiments each performed in triplicate.

Electrolyte concn (M-KCl)	10 ⁻⁸ $\times$ No. of adsorbed cells with untreated hydroxyapatite			10 ⁻⁸ $\times$ No. of adsorbed cells with saliva-treated hydroxyapatite*		
	<i>S. mutans</i> FA-1	<i>S. mutans</i> KPSK2	<i>S. sanguis</i> T175-1	<i>S. mutans</i> FA-1	<i>S. mutans</i> KPSK2	<i>S. sanguis</i> T175-1
0.001	16.90 $\pm$ 0.30	21.38 $\pm$ 0.50	19.59 $\pm$ 0.72	0.09 $\pm$ 0.04	0.47 $\pm$ 0.04	0.61 $\pm$ 0.10
0.005	ND	19.54 $\pm$ 0.60	20.08 $\pm$ 0.68	0.62 $\pm$ 0.04	0.75 $\pm$ 0.06	1.58 $\pm$ 0.08
0.01	17.50 $\pm$ 0.35	20.67 $\pm$ 0.90	19.79 $\pm$ 0.59	0.69 $\pm$ 0.04	0.78 $\pm$ 0.09	1.62 $\pm$ 0.12
0.05	17.42 $\pm$ 0.25	20.44 $\pm$ 0.30	19.82 $\pm$ 0.41	0.79 $\pm$ 0.09	1.28 $\pm$ 0.20	1.71 $\pm$ 0.05
0.1	17.38 $\pm$ 0.40	19.92 $\pm$ 0.25	20.61 $\pm$ 0.37	0.72 $\pm$ 0.23	1.25 $\pm$ 0.09	1.69 $\pm$ 0.18

ND, Not determined.

* A.A. saliva.

Wettability

The relative wettabilities of salivary films on hydroxyapatite discs varied with the source of saliva and ranged from 2.09–3.41 mm with mean droplet diameter of 2.98 mm (SD = 0.38) for the eight subjects. Each was significantly greater ($P < 0.05$) than a hydrophobic control using parafilm (2.45 mm, SD = 0.02, $n = 10$) except for subject B.L.S. (2.09 mm, SD = 0.27, $n = 10$). There was no significant relationship between wettability and the affinity of *S. mutans* or *S. sanguis* to saliva-treated hydroxyapatite powders (Table 1).

Ionic strength

Adsorption to untreated apatite was independent of the ionic strength of the suspending fluid but the number of bacteria adsorbing to saliva-treated powders was lowest from the 0.001 M-KCl suspension (Table 2). This varied between organisms, with *S. mutans* FA-1 being most susceptible. Measurements of the zeta potentials of the bacteria and the saliva-coated powder showed a decrease in negative potential with increase in ionic strength in accordance with known effects of increasing electrolyte concentration on the compression of the electrical double layer surrounding charged particles (data not shown, but similar to Fig. 2 of Abbott *et al.*, 1983). Potentiometric titrations confirmed the negatively charged nature of saliva-treated hydroxyapatite and indicated untreated hydroxyapatite to be positively charged at pH 6.5 at all the electrolyte concentrations studied.

Influence of saliva-treatment of bacteria on their adsorption of saliva-coated hydroxyapatite

Treatment with, or growth in, saliva from eight subjects enhanced the adsorption of *S. mutans* FA-1 to hydroxyapatite powder coated with saliva from the same subject (Table 3) when compared to untreated cells (Table 1). Group mean values were significantly greater at $P < 0.05$. No consistent differences were observed between the adsorption of saliva-treated and saliva-grown organisms.

Aggregation

Treatment with saliva can aggregate organisms in suspension and may thus reduce their adsorption (Rosan *et al.*, 1982). Experiments with *S. mutans* FA-1 indicated little or no aggregation; cells treated with saliva from the eight subjects showed a ΔA_{540} min⁻¹ of less than 1.1×10^{-3} . Any aggregates that occurred during growth in saliva were readily dispersed by sonication. In contrast, *S. sanguis* T175-1 showed varying degrees of aggregation, with ΔA_{540} min⁻¹ values ranging from 1.6×10^{-3} – 10.9×10^{-3} . However, calculations showed that there was no significant correlation between aggregation and the affinity of these cells for saliva-treated hydroxyapatite (Table 1).

Table 3. Influence of treatment with, or growth in, saliva on the affinity of *S. mutans* FA-1 for hydroxyapatite surfaces

Suspensions of *S. mutans* FA-1 were either treated or grown in a subject's saliva and the affinity for hydroxyapatite coated with the same saliva measured. The affinities were calculated as in Table 1; 95% confidence limits are given. The affinity of *S. mutans* FA-1 grown in A.A. saliva for untreated hydroxyapatite for comparison was 0.720 ± 0.038 ($n^* = 18$).

Subject	Saliva-treated cells		Saliva-grown cells	
	<i>b</i>	<i>n</i> *	<i>b</i>	<i>n</i> *
A. A.	0.056 ± 0.007	28	0.080 ± 0.023	15
B. L. S.	0.135 ± 0.014	15	0.120 ± 0.030	18
C. M. W.	0.235 ± 0.027	15	0.634 ± 0.017	15
R. P. S.	0.030 ± 0.005	15	0.029 ± 0.002	15
D. J.	0.037 ± 0.012	15	0.060 ± 0.014	15
J. R.	0.122 ± 0.020	15	0.065 ± 0.007	15
J. E. T.	0.037 ± 0.012	15	0.037 ± 0.012	15
M. K. C.	0.022 ± 0.007	15	0.065 ± 0.007	15
	$\bar{b} = 0.084$ $n^\dagger = 8$		$\bar{b} = 0.136$ $n^\dagger = 8$	

* No. of samples.

† No. of subjects.

Table 4. Influence of growth in saliva on the zeta potential of *S. mutans* FA-1

Zeta potentials were derived from electrophoretic mobility data using the tables of Ottowill & Shaw (1972) based on computations by Wiersema *et al.* (1966). Electrophoretic mobility measurements were made in 0.05 M-KCl suspensions, pH 6.5 at 20 °C using a Rank Bros. microelectrophoresis apparatus. The radius of the bacteria used to determine zeta potentials was 0.4 µm. This was measured using a Vickers-AE1 image splitting eyepiece on a conventional Wild microscope. Means and ranges of zeta potentials calculated from 20 measurements are shown.

Saliva	Negative zeta potential (mV)
None	26.6 (22.4–32.9)
A. A.	20.7 (14.6–25.6)
B. L. S.	17.8 (14.5–23.0)
C. M. W.	12.6 (9.5–19.5)
R. P. S.	22.6 (19.0–28.2)
D. J.	24.0 (21.2–27.5)
J. R.	23.0 (20.0–27.4)
J. E. T.	22.6 (19.9–26.5)
M. K. C.	20.8 (17.8–25.0)

Zeta potentials

The possession of a relatively high negative zeta potential (–26.6 mV) by *S. mutans* FA-1 is important in preventing its attachment to negatively charged surfaces (Abbott *et al.*, 1980, 1983). The influence of growth in saliva from eight subjects on the zeta potential of *S. mutans* FA-1 is shown in Table 4. This seemed to lower the zeta potential but the effect was only significant with the saliva from subject C. M. W. There was no significant change in the zeta potentials of *S. mutans* KPSK2 (–17.6 mV) and *S. sanguis* T175-1 (–11.4 mV) after growth in medium containing A.A. saliva compared with untreated cells. Calculations showed a significant ($P < 0.01$) negative correlation ($r = 0.91$) between the affinities of saliva-grown bacteria for saliva-treated hydroxyapatite (Table 3) and the zeta potentials for *S. mutans* FA-1 after growth in saliva (Table 4).

Table 5. *Comparison of effect of a 'hydrophilic' and a 'hydrophobic' saliva on the affinity of streptococci for saliva-treated hydroxyapatite*

Hydroxyapatite powder was coated with the saliva, diluted 1:1 with water, from two subjects. A.A. saliva had a 'hydrophilic' character (mean drop diameter 3.14 mm) and B.L.S. saliva had a 'hydrophobic' character (2.09 mm) (see Results). The adsorption affinity of untreated and saliva-grown organisms was measured and is expressed as for Tables 1 and 3; 95% confidence limits are given, $n = 15$.

Saliva	Organism	<i>b</i> value	
		Untreated cells	Saliva-grown cells
A.A.	<i>S. mutans</i> FA-1	0.035 ± 0.006	0.100 ± 0.005
	<i>S. mutans</i> KPSK2	0.050 ± 0.010	0.094 ± 0.004
	<i>S. sanguis</i> T175-1	0.144 ± 0.015	0.132 ± 0.010
B.L.S.	<i>S. mutans</i> FA-1	0.073 ± 0.021	0.169 ± 0.022
	<i>S. mutans</i> KPSK2	0.122 ± 0.010	0.122 ± 0.015
	<i>S. sanguis</i> T175-1	0.141 ± 0.029	0.287 ± 0.035

Relative hydrophobicity

All three organisms showed a low affinity for hexadecane with only 3% of the total cell numbers of the *S. mutans* strains and 10% of the *S. sanguis* strain, partitioning into hexadecane. The more hydrophobic nature of the *S. sanguis* was confirmed by hydrophobic interaction chromatography when 70% of the cells were retained on gels of octyl- or phenyl-Sepharose compared with 40% of the *S. mutans* strains. Growth in saliva from subject A.A. had no effect on the hydrophobicities of any of the strains.

An experiment was carried out to compare differences in affinity between strains and between saliva samples from two subjects (Table 5). Saliva from subject B.L.S. was shown above to be more hydrophobic than saliva from subject A.A. (mean droplet diameter 2.09 mm, SD = 0.27, $n = 10$). Each saliva sample was diluted 1:1 with water and used to prepare saliva-hydroxyapatite and saliva-grown cells as described. Saliva from both subjects enhanced the affinity of the saliva-grown *S. mutans* FA-1 compared to untreated cells. Enhanced affinity was associated with a decrease in the zeta potential of the cells. In contrast, the salivas had no effects on the zeta potentials of the other organisms. It is interesting that only the hydrophobic saliva, B.L.S., promoted binding by the relatively hydrophobic *S. sanguis* and the hydrophilic saliva, A.A., only affected the relatively hydrophilic *S. mutans* KPSK2. In general, the hydrophobic environment provided by saliva B.L.S. favoured binding by all saliva-grown organisms.

DISCUSSION

Saliva may influence bacterial attachment to teeth by modification of either the enamel surface or the bacterial cell surface (Orstavik, 1980). In the present study treatment of hydroxyapatite surfaces with saliva reduced the binding of both untreated ($\bar{b} = 0.024$; Table 1) and saliva-grown ($\bar{b} = 0.136$; Table 3) cells of *S. mutans* FA-1 when compared to direct cell-apatite interactions ($b = 0.944$; Table 1). Similarly, growth in saliva reduced the affinity of these cells for untreated apatite surfaces ($b = 0.720$; Table 1). However, growth in saliva tended to increase the affinity of *S. mutans* FA-1 for saliva-treated hydroxyapatite (Table 3). Analogous results were obtained using *S. sanguis* T175-1 (Tables 1 and 5). The affinity of *S. mutans* FA-1 was reduced more substantially by saliva-treatment of the hydroxyapatite than was the affinity of *S. sanguis*, corroborating previous reports (McGaughey *et al.*, 1971; Rolla *et al.*, 1977; Clark *et al.*, 1978). The source of saliva was also important as noted by Orstavik *et al.* (1982) and Nesbitt *et al.* (1982).

Jedresen *et al.* (1981) suggested that modification of the hydroxyapatite surface by the acquisition of a salivary pellicle is a biological mechanism which protects and maintains the tooth surface in a relatively low state of surface free energy. Similarly, in the marine

environment, macromolecular adsorption inhibits attachment of organisms by converting the substrate from a favourable hydrophobic state into an unfavourable, hydrophilic state (Fletcher & Loeb, 1979; Pringle & Fletcher, 1983). Our experiments showed that the salivary film converted the apatite surface from a favourable, positively charged state to an unfavourable, negatively charged state, thus reducing attachment by negatively charged cells. All saliva samples produced much the same reduction in affinity for each organism and this appeared to be independent of the hydrophobic nature of the salivas as judged by the wettability of the saliva-apatite films. Enhancement of binding by compression of the electrical double layer at higher ionic strengths confirmed the predominantly electrostatic nature of the interaction, particularly for *S. mutans* FA-1, and supports the conclusions of Abbott *et al.* (1983), who noted that the rates of deposition of *S. mutans* FA-1 and KPSK2 to glass and protein surfaces also depended on electrolyte concentrations and the zeta potentials of the bacteria. There was considerable variation between the different organisms, however, as the attachment of bacteria to saliva-apatite seemed to depend on their hydrophobicity. *Streptococcus sanguis* adhered more firmly than did *S. mutans* FA-1, whereas KPSK2 was intermediate in this respect. Presumably the bound saliva imparted some degree of hydrophobicity to the apatite surface as well as conferring an overall negative charge.

Gibbons (1980) argued that a salivary pellicle imparts a specificity to bacterial attachment to tooth surfaces; specific molecules in saliva, which are known to interact with and sometimes aggregate oral organisms, would, when present in the pellicle, help to direct organisms onto the surface. Our results, however, showed no relationship between aggregation by saliva and binding to saliva-treated apatite and are consistent with those of Liljemmark *et al.* (1981) and Rosan *et al.* (1982) who regarded aggregation and adsorption to pellicle as independent traits.

Growth of the organisms in saliva reduced binding by *S. mutans* FA-1 and by *S. sanguis* to the untreated, positively charged apatite, probably by reducing the overall negative charge on the cell surface. This reduction was made more apparent when the apatite was also treated with saliva and results for *S. mutans* FA-1 showed an inverse relationship with the zeta potential of the cell (Table 4), confirming the importance of charge repulsion in preventing binding by this organism. There was considerable variation, however, not only between the effects of different salivas but also between strains; this may be because the hydrophobicity of the cell is just as important in the adsorption of these streptococci (Ollson & Westergen, 1982). The relatively hydrophobic *S. sanguis* bound more readily to saliva-treated apatite and it is possible that there is some interaction between the hydrophobicity of the saliva and of the cell which determines adhesion. Results (Table 5) showed that a hydrophobic saliva enhanced the affinity of all saliva-grown organisms, particularly *S. sanguis*.

In conclusion, it appears that treatment of apatite with saliva produces a large, relatively non-specific decrease in binding which depends on the conversion of a positive to a negative charge and is independent of the source of saliva. In the mouth, however, the enamel is nearly always covered by a salivary pellicle and cell growth is determined by saliva so that saliva-saliva interactions become the dominant feature. Hydrophilic salivas would reduce binding by *S. mutans* FA-1 and other organisms that depend on electrostatic interactions whereas hydrophobic salivas could promote binding by hydrophobic cells such as *S. sanguis*. It is relevant to note that hydrophobic streptococci such as *S. sanguis* and *S. mitior* are known to be early colonizers of the teeth whereas the hydrophilic *S. mutans* and *S. salivarius* colonize less easily, if at all.

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