Fluoridated dentifrices reduce dental caries in subjects who perform effective oral hygiene. *Actinomyces naeslundii* increases in teeth-adherent microbial biofilms (plaques) in these subjects, and a well-characterized serum immunoglobulin G (IgG) antibody response (*Actinomyces* antibody [A-Ab]) is also increased. Other studies suggest that a serum IgG antibody response to streptococcal D-alanyl poly(glycerophosphate) (S-Ab) may indicate caries experience associated strongly with gingival health and exposure to fluoridated water. The aim of this study was to investigate relationships between A-Ab response, oral hygiene, S-Ab response, and caries experience. Measurements were made of A-Ab and S-Ab concentrations, caries experience (number of decayed, missing, and filled teeth [DMFT], number of teeth surfaces [DMFS], and number of decayed teeth needing treated [DT]), exposure to fluoridated water (Flu), mean clinical pocket depth (PD; in millimeters), and extent of plaque (PL) and gingival bleeding on probing (BOP). A-Ab concentration, the dependent variable in a multiple regression analysis, increased with S-Ab concentration and decreased with PL and DMFT adjusted for Flu ($R^2 = 0.51$, $P < 0.002$). Residual associations with age, DMFS, DT, and BOP were not significant. In addition, an elevated A-Ab response, defined from immunoprecipitation and immunoassay measurements, indicated a significant, 30% reduction in DMFT after adjustment for significant age and Flu covariance (analysis of variance with covariance $F$ statistic = $10.6$, $P < 0.003$; S-Ab response and interactions not significant). Thus, an elevated A-Ab response indicates less caries in subjects performing effective oral hygiene using fluoridated dentifrices. Conversely, a low A-Ab response is suggestive of decreased *A. naeslundii* binding to saliva-coated apatite and greater caries experience, as reported by others.

Microbial biofilms metabolize dietary carbohydrate to acids that cause dental caries (34). The pH in these biofilms (plaques) drops from about 7 to about 4 within 3 min of ingesting sucrose and other carbohydrates but takes 30 to 60 min to return to the starting pH (18). Critical bacteria, *Streptococcus mutans* and certain non-*S. mutans* streptococci, are acid tolerant (39); they survive and grow in acidic environments. Fluoride slows enamel apatite dissolution as the pH falls and increases remineralization as it returns to neutral (12). Fluoride also inhibits the extent of the pH drop by inhibiting enolase during glycolysis (19). Yet neither increased colonization of biofilms by *S. mutans* nor decreased systemic and local intake of fluoride explain why 60% of cavities occurs in only 20% of western European and U.S. populations (37). Some factors may not yet be recognized as contributing to caries experience.

Dental caries is less severe in populations from water-fluoridated regions (32). The longer a subject has lived in a water-fluoridated area during childhood and early adolescence, the less severe the caries (43, 44). By contrast, fluoridated dentifrices reduce caries only in subjects that maintain effective oral hygiene (2, 11, 38). A significant relationship between caries experience and oral hygiene has been apparent in the United States and western Europe since 1980 (3, 6), a generation following the introduction of fluoridated dentifrices in 1965 (40). Adults who are older and grew up using nonfluoridated dentifrices showed no association of caries experience with oral hygiene.

*Actinomyces naeslundii* is prominent in subjects practicing exceptionally effective oral hygiene (16, 45). Each time a tooth is thoroughly cleaned, its enamel acquires a tightly attached pellicle composed of salivary proteins that attach *A. naeslundii* (15, 31) and *Streptococcus gordonii* (20). *S. gordonii* adheres to *A. naeslundii* and also to other viridans streptococci by inter- and intrageneric coaggregations, respectively (9). The mixed biofilms grow better than individual bacteria on saliva in vitro (35) and coaggregate additional bacteria, including *S. mutans* (20, 21). Cell membrane and wall teichoic acids of most *S. viridans* and *S. mutans* streptococci possess poly(glycerophosphate) that is esterified to *D*-alanine (33). Mutations that inhibit esterification of *D*-alanine to the poly(glycerophosphate) prevent intrageneric coaggregation by *S. gordonii* (10) and destroy acid tolerance in *S. mutans* (7).

*D*-alanyl esters of poly(glycerophosphate) rapidly hydrolyze above pH 7 but are especially stable around pH 6 (8, 22). The pH of subgingival fluid is about 7 at healthy sites and above 7 at inflamed sites (5). The stability and immunogenicity of *D*-alanyl esters may therefore depend on the pH of supra- and subgingival biofilms and how inflamed the sulcus is. Human serum immunoglobulin G (IgG) antibodies precipitate with *D*-alanine esterified to poly(glycerophosphate) from biofilms (27). An elevated IgG antibody response to this epitope from
S. mutans (S-Ab response) indicates caries experience that associates strongly with gingival health and exposure to fluoridated water (24).

Human serum from many different individuals also precipitates with a group of antigens from *A. naeslundii* spent culture fluid and cells after growth to late log and stationary phase (26). The serum precipitins are detected by their reacting with a recurrent, ornithine-rich epitope on the precipitating antigens (28). A preliminary study indicated that the IgG response to this epitope (*Actinomyces* antibody [A-Ab]) is increased in adults in whom the extent of colonization is increased (30). Another preliminary study indicated that subjects who were exposed to water fluoridation from birth through age 14 and whose serum precipitated actinomyces antigen had significantly less caries than those whose serum did not precipitate the antigen (29). The aim of this study was to investigate relationships between A-Ab response, oral hygiene, S-Ab response, and caries experience. A-Ab response was quantified by enzyme immunoassay. The subjects varied more in caries experience, oral hygiene, and childhood water fluoridation exposure than in the serum precipitin study and were the same individuals as in the elevated S-Ab response study.

**MATERIALS AND METHODS**

**Subject sources.** Antibody was obtained from blood samples from 105 dental students (ages 23 to 38), 147 dental patients in good general health (ages 15 to 71), and 145 voluntary blood donors attending tissue-typing centers (Johns Hopkins University, Baltimore, MD, and University of Minnesota Medical School, Minneapolis, MN; ages 16 to 72). The dental students and patients were attending the University of Oklahoma Health Sciences Center between 1985 and 1988. All these subjects, and at least one of their parents in the case of seven minors (ages 15 to 18 years), consented to provide blood for antibody analysis according to local institutional review board procedures. The findings permitted the incidence of A-Ab in Oklahoma dental students and patients and in out-of-state volunteers to be determined. They also permitted an elevated antibody response to be defined and age and sex associations to be examined as in a study of S-Ab response described previously (24). Nineteen dental students provided a second sample of blood for determining the variation in A-Ab content after 6 months.

The clinical study participants were a subset of the dental students and patients: 67 dental students (86% male) and 41 patients (44% male). Subjects who were more than 40 years of age at examination in 1985 and 1986 were excluded. They were not exposed to fluoridated dentifrices before age 25 and exhibited no significant relationship of oral hygiene to caries experience, unlike the younger subjects. Other excluded subjects were medically unhealthy or had not used the U.S. public water supply for 18 months or longer as a child, had fewer than 18 teeth, had 4 or more crowned teeth, or were wearing orthodontic bands or dental prostheses.

**Clinical measurements.** Examinations were performed during the early to mid-afternoon by experienced clinicians who were calibrated as reported previously (24). Caries experience was measured as the number of decayed, missing, and filled teeth (DMFT), teeth surfaces (DMFS), and decayed teeth (DT). Third molars were excluded from the count if all posterior teeth were present, as also were missing teeth not lost from caries. Sensitive staining for biofilm (plaque) accumulation (36) was used with measures of gingivitis and pocket depth at four sites (mesio-buccal, center-buccal, disto-lingual, and center-lingual) around six indicator teeth: right first molar, right second incisor, and left first premolar in the upper arch, and left first molar, right second incisor, and right first premolar in the lower arch. Adjacent teeth were substituted as necessary (41).

Plaque was detected after a subject had rinsed with water, chewed a disclosing tablet for 30 s, and rinsed again. Each site around each indicator tooth that had plaque present exhibited a red stain that was recorded as present (1) or absent (0). The sites were first probed for clinical pocket depth and bleeding on probing, which was recorded as present (1) or absent (0) within 30 s. For each subject, the number of sites that stained for plaque (PL) and the number that bled on probing (BOP), a measure of gingivitis, were calculated along with mean pocket depth (PD). In a few subjects, measurements were obtained from 5 teeth (20 sites) and adjusted to a fraction of 24 sites. Plaque measurements were excluded from nine dental students who had inadvertently brushed their teeth shortly before the examination.

The dental students exhibited fewer teeth that were decayed, filled, or missing due to caries (mean DMFT, 7.90; standard deviation, 5.58) or sites that bled on probing (mean BOP, 2.87; standard deviation, 2.43) than the patients (mean DMFT, 12.46; standard deviation, 5.71; mean BOP, 7.99; standard deviation, 5.15). Using a mixture of dental students and patients in the clinical study (clinical subset in Table 1) provided more stable estimates (narrower confidence intervals) of regression coefficients (B) than would be obtained from a similar number of subjects from a random sample of the general population. Regression lines are more robust when a greater range of measurements is used (13).

**Fluoride exposure.** Fluoride exposure was estimated from a subject’s date of birth over each of five periods (birth to 2, 3 to 5, 6 to 8, 9 to 11, and 12 to 14 years). A subject’s address at these times was scored as listed (1) or not listed (0) in the 1980 Fluoridation Census (24). Flu was the sum of these scores and had a value of 0 (address not listed for any period) through 5 (listed for all periods).

**Antigen purification.** *Actinomyces* antigen was obtained from the spent culture fluid of stationary-phase *A. naeslundii* ATCC 19246 cells (26). This antigen forms a fused double precipitin arc on immunoelectrophoresis against many human sera (28). Purification is described in detail elsewhere (28, 30). Briefly, isopropanol (75%, vol/vol) precipitated the antigen from concentrated, spent culture fluid. After redissolution in 0.06 M Tris-HCl, pH 8, the antigen bound to a DEAE Sephadex column and eluted as the NaCl concentration increased to 0.2 M. The antigen-containing fractions were all combined, dialyzed into 5 mM Na formate (pH 3.0), and applied to a phosphocellulose column (Celite C, Bio-Rad Inc.). A slow migrating are absent in the void volume, but a faster one eluted only after increasing the Na formate concentration in a gradient to 5.0 M (antigen A2). The actinomyces antigens all contained sugar and amino acids but no detectable protein (28). Of the antigens purified from culture filtrate or from intact cells, antigen A2 was the smallest (molecular mass, 10 kDa) and fastest migrating. All the antigens possess an epitope (maximal molecular mass, 2 kDa) required for serum antibody precipitation (28).

The streptococcal antigen was obtained from the spent 3-day culture filtrate of stationary-phase *S. mutans* GS5 and identified by its precipitating with many human sera after immunoelectrophoresis (26). It eluted from Sephacryl S200 as a single peak, approximately 30 kDa in molecular mass, and bound to AGMP1 resin at pH 5 or 6 (30). It eluted with 0.4 M NaCl and was identified as poly(glycerophosphate) containing fatty acids and o-alanine (22, 24, 26, 27). Precipitation is due to large amounts of IgG reacting with esterified o-alanine (24, 25).

**Enzyme immunoassay.** A-Ab was assayed using 96-well F.A.S.T. plates (Becton Dickinson, Lincoln Park, NJ) as previously described for S-Ab (23, 24). Pegs on a F.A.S.T. plate lid were coated by immersion for 2 h in troughs containing purified A-Ab antigen (14 ml of 10 µg/ml in 50 mM Na carbonate buffer, pH 10), but a set of 8 pegs was left uncoated as a control. All the pegs were then blocked by immersion for 10 min in 2% (vol/vol) Tween 20 (Sigma Chemical Co., St. Louis, MO) to prevent nonspecific IgG antibody binding. A set of 8 wells containing standard serum diluted 1:100, six additional serial fivefold dilutions, and serum dilution buffer only (phosphate-buffered saline [PBS] containing 1%
[wt/vol] albumin and 0.05% [vol/vol] Tween 20), was reacted with coated pegs and a second set with uncoated pegs as a control. The remaining pegs were immersed in wells containing 0.1 ml of four serial fivefold dilutions of a subject’s serum, starting at 1:100. Each serum was assayed in duplicate on at least two plates (quadruplicate assay of each serum).

After 16 h of incubation at room temperature, the pegs were washed with serum-diluting buffer without albumin, incubated for 2 h with anti-human IgG F(ab’)2 fragment covalently conjugated to alkaline phosphatase, and developed with paranitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO). The amount of nitrophenol produced was measured by its absorbance at 410 nm using a MR600 Double Beam Spectrophotometer (DYNEX Technologies, Inc., Chantilly, VA). Blanks on each plate were taken as the average absorbance from antigen-coated pegs exposed to diluting buffer only and uncoated pegs exposed to standard serum dilutions.

Standardizing antibody concentration measurements. One milliliter of the standard serum precipitated 0.65 mg of IgG antibody when mixed with actinomyces antigen (29). A standard curve was therefore obtained from graphing absorbance against concentration of IgG antibody (A-Ab) in the standard serum dilutions on each plate (Fig. 1). The greatest range of absorbance was observed when the sera of 397 subjects were each assayed at a dilution of 1:500. A-Ab concentrations were obtained from the respective absorbances and transformed to micrograms of A-Ab/milliliter of undiluted serum.

Defining high and low actinomyces antibody responses. Figure 2, a graph of ranked A-Ab concentrations, resembles the ranked S-Ab concentrations reported previously (24). After clustering by the unweighted pair group method with arithmetic averages, the lowest S-Ab cluster had a supremum (7.26 μg/ml) virtually identical to the mean plus standard deviation of the sera not precipitating homologous antigen (24). In this study, the mean plus standard deviation of A-Ab concentration in sera not precipitating homologous antigen was 15.0 μg IgG/ml. A high A-Ab response was therefore greater than this amount of actinomyces antigen-specific IgG in a serum.

Statistical analyses. The relationships of A-Ab to S-Ab concentration and clinical variables were examined using Statistical Analysis Systems (SAS) computer programs (General Linear Models Program). DMFT or DMFS (dependent variable) was regressed on A-Ab, age, Flu, S-Ab, PL, BOP, and PD using single- and multiple-regression analysis methods. However, stronger associations were obtained when A-Ab (in log nanograms/milliliter) was the dependent variable and regressed on caries severity along with the other independent variables in a multiple regression. In addition, the effects of elevated (high) and low A-Ab and S-Ab responses (class variables) were examined in a two-way analysis of variance of DMFT (dependent variable) adjusted for age and Flu covariates (ANCOVA).

RESULTS

A-Ab response associations. The mean A-Ab concentration (in log nanograms/milliliter of serum) was greatest in dental student serum, less in volunteer serum, and least in patient serum (Table 1). These different concentrations were not associated with sex or age differences between the groups. The mean A-Ab concentration in serum from the clinical study participants resembled that of volunteer serum, which likely reflects the U.S. population. A-Ab concentrations correlated weakly but significantly with S-Ab concentration (24). This finding suggests a tendency for A-Ab concentration to be in-

FIG. 1. Aggregate of standard curves from each plate. Actinomyces antibody concentration (nanograms/milliliter) was calculated from the dilution of standard serum on each plate and graphed against the absorbance obtained (see Materials and Methods). Vertical lines indicate the standard deviation of measurements across all plates.

FIG. 2. Ranked A-Ab concentrations from the 397 subjects. The low response supremum was the mean plus standard deviation of individuals whose serum did not precipitate antigen (see the text). Dotted line parallel to the x axis indicates the supremum (15 μg of A-Ab reactive IgG). The vertical arrow indicates that the supremum occurs just before the ranked A-Ab concentrations (○) curve away from the x axis. A similar figure and related statistics were described in detail for the S-Ab response (24).
Table 2: Multiple regression of A-Ab: regression equation \( \alpha \) and \( \beta \) estimates, standard error, and significance (\( P \))

| Variable | \( \alpha \) or \( \beta \) estimate | SE | "t" | \( P \) |
|----------|-------------------------------------|----|-----|------|
| INTERCEPT\( ^a \) | 5.057\( ^a \) | 0.3566 | 14.18 | 0.0001 |
| DMFT | -0.038 | 0.0108 | -3.56 | 0.0006 |
| PL | -0.081 | 0.0132 | -6.18 | 0.0001 |
| Flu | -0.076 | 0.0256 | -2.95 | 0.0040 |
| S-Ab | 0.289 | 0.0677 | 4.27 | 0.0001 |

\( ^a \) Regression equation is A-Ab = 5.057 - 0.038 DMFT - 0.081 PL - 0.076 Flu + 0.289 S-Ab (units described in Materials and Methods).

A-Ab concentration associated most strongly and significantly (\( P < 0.005 \)) with plaque accumulation (PL, \( r^2 = 0.30 \)), less strongly with number of decayed, missing and filled teeth (DMFT, \( r^2 = 0.18 \)), teeth surfaces (DMFS, \( r^2 = 0.15 \)), decayed teeth requiring filled (DT, \( r^2 = 0.12 \)), or the incidence of bleeding of probing (BOP), a measure of gingivitis (\( r^2 = 0.12 \)).

Age did not associate with A-Ab concentration within 386 of the subjects who had their age recorded (range, 15 to 72 years). A-Ab concentrations from a second blood sample after 6 months correlated with those from the original sample (correlation coefficient, \( r = 0.92 \)). A-Ab stability over time was reported for other subjects before and after oral hygiene therapy to treat gingivitis (30).

Multivariate regression analysis. The number of decayed, missing, and filled teeth (DMFT) or teeth surfaces (DMFS) were poor dependent variables because of multiple covariance, whereas A-Ab concentration associated significantly with plaque accumulation (PL), decayed, missing, and filled teeth (DMFT), length of fluoride exposure (Flu), and S-Ab concentration (multiple \( R^2 = 0.51 \), \( P < 0.0001 \)). There were no residual associations with decayed, missing, and filled teeth surfaces (DMFS), decayed teeth (DT), bleeding on probing (BOP), pocket depth (PD), or age. These observations were confirmed using stepwise regression. The multiple-regression equation (Table 2) indicates that A-Ab concentration increased as S-Ab concentration increased and decreased as PL, DMFT, and exposure to water fluoridation (Flu) increased. A lower PL (less plaque) may reduce caries by exposing more teeth surfaces to the fluoride in dentifrices. Replacing DMFT with DMFS gave essentially the same results.

Figure 3 compares the observed A-Ab concentration with that calculated from the multiple-regression equation. It shows that the above relationships did not differ by whether the subject was a patient or dental student and that outliers comprised a similar small fraction of each group. The strength of the regression coefficients for PL and S-Ab on A-Ab (\( r^2 = 0.41 \)) was approximately the sum of the respective univariate regressions (0.10 plus 0.30). Adding DMFT and Flu as covariates after PL and S-Ab increased the strength of the A-Ab association by 10% (partial \( r^2 = 0.10 \)), of which Flu contributed 5% (partial \( r^2 = 0.05 \) when added last to adjust for DMFT. Flu did not otherwise associate significantly with A-Ab.

Relationship of high A-Ab response to caries. The above results suggest that a high A-Ab response associated with less DMFT adjusted for individual differences in S-Ab response, water fluoridation (Flu), and age. In ANCOVA, high A-Ab response associated with a 30.5% lower mean DMFT than low A-Ab response (F statistic = 10.63, \( P < 0.003 \)). By contrast, high or low S-Ab response did not associate or interact with mean DMFT. Flu associated with decreased DMFT (covariate F statistic = 7.54, \( P < 0.01 \)) and age with increased DMFT (covariate F statistic = 5.39, \( P < 0.03 \)). These relationships were similar in all combinations of A-Ab/S-Ab response (identified in Table 3); tests for inequality were not significant (\( P > 0.62 \) for age and \( P > 0.30 \) for Flu). Table 3 shows the respective
subjects was 9.69 (standard deviation, 4.56).

Previous studies have shown that individuals with 40% or more of gingival sites containing at least 10⁵ *A. naeslundii* cells had a greater A-Ab concentration than those with fewer such colonized sites (30). Moreover, individuals who maintain effective oral hygiene increase gingival *A. naeslundii* colonization (16, 45). In this study, the subjects displayed a strong increase in A-Ab response as plaque accumulation decreased independently of age. These findings suggest that young adults who have established and maintained efficient plaque removal are enriched in A-Ab response as plaque accumulation decreased independently of age. Despite nonidentity with S-Ab response (26), high A-Ab responders were nearly three times more likely to exhibit a high S-Ab response (Table 3); 55% (22 out of 40) compared with 21% (14 out of 68). An increased A-Ab response may reflect increased actinomyces colonization associated with effective oral hygiene and a concomitantly increased expression of β-alanyl poly(glycerophosphate) from coaggregating *S. viridans* streptococci. Thus, the conditions favoring a high A-Ab response apparently also favor a high S-Ab response. Previously, a significant interaction of caries experience with age and water fluoridation was detected in high but not low S-Ab responders (24). In this study, adjusting the high and low S-Ab response to account also for high and low A-Ab response resulted in DMFT decreasing with exposure to fluoridated water and increasing with age irrespective of S-Ab response (Table 3).

Enhanced attachment of *S. mutans* and the associated increase in caries experience appears accompanied by less actinomyces in biofilms (42). *A. naeslundii* binding to saliva-coated apatite appears mostly controlled by acidic proline-rich proteins (31); a group of salivary proteins that initially bind to freshly cleaned teeth (4). Two genes encode the acidic proline-rich proteins, PRH1 and PRH2 (1). PRH2 has two alleles and PRH1 has three. All five alleles are very similar in sequence, except for one of the three alleles encoded by PRH1, acidic proline rich protein (Db). In Db, a 63-bp sequence in exon 3 is repeated (1), making the protein about 12% longer. *A. naeslundii* attachment to apatite coated with Db-positive saliva is inhibited, whereas that of *S. mutans* is enhanced and the subjects have a significantly greater caries experience (14, 42).

The gene frequency of Db is approximately 16% in the U.S. population (17) but 39% in adolescents with severe caries in an adolescent Swedish population (42). Decreased *A. naeslundii* binding to saliva-coated apatite associated with Db expression may explain why some of the low A-Ab responders had increased caries despite apparently effective oral hygiene. Salivary Db expression needs to be investigated more thoroughly for its possible role in determining A-Ab response and caries experience.

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**REFERENCES**

1. Azen, E. A., K. S. Kim, P. Goodman, S. Flynn, and N. Maeda. 1987. Alleles of the PRH1 locus coding for the human salivary-acidic proline-rich proteins. Am. J. Hum. Genet. 41:1035–1047.

2. Bagramian, R. 1982. A 3-year school-based comprehensive preventive program in Michigan, USA. Comm. Dent. Oral Epidemiol. 10:234–237.

3. Bellini, H. T., P. Arnesberg, and F. R. von der Feur. 1981. Oral hygiene and caries. a review. Acta Odontol. Scand. 39:257–265.

4. Bennick, A., G. Chau, R. Goodlin, S. Abrams, D. Tustian, and G. Madapal-
Elevated antibody to D-alanyl lipoteichoic acid indicates caries experience: antibody responses. J. Immunol. Methods 1989. Fast ELISA for measuring serum antibody responses. J. Immunol. Methods 1994. Changing trends in dental caries. Int. J. Epidemiol. 1998. Caries prediction: a review of the literature. Comm. Dent. Oral Epidemiol. 2005–2010. vol. 12, 2005

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**Levine, M.**, R. L. Brumley, and T. S. Burks. 1985. Defined antibody re-

**Levine, M., and B. F. Movafagh.** 1986. Analysis of the specificity of natural human antibody reactive to *Actinomyces*. Mol. Immunol. 23:255–261.

**Levine, M., D. E. Parker, and J. A. Stober.** 1984. Human serum precipitins to oral bacteria related to dental caries. Arch. Oral Biol. 29:191–194.

**Levine, M., S. Ronda-Lapolla, W. L. Owen, and S. S. Soeransky.** 2002. Antibody-based diagnostic for ‘refractory’ periodontitis. J. Clin. Periodontol. 29:935–945.

**Miller, A. J., J. A. Brunelle, J. P. Carlos, L. J. Brown, and H. Loe.** 1987. Oral health of United States adults, p. 3–168. U.S. Department of Health and Human Services, Bethesda, Md.

**Neuhaus, F. C., and J. Buddiley.** 2003. A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria. Microbiol. Mol. Biol. Rev. 67:856–872.

**Newbrun, E.** 1982. Sugar and dental caries; a review of human studies. Science 217:418–423.

**Palmer, R. J., S. M. Gordon, J. O. Cisar, and P. E. Kolenbrander.** 2003. Coaggregation-mediated interactions of streptococci and actinomyces detected in initial human dental plaque. J. Bacteriol. 185:3400–3409.

**Podshadley, A. G., and J. V. Haley.** 1968. A method for evaluating oral hygiene performance. Publ. Health Rep. 83:259–264.

**Powell, L. V.** 1998. Caries prediction: a review of the literature. Comm. Dent. Oral Epidemiol. 26:361–371.

**Rule, J. T., M. R. Smith, R. B. Truelove, D. J. Macko, and C. R. Castaldi.** 1984. Caries inhibition of a dentifrice containing 0.76% sodium monofluorophosphate in a silica base. Comm. Dent. Oral Epidemiol. 12:213–217.

**Sansone, C., J. Van Houte, K. Joshupira, R. Kent, and H. C. Margolis.** 1993. The association of mutants streptococci and non-mutans streptococci capable of acidogenesis at a low pH with dental caries on enamel and root surfaces. J. Dent. Res. 72:508–516.

**Sheilam, A.** 1984. Changing trends in dental caries. Int. J. Epidemiol. 13:142–147.

**Stallard, R. D., A. R. Volpe, J. E. Orban, and W. J. King.** 1969. The effect of an anti-microbial mouth rinse on dental plaque, calculus and gingivitis. J. Periodontol. 40:683–694.

**Stenudd, C., A. Nordlund, M. Ryberg, I. Johansson, C. Kallestal, and N. Stromberg.** 2001. The association of bacterial adhesion with dental caries. J. Dent. Res. 80:2005–2010.

**Stephen, K. W., L. M. D. Macpherson, W. H. Gilmour, R. A. M. Stuart, and M. C. W. Merrett.** 2002. A blind cases and fluorosis prevalence study of schoolchildren in naturally fluoridated and nonfluoridated townships of Morayshire, Scotland. Comm. Dent. Oral Epidemiol. 30:70–79.

**Stephen, K. W., D. R. McCall, and I. T. Tullis.** 1987. Caries prevalence in Northern Scotland before and 5 years after water de-fluoridation. Br. Dent. J. 163:324–326.

**Ximenez-Fyvie, L. A., A. D. Hauffman, S. Son, M. Thompson, G. Torrey, and S. S. Soeransky.** 2000. The effect of repeated professional supragingival plaque removal on the composition of the supra- and subgingival microbiota. J. Clin. Periodontol. 27:637–647.