In Vitro Sorption of Albumin, Immunoglobulin G, and Lysozyme to Enamel and Cementum from Human Teeth

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Sorption of three ¹²⁵I-labeled human proteins (albumin, immunoglobulin G, and lysozyme) to enamel and cementum was investigated. All three proteins sorbed most when suspended in 0.0005 M solution of phosphate or calcium chloride where the least competition between solute ions and label occurred. The addition of human serum to labeled proteins caused a decrease in their sorption which could be partially reversed by increasing the concentration of label. Kinetic experiments demonstrated that sorption was dependent on protein concentration and incubation time and that most of the sorption occurred within the first minute of the reaction. In conclusion, the binding of the three labeled proteins was affected by the charge of the solute ions and was dependent on ion concentration and reaction time. Sorption correlated for the most part with the pK values of the proteins and thus lysozyme, the most basic protein, sorbed more than immunoglobulin G, which sorbed more than albumin. In all cases, cementum bound more basic protein than did enamel. Increased levels of albumin sorption to enamel occurred when the protein was suspended in the CaCl₂ solution rather than in phosphate. In addition, based on Scatchard analysis, approximately twice as many potential protein binding sites were found for cementum versus enamel.

The attachment of bacteria to tooth surfaces is undoubtedly important in the etiology and progress of dental disease (7, 10). Colonization of supragingival tooth surfaces by gram-positive bacteria is often mediated by the selective adsorption of salivary proteins to enamel (1). Modification of this supragingival surface can strongly influence the pattern of bacterial colonization and the resulting pattern of dental disease (6). Although salivary interactions with enamel and with attached gram-positive bacteria are understood to be critical to the caries process (12, 22), little is known about the sorption of proteins to cementum. Furthermore, the effect of cemental acquisition of serum proteins on the subsequent bacterial colonization of cementum is poorly understood (2). Nevertheless, it seems reasonable to postulate that sorption of serum proteins to root surfaces may influence the nature (25), and perhaps also the quantity (9), of subgingival bacterial attachment to cementum and may thus affect the progression of periodontal disease. The study that follows constitutes part of a series of experiments designed to explore the characteristics of the interactions of subgingival serum proteins with cementum and to define the conditions that promote or interfere with these interactions.

MATERIALS AND METHODS

Preparation and handling of tooth samples. Normal teeth extracted for orthodontic reasons were frozen and stored at -10°C until needed. Roots were scaled carefully to remove periodontal ligament remnants with minimal impingement on adjacent cementum. Rectangular root segments of approximately 4 mm by 3 mm by 200 to 250 µm in thickness were prepared from the midportion of the root containing acellular cementum. These segments were affixed to one end of a 20-µl borosilicate capillary pipette (Fisher Scientific Co., King of Prussia, Pa.), the opposite end of which was mounted to the polypropylene cap of a 2-ml Technicon autoanalyzer sample cup (VWR Scientific Co., So. Plainfield, N.J.). For purposes of comparison, rectangular segments of enamel of

the same approximate size were also prepared. The lateral borders and underside of each of the prepared segments were carefully blocked with wax so that, when each tooth segment was immersed in test solution contained in the autoanalyzer cup, only the enamel or cemental surface and its wax coating were exposed to the test solution. For testing, 200 µl of an appropriate test solution was placed in an autoanalyzer tube. The vial cap, bearing its attached tooth segment, was placed in its tube, thus immersing the tooth segment in labeled protein. After the appropriate time period, the tooth segment was removed from the solution containing labeled protein, washed for 15 to 30 s in buffer, cleaned free of all wax, and assayed for tooth-bound label.

Radiolabeling of proteins. All proteins to be tested were iodinated by a modification of the method of Hunter and Greenwood (8). To a vial containing 1 mCi of high-concentration ¹²⁵I, having an activity of 350 mCi/ml (New England Nuclear, Boston, Mass.), the following was added: 1.25 mg of chromatographically purified human immunoglobulin G (IgG) in 0.05 M phosphate buffer, pH 7.5; two aliquots of 40 µg of chloramine-T (Sigma Chemical Co., St. Louis, Mo.) in 0.05 M phosphate buffer; 250 µg of Na₂S₂O₅ in 0.05 M phosphate buffer; and 1 mg of KI. The iodinated IgG was then added to a column (10 by 30 cm) packed to a height of 20 cm with Sephadex G-25 Superfine (Pharmacia Fine Chemicals, Uppsala, Sweden). One-milliliter fractions were collected from the column, and 10-µl aliquots of these fractions were assayed for gamma radioactivity. Aliquots with high levels of radioactivity were precipitated in 10% trichloroacetic acid. Iodination was considered to be successful if more than 95% of the radioactivity of the most active fractions was present in the trichloroacetic acid-precipitated protein. Similar labeling procedures were used for human serum albumin (Sigma) and immunoabsorption affinity chromatography-purified human urinary lysozyme (supplied by J. J. Pollack, State University of New York, Stony Brook).

Experimental protocol. (i) Initial observations of sorption of label to enamel and cementum. (a) Frequency distribution of

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bound label. Twenty-eight enamel and cemental segments were immersed in 200 μ l of a 100- μ g/ml amount of [125 I]IgG in phosphate-buffered saline (7.65 g of NaCl, 0.724 g of Na_2HPO_4 , 0.21 g of KH_2PO_4 per liter of distilled water), pH 7.2, for 1 h at room temperature. The fragments were washed in phosphate-buffered saline, separated from their wax supports, and assayed for gamma radioactivity. Similar experiments were employed with 10 enamel and 10 cemental fragments immersed in [125 I]albumin at a concentration of 100 μ g/ml. The calculation of bound protein per segment was derived as follows: $[(T \times 1 \mu\text{g})/L \times S] Q$ (quantity of bound label in micrograms per square millimeter), where L = counts of gamma activity in 1 μ g of label, T = counts of label remaining bound to the tooth fragment, and S = size of the tooth fragment in square millimeters. Protein sorption by enamel and cemental segments was compared by Student's t test. Data were also analyzed by correlation analyses of protein concentration to amount of bound protein.

(b) Effect of time and concentration. The sorption kinetics resulting from exposure of cemental and enamel segments to 10, 33, 130, and 650 μ g of [125 I]IgG per ml in phosphate-buffered saline pH 7.2, were determined in triplicate at each concentration and at 1-, 5-, 15-, 30-, and 60-min intervals. Segments were suspended in 200 μ l of the test solution, removed at the appropriate time and washed in phosphate-buffered saline, pH 7.2, for 15 to 30 s, cleaned free of wax, and assayed for gamma radioactivity. In similar experiments, [125 I]albumin, at a concentration of either 33 or 650 μ g/ml, was reacted with cemental and enamel segments for 1, 5, 15, 30 and 60 min. From average values at each time interval and concentration of [125 I]IgG or [125 I]albumin, kinetic curves were plotted for enamel and cemental sorption.

In other experiments enamel and cemental tooth segments were exposed to [125 I]IgG at concentrations of 130, 325, and 650 μ g/ml and 1.3 mg/ml for 15 min at room temperature. Data of the relationship between sorption (micrograms per square millimeter) and concentration of [125 I]IgG (micrograms per milliliter) were analyzed by the method of Scatchard (19) to estimate the number of potential binding sites available for cementum as compared with enamel segments and to estimate the affinity of IgG for those sites. In this analysis the ratio of the amount of label bound to the solid surface (Q) over the amount remaining free in solution (C) was plotted against the amount of label bound (Q) (Q/C versus Q).

(c) Effect of temperature and pH. Sets of five enamel and five cemental segments were suspended in 200 μ g of [125 I]IgG per ml in 0.0005 M phosphate buffer at pH 5.0, 7.2, and 9.5 for 30 min at room temperature, washed in distilled water, and assayed for activity. Experiments were replicated at 3 or 37°C in 0.0005 M phosphate buffer, pH 7.2, to determine the effect of temperature on protein sorption.

(ii) Comparative binding of three proteins to tooth segments as affected by calcium and phosphate ions. For comparative binding procedures, the labeled proteins were suspended in the following solutions: (i) 0.0005 M phosphate solution, pH 7.2; (ii) 0.5 M phosphate solution, pH 7.2; (iii) 0.0005 M calcium chloride solution, pH 7.2; and (iv) 0.5 M calcium chloride solution, pH 7.2. [125 I]-labeled proteins were added to each of the solutions (i to iv) in the following manner: albumin at a concentration of 650 μ g/ml (specific activity, 3.5 Ci/mmol); IgG at a concentration of 200 μ g/ml (specific activity, 7.8 Ci/mmol); and lysozyme at a concentration of 180 μ g/ml (specific activity, 8.8 Ci/mmol). Five cemental and five enamel segments were suspended in each of the labeled

proteins in each solution (i to iv) for 30 min at room temperature.

(iii) Effects of combining labeled and unlabeled (cold) protein on sorption. In these experiments an effort was made to determine the effect of unlabeled serum protein on sorption of the label. Labeled albumin and lysozyme were suspended in human serum diluted 10 times in 0.0005 M phosphate solution, pH 7.2. Albumin was used at a concentration of 650 μ g/ml as a result of the amount of label available. The concentrations of lysozyme were based on amounts found in the crevicular fluid of patients with juvenile periodontitis (180 μ g/ml) as compared with patients with gingivitis (30 μ g/ml) as determined by Friedman et al. (4). Three experiments were performed. For each experiment five cemental segments were analyzed for sorption. All reactions were conducted in autoanalyzer tubes for 30 min at room temperature as follows.

(a) Labeled albumin or lysozyme in 0.0005 M phosphate was added to cemental segments in the presence of diluted serum to quantitate inhibition of binding or competition for binding of label by unlabeled serum proteins.

(b) Labeled albumin or lysozyme was added to root segments first, followed by addition of diluted human serum to ascertain any reversal of label binding caused by the addition of unlabeled serum proteins.

(c) The diluted serum was added to the root segment and was followed by addition of labeled albumin or lysozyme to determine the ability of unlabeled serum proteins to block the sorption of label.

The results of (a) inhibition, (b) reversal, and (c) blocking experiments were compared with those obtained by the addition of the labeled protein alone to tooth segments.

(iv) Data analysis. Where indicated in each experiment, mean values were compared for statistically significant differences, using the unpaired Student t test at $P \leq 0.05$.

RESULTS

Initial observations of binding of labeled proteins to tooth fragments. The sorption of [125 I]IgG to enamel and cemental segments was assayed and calculated as described above. Thus, the quantity of IgG bound to enamel was

$$\frac{(1,035 \pm 379) \times 1.0 \mu\text{g}}{(16,517 \pm 1,495) \times 12.20 \text{ mm}^2} = 0.0051 \pm 0.019 \mu\text{g/mm}^2$$

The quantity of IgG bound to cementum was

$$\frac{(1,705 \pm 513) \times 1.0 \mu\text{g}}{(16,517 \pm 1,495) \times 10.94 \text{ mm}^2} = 0.0094 \pm 0.0030 \mu\text{g/mm}^2$$

Enamel sorption was clearly less than that of cementum. In spite of this significant difference between enamel and cementum in the quantity of IgG bound, histograms revealed a similar pattern of distribution of bound IgG in enamel and cemental segments. No such difference was observed when enamel sorption of albumin was compared with cemental sorption. After exposure of 100 μ g of [125 I]albumin per ml, enamel sorption was $0.00053 \pm 0.00010 \mu\text{g/mm}^2$ and cemental sorption was $0.00055 \pm 0.00022 \mu\text{g/mm}^2$.

Effect of concentration, time, and temperature. The sorption of [125 I]IgG to cementum can be linearly expressed (Fig. 1). By Scatchard analysis, binding to cementum had a slope of 0.00136 ml/ μ g and an X-intercept of 0.0163 $\mu\text{g/mm}^2$. As calculated by the Scatchard method, the number of binding sites for cementum was 1.02×10^{11} sites per mm^2 and that for enamel was 6.56×10^{10} sites per mm^2 , whereas satura-

FIG. 1. Demonstration of the linearity of the relationship between the concentration of label added (IgG) to the quantity of label bound to the cemental segment.

tion binding occurred at $0.0255 \mu\text{g}$ of IgG per mm^2 for cementum and at $0.0163 \mu\text{g}$ of IgG per mm^2 for enamel (Table 1, Fig. 2). Figure 3 clearly shows the similarity of the shapes of the kinetic curves for cementum and enamel. This similarity further confirmed the initial observation that cementum binding was almost two times greater than enamel binding.

TABLE 1. Sorption of IgG by cementum and enamel (Scatchard calculation)^a

Tooth surface	Quantity of IgG in soln (μg)	Amt bound (Q) ($\mu\text{g}/\text{mm}^2$)	Amt remaining free in soln (C) ($\mu\text{g}/\text{mm}^2$)	Q/C (ml/mm^2)
Cementum	1,300	0.0175 ± 0.0027	1,299.2	1.35×10^{-5}
Enamel		0.0111 ± 0.0015	1,299.5	8.54×10^{-6}
Cementum	650	0.0092 ± 0.0017	649.6	1.42×10^{-5}
Enamel		0.0054 ± 0.0021	649.8	8.31×10^{-6}
Cementum	325	0.0079 ± 0.0009	324.6	2.43×10^{-5}
Enamel		0.0036 ± 0.0005	324.8	1.11×10^{-5}
Cementum	130	0.0043 ± 0.0008	129.8	3.31×10^{-5}
Enamel		0.0037 ± 0.0006	129.8	2.85×10^{-5}

^a Cementum: Slope ($-K$) = $-0.00135 \text{ ml}/\mu\text{g}$; X-intercept (saturation) = $0.0255 \mu\text{g}/\text{mm}^2$ = number of binding sites per square millimeter = $[(0.0255 \times 10^{-6} \times 6.02 \times 10^{23})/150,000] = 1.02 \times 10^{11}$. Enamel: Slope ($-K$) = $-0.00136 \text{ ml}/\mu\text{g}$; X-intercept (saturation) = $0.0163 \mu\text{g}/\text{mm}^2$ = number of binding sites per square millimeter = $[(0.0163 \times 10^{-6} \times 6.02 \times 10^{23})/150,000] = 6.56 \times 10^{10}$. (K = intrinsic binding constant).

Kinetic experiments indicated that $10 \mu\text{g}$ of IgG per ml yielded a gentle slope; a plateau was reached within 15 min of incubation of both enamel and cemental segments (Fig. 3). However, at IgG concentrations of $>33 \mu\text{g}/\text{ml}$, saturation and plateau values were seen within 1 min of incubation. In the case of albumin, binding at concentrations of $33 \mu\text{g}/\text{ml}$ occurred gradually, requiring 15 min of incubation for maximum saturation of albumin binding, $0.62 \pm 0.21 \text{ ng}/\text{mm}^2$, to be reached. This differed from the rapid saturation by albumin at a concentration of $650 \mu\text{g}/\text{ml}$, where 90% of the maximum sorption ($1.8 \pm 0.3 \text{ ng}/\text{mm}^2$) took place within 1 min of incubation.

Table 2 shows that both cementum and enamel bound significantly more IgG at 37°C than at 3°C . At either 3 or 37°C , cementum bound significantly more IgG than enamel.

Comparison of binding of three proteins as affected by calcium and phosphate ions, charge, and pH. A consistent decrease in the sorption of labeled protein was seen as the molarity of the phosphate was increased from 0.0005 M to 0.5 M (Fig. 4). The decrease was most evident with respect to lysozyme sorption, whereas the least evidence of change was seen with albumin sorption. Lysozyme sorption to cementum in low phosphate (0.0005 M) was $110 \pm 26 \text{ ng}/\text{mm}^2$. However, there was a 12- to 24-fold decrease in bound lysozyme (to $4.6 \pm 0.4 \text{ ng}/\text{mm}^2$) when the high-molarity phosphate (0.5 M) was used. This pattern of lysozyme sorption held true for enamel as well as cementum, although even in the low-phosphate solution enamel sorption at $18.9 \pm 6.8 \text{ ng}/\text{mm}^2$ was consistently lower than cemental sorption ($110 \pm 26 \text{ ng}/\text{mm}^2$) in the same phosphate concentration (Fig. 4). IgG sorption demonstrated a similar pattern of sorption, and thus increasing the phosphate concentration caused a precipitous drop in cemental sorption from 56.4 ± 11.5 to 2.0

FIG. 2. Scatchard plot comparing cementum and enamel binding of $[^{125}\text{I}]\text{IgG}$. The amount bound to the solid surface (micrograms per square millimeter) is plotted against the ratio of the amount bound (Q) to the amount remaining in solution (C) (Q/C , in milliliters per square millimeter). Saturation binding is seen on the X-intercept and is $0.0255 \mu\text{g}/\text{mm}^2$ for cementum (solid line) and $0.0163 \mu\text{g}/\text{mm}^2$ for enamel (broken line).

FIG. 3. Comparison of the kinetic curves for enamel and cementum binding of [125 I]IgG. At all time periods cementum bound greater quantities than did enamel.

± 0.2 ng/mm 2 . Similar decreases were found in enamel sorption of IgG (Fig. 4). On the other hand, enamel sorption of albumin in the high-molarity (0.5 M) solution decreased only very slightly from the level sorped in the low-molarity buffer (1.3 ± 0.4 to 1.1 ± 0.2 ng/mm 2). These findings were also seen in studies of cemental binding (Fig. 4).

Increasing the concentration of the calcium chloride in the incubating solution had an effect similar to that of the high-molarity phosphate solution so that binding of all labeled proteins was decreased at the high calcium chloride concentration (Fig. 5). At low calcium chloride concentration (0.0005 M), each of the three proteins demonstrated a greater binding to cementum than to enamel (Fig. 5). The greatest sorption per square millimeter of surface in the low calcium chloride solution took place when lysozyme was added to cementum, yielding 83 ± 8.0 ng of bound lysozyme per mm 2 . Of the proteins tested, the least cemental sorption, 5.0 ± 0.6 ng/mm 2 , occurred with albumin suspended in the 0.0005 M calcium chloride solution (Fig. 5). However, suspension of albumin in the 0.5 M calcium chloride solution caused a 41% reduction in binding to cementum (5.0 ± 0.6 to 2.8 ± 0.4 ng/mm 2 ; $P \leq 0.05$), but only a slight reduction in albumin sorption to enamel (3.5 ± 0.7 to 2.0 ± 1.4 ng/mm 2) (Fig. 5). In fact, albumin sorption by both enamel and cementum was greater in calcium chloride than in the phosphate solution. This solution/sorption relationship was unique among the labeled proteins tested.

At comparable pH levels cementum bound more IgG than did enamel. Both enamel and cementum bound the greatest amount of IgG at pH 7.2 and considerably less at pH 9.5 (Table 3).

Effect of combining labeled and unlabeled serum proteins on sorption. Several experiments were designed to determine the level of sorption of labeled test proteins in a solution that resembles the gingival crevicular fluid (Tables 4 and 5). In these experiments cemental strips were studied. The presence of serum proteins significantly reduced the sorption of

TABLE 2. Sorption of IgG by enamel and cementum at 3 and 37°C^a

Temp (°C)	Sorption (ng/mm 2)	
	Enamel	Cementum
3	7.9 ± 1.0	15.5 ± 2.5
37	12.2 ± 1.8	21.2 ± 3.3

^a Each measurement is derived from sorption on five tooth fragments. At both temperatures, cementum sorped significantly more IgG than enamel ($P \leq 0.05$). Both cementum and enamel sorped significantly more IgG at 37 than at 3°C ($P \leq 0.05$).

albumin (from 0.90 to 0.68 ng/mm 2) and lysozyme (from 89.0 to 52.0 ng/mm 2) (Table 4). In addition, when lysozyme was added at a concentration of 30 µg/ml, a reduction of sorption from 89 ± 16 to 5.9 ± 0.7 ng/mm 2 occurred (Tables 4 and 5). Furthermore, when this lower concentration of lysozyme was suspended in diluted human serum, a 77% reduction in binding ensued (Table 5). Precoating tooth segments with human serum appeared to block cemental binding sites and thus decreased the binding of labeled proteins (Table 5). Similarly, the addition of unlabeled human serum to label-precoated cemental segments displaced previously bound label (Table 5).

DISCUSSION

Periodontal disease is an extremely complex infectious disease of multiple origin (5, 11, 16, 26). Cementum is an integral part of the attachment of tooth to bone and, during the life of the tooth, is subject to the adherence of mammalian or bacterial cells or their products (13, 27). Recent evidence suggests that adsorption of specific host-derived proteins can influence subsequent interactions of either mammalian cells (23) or bacteria (3) with the cemental surface. Thus, laminin sorption to cementum would favor colonization by epithelial cells, whereas fibronectin sorption would favor interaction between fibroblasts and cementum (23). Moreover, polymorphonuclear leukocytes, the dominant inflammatory cellular element in the subgingival crevicular region, may contribute to the elevated enzyme levels found in crevicular fluid adjacent to cementum (4), and these enzymes may sorp to cementum. In fact, preliminary evidence indicates that protease-like substances derived from host cells or subgingival bacteria can be eluted from the root surfaces of teeth obtained from patients with juvenile periodontitis (D. H. Fine and R. Oshrain, Abstr. Annu. Meet. Am. Assoc. Dent. Res. 1983, no. 694, p. 246). At this time, the parameters that regulate the sorption of these or other proteins to cementum is unknown. Furthermore, the results obtained in this study suggest that extrapolation of data based on sorption of substances to hydroxyapatite or enamel or both appears ill advised (see Fig. 4 and 5).

By the Scatchard method, an estimate of the intrinsic binding constant, or the inherent affinity of the specific protein for the specific interacting surface, can be determined. Comparison of the slopes for enamel and cementum by means of the Scatchard plot revealed a similar intrinsic binding constant for the two surfaces, and this suggests a similar mechanism of interaction. However, the Scatchard

TABLE 3. Effect of pH on sorption of [125 I]IgG^a to tooth surfaces^b

Tooth surface	pH	IgG bound (ng/mm 2)	
Cementum	5.0	30.1 ± 5.1	NS
	7.2	33.6 ± 11.6	
	9.5	21.9 ± 2.7	
Enamel	5.0	11.7 ± 2.5	NS
	7.2	12.8 ± 1.9	
	9.5	8.4 ± 1.6	

^a [125 I]IgG = 200 µg/ml in 0.0005 M phosphate buffer.

^b Each measurement is derived from sorption on five samples. At any pH cementum sorped significantly more IgG than did enamel. All comparisons between measurements at different pH values show significant differences except those marked NS (not significant; Student's *t* test, $P < 0.05$).

FIG. 4. Comparison of enamel and cemental binding of three labeled proteins (a, b, c) suspended in phosphate solution. Differences were seen when the more basic proteins lysozyme (c) and IgG (b) were compared with albumin (a) in low-ionic-strength phosphate solution. Also, cementum bound more basic protein than did enamel: (b) 5 versus 6; (c) 9 versus 10. Furthermore, greater binding of basic proteins took place in low-molarity phosphate than in high-molarity phosphate solution (5 versus 7, 6 versus 8; 9 versus 11, 10 versus 12). All differences were significant at the 0.05 level. Comparisons between enamel and cementum with respect to albumin (a) binding were not significant (1 versus 2, 3 versus 4).

analysis also shows that approximately twice as many of these potential protein binding sites were found for cementum versus enamel (Fig. 2; Table 1).

The differences found in this study between the sorptive

capacities of cementum and enamel could be due to differences in the inorganic content of these surfaces, differences in their organic matrix proteins, or differences in their surface areas. Two distinctions between enamel and cemen-

FIG. 5. Comparison of enamel and cemental binding of three labeled proteins (a, b, c) suspended in calcium chloride solution. In a lower concentration of calcium chloride, cementum binding was significantly greater than enamel binding in the case of each protein (a, b, c) ($P \leq 0.05$). As in Fig. 4, lysozyme binding was significantly greater than IgG or albumin with respect to cementum ($10 > 6 > 2$). However, in a higher concentration of calcium chloride, albumin binding to enamel surpassed IgG and lysozyme ($[3] = 3.5 \pm 0.7 \text{ ng/mm}^2$; $[7] = 1.7 \pm 0.3 \text{ ng/mm}^2$; $[11] = 1.1 \pm 0.16 \text{ ng/mm}^2$; $P \leq 0.05$).

TABLE 4. Inhibition of sorption of labeled proteins to cementum by human serum^a

Protein(s) added	Labeled protein ^b or ^c bound to cementum (ng/mm ²)	
Albumin ^b	0.90 ± 0.20	} $P \leq 0.05$
Albumin + human serum ^c	0.68 ± 0.12	
Lysozyme ^d	89.0 ± 16.0	} $P \leq 0.05$
Lysozyme + human serum	52.0 ± 13.0	

^a Each mean and its standard deviation is derived from measurement of five cemental strips. As indicated, mean values were tested for statistically significant differences, using Student's *t* test.

^b Albumin = 650 µg/ml.

^c Human serum = 7.2 mg/ml; diluted 1:10 in 0.0005 M phosphate.

^d Lysozyme = 180 µg/ml.

tum that could influence their respective sorption of protein are (i) the difference in the relative proportion of organic to inorganic components and (ii) the differences in the size of the inorganic crystals. Enamel consists almost entirely of mineral (18), whereas cementum contains a more equal distribution of inorganic and organic substances (15). In addition, the mineral crystals in enamel are larger than those of cementum and thus the area of crystal surfaces available for sorption in enamel is less than in cementum (18, 21). Furthermore, recent evidence derived from Laser Raman Spectra analysis indicates that there are also qualitative differences in the inorganic components when cementum and enamel are compared, as demonstrated, by a more highly ordered cemental crystal structure on an atomic scale and by an extra cemental peak at the 1,115-cm⁻¹ position (V. Ryan, V. Lopes, and D. H. Fine, personal communication). The effect of these differences on net charge are currently under investigation, and distinctions in zeta potential have been found when enamel and cementum are compared. Whereas the surface charge of each species was negative in these studies, cementum consistently demonstrated a greater net negative charge (V. Ryan and R. Schiller, personal communication).

Calcium chloride and phosphate solutions were chosen for suspension of the labeled proteins because these are the two fundamental ions in the structure of cementum and enamel. Despite similarities in cementum and enamel calcium and phosphate concentrations, the ratio of calcium to phosphate is higher in enamel (15, 24). Thus, relative to enamel, cementum has more negatively charged phosphate groups available for interaction with basic proteins such as lysozyme. In our experiments these root surface protein interactions were most obvious when labeled proteins were added to solutions of low salt concentrations where charge interactions between the labeled protein and solute ions were

minimal. To illustrate this point, most lysozyme bound to roots in either 0.0005 M phosphate or calcium chloride solutions. The dramatic reduction in lysozyme binding to teeth segments in solutions of increased concentrations of calcium chloride or phosphate may be due to interaction between the solute ions and the labeled protein, thus inhibiting interaction with the tooth surface. In fact, this situation was true for all proteins studied. In one set of studies, binding of lysozyme decreased in the presence of CaCl₂, whereas albumin sorption increased 2.7 to 4.5 times (Fig. 5). Based on the results, sorption of albumin may be assisted by the presence of positively charged calcium ions. On the other hand, the decrease in lysozyme binding in the presence of CaCl₂ could be due in part to calcium ion competition for surface sites with lysozyme since both are positively charged at pH 7.2.

With regard to comparison of the labeled proteins, albumin, IgG, and lysozyme were chosen as reference proteins because of their high concentrations in gingival fluid and because of the wide variation in their isoelectric points relative to the neutral pH of gingival fluid in its natural state (20). At 0.0005 M phosphate solution, pH 7.2, cementum bound 100 times more lysozyme, the basic protein, than albumin, the acidic protein, whereas enamel bound 17 times more lysozyme than did albumin (Fig. 4). In comparison, binding of IgG, a protein whose charge is intermediate to that of albumin and lysozyme, was neither as great as the binding of lysozyme nor as poor as the binding of albumin. Taken together, these findings demonstrate that, in a neutral buffer, basic substances such as lysozyme and IgG bind more readily to tooth surfaces than do acidic substances and, in addition, cementum binds significantly more basic protein than does enamel. Further support for this conclusion comes from experiments in which the pH of the buffer was higher than the isoelectric point of the label. The low level of binding of IgG at pH 9.5 was not unexpected (Table 3). IgG, with an isoelectric point of approximately 8.0, has a net negative charge at pH 9.5, as does the tooth surface, and as anticipated these two negatively charged species are less likely to interact than when IgG is suspended in a pH 7.2 buffer (Table 3).

Several experiments underscore the complexity of the interactions observed. In one group of studies, the effect of unlabeled serum proteins on the sorption of labeled proteins to cementum was assessed (Tables 4 and 5). Although there was only a minor decrease in albumin sorption by cementum when labeled albumin was present in diluted human serum, binding of lysozyme at a low concentration (30 µg/ml) was greatly reduced when added to tooth fragments in the presence of diluted human serum. This reduction in binding was modest when lysozyme was used at a higher concentra-

TABLE 5. Interference with sorption of labeled proteins to cementum by human serum (HS)^a

Sequence of addition of proteins to cementum	Cementum-bound albumin (ng/mm ²) ^b	Sequence of addition of proteins to cementum	Cementum-bound lysozyme (ng/mm ²) ^c	
Albumin	1.2 ± 0.2*	Lysozyme	5.9 ± 0.7	} NS
HS, ^d then albumin	0.52 ± 0.13	HS, then lysozyme	4.5 ± 0.9	
Albumin, then HS	0.51 ± 0.07	Lysozyme, then HS	0.77 ± 0.2	
Albumin + HS	0.68 ± 0.12	Lysozyme + HS	1.2 ± 0.6	

^a Each measurement is derived from sorption studies of five samples. In the measurement of cementum-bound albumin, the mean marked * is significantly different from other means in that group. The other three means do not differ from each other. In the measurement of cementum-bound lysozyme, each mean value is significantly different from all other means except as indicated by NS (not significant).

^b Albumin = 650 µg/ml.

^c Lysozyme = 30 µg/ml.

^d HS = 7.2 mg/ml; diluted 1:10 in 0.0005 M phosphate buffer, pH 7.2.

tion (180 µg/ml). Thus, the effect seen in these aforementioned experiments with human serum may reflect the relationship of concentration to sorption. Similar concentration-dependent sorption was found in in vitro studies of cemental sorption when [¹²⁵I]IgG was added to human serum deficient in IgG (D. H. Fine et al., Abstr. Annu. Meet. Int. Assoc. Dent. Res. 1982, no. 1262, p. 318).

At this point, the relationship of these findings to the in vivo situation is not clear. However, sorption of serum proteins such as albumin (14) or IgG (17) or enzymes such as lysozyme (9) is likely to affect subgingival bacterial colonization of cementum, just as selective adsorption of salivary proteins influences supragingival enamel colonization (1, 12). Recent evidence suggests that certain bacteria (cytobacteria) have a preferential affinity for cementum as compared with enamel (3), implying that surface chemistry or configuration is an important determinant in the adherence of substances to specific tooth areas. The results of our experiments are consistent with the observations of Celesk et al. (3) of tooth surface selectivity for specific bacteria and further point out differences between enamel and cemental sorption of IgG, albumin, and lysozyme. Furthermore, proteins such as laminin and fibronectin are prominent in mammalian cell attachment to cementum (23). It is likely that the criteria advanced in this paper apply to these proteins as well.

Perhaps an observation of significant clinical relevance is that at a high concentration (180 µg/ml) high levels of lysozyme sorped to cementum. This elevated lysozyme binding could have profound implications, especially in localized juvenile periodontitis, in which an elevated concentration of lysozyme is known to exist (4). The effect of lysozyme on the viability of suspected localized juvenile periodontitis pathogens has been reported recently and may be of clinical significance (9). The effect of cemental or enamel lysozyme sorption on adherence or clearance of bacteria or their products to those surfaces is not known, although preliminary evidence suggests that interference with the binding of bacteria to glass surfaces is modified by the lysate obtained from polymorphonuclear leukocytes (25). In fact, electron microscopy reports indicate that patients with juvenile periodontitis have a minimal amount of subgingival root-adherent bacteria as compared with chronic patients (13).

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