Initial Acquisition of Mutans Streptococci by Infants: Evidence for a Discrete Window of Infectivity
P.W. Caufield, G.R. Cutter and A.P. Dasanayake
J DENT RES 1993 72: 37
DOI: 10.1177/00220345930720010501

The online version of this article can be found at:
http://jdr.sagepub.com/content/72/1/37

Published by:
SAGE
http://www.sagepublications.com

On behalf of:
International and American Associations for Dental Research

Additional services and information for Journal of Dental Research can be found at:

Email Alerts: http://jdr.sagepub.com/cgi/alerts
Subscriptions: http://jdr.sagepub.com/subscriptions
Reprints: http://www.sagepub.com/journalsReprints.nav
Permissions: http://www.sagepub.com/journalsPermissions.nav
Citations: http://jdr.sagepub.com/content/72/1/37.refs.html
Initial Acquisition of Mutans Streptococci by Infants: Evidence for a Discrete Window of Infectivity

P.W. CAUFIELD, G.R. CUTTER\(^1\), and A.P. DASANAYAKE\(^1\)

University of Alabama at Birmingham, Schools of Dentistry and \(^1\)Public Health, Department of Oral Biology, Birmingham, Alabama 35294

Oral bacterial levels of 46 mother-child pairs were monitored from infant birth up to five years of age so that the acquisition of mutans streptococci (MS) by children could be studied. The initial acquisition of MS occurred in 38 children at the median age of 26 months during a discrete period we designated as the "window of infectivity". MS remained undetected in eight children (17%) until the end of the study period (median age of 56 mo). The levels of both MS and lactobacilli in saliva of mothers of children with and without MS were not significantly different. Comparisons between a caries-active cohort colonized by MS (nine of 38) and children without detectable MS revealed similar histories in terms of antibiotic usage, gestational age, and birth weight. Interestingly, half of the children between the ages of one and two years who were not colonized by MS were attended by caretakers other than the mother, while all of the caries-active children during this same time period were cared for by their mothers; the difference was statistically significant. Here we report for the first time that MS is acquired by infants during a defined period in the ontogeny of a child. Support for the notion of a discrete window of infectivity comes from other sources, including animal models.


Introduction.

The notion that dental caries in animals is an infectious, transmissible disease was first demonstrated by Keyes (1960). Since that time, a group of phenotypically similar bacteria, collectively known as the mutans streptococci (MS), has been implicated as the principal bacterial component responsible for dental caries in humans (Gibbons and van Houte, 1975; Loesche et al., 1975). Infants do not harbor this organism until sometime after teeth emerge; MS require the presence of a hard, non-desquamating surface for their colonization (Berkowitz et al., 1975; Carlsson et al., 1975; Catalanotto et al., 1975; Stiles et al., 1976). The major source from whence infants acquire MS is thought to be their mothers; the evidence for this comes from several studies showing that isolates of MS harbored by mothers and their children exhibit similar or identical bacteriocin profiles (Berkowitz and Jordan, 1975; Davey and Rogers, 1984) and identical plasmid or chromosomal DNA patterns (Caufield et al., 1982, 1985, 1986, 1988; Caufield and Walker, 1989; Hagan et al., 1989; Kulkarni et al., 1989).

Several studies suggest that the extent of MS colonization and, to some degree, subsequent caries activity experienced by a child may be correlated with the mother's salivary level of MS; mothers with high levels of MS tend to have children with high levels; those with low levels tend to have children with low levels (Kohler and Bratthall, 1978; van Houte et al., 1981; Kohler et al., 1984; Caufield et al., 1988). While correlations between caries or MS levels in mothers and those in their children may be explained in part by common genetic or environmental factors, others have suggested that a child's degree of colonization or disease may be dictated by the mother's levels of MS at the time of transmission. For example, in a landmark study, Kohler and co-workers (1983, 1984) selected mothers with initially high levels of MS in saliva and determined the effects of various preventive and treatment regimens aimed at reducing MS below a predetermined threshold level. Children of these mothers were monitored for initial acquisition of MS and, subsequently, for caries activity over a three-year period. A statistically significant difference was observed between control and experimental groups in terms of when a child acquired MS, the levels of MS harbored by the mother and child, and the child's caries outcome.

In the present investigation, the oral bacterial populations of mothers and their children were monitored longitudinally from birth to between three and six years of age. Several environmental and biological factors associated with two subsets of the study population (MS-colonized children who developed caries vs. non-MS-colonized children) were monitored to assess why some children acquired MS and others did not.

Materials and methods.

Study population and study design.—Mother-infant pairs were recruited in this study for evaluation of intervention strategies aimed at preventing or delaying the transmission of mutans streptococci from mother to child. A more detailed description of the study design and population demographics is available elsewhere (Dasanayake et al., 1992; Wright et al., 1992). Informed consent was approved and implemented under the guidelines of the Institutional Review Board of the University of Alabama at Birmingham. First- or second-time mothers in their third trimester of pregnancy were screened for having relatively high levels of MS in unstimulated saliva (≥ 2.5 x 10^6 colony-forming units per mL; CFU/mL) in two of three samples taken on different days. These expectant mothers were asked to participate pending a satisfactory delivery. All participants resided in a municipality with fluoridated water and in households in which there was no more than one other adult and/or child. None of the mothers...
meeting eligibility requirements breast-fed her infant. The final study population consisted of 46 mother-infant pairs who remained in the study until at least the child's third birthday.

Following birth of their infants and during the first six months post partum, mothers received comprehensive dental treatment as described elsewhere (Wright et al., 1992). After completion of restorative treatment, mothers were randomized into two groups and received, in addition to a dental prophylaxis, a two-week series of topical applications of either an iodine-NaF disinfectant or a placebo agent (Caufield and Gibbons, 1979; Dasanayake et al., 1992). The intention of the intervention trial was to examine whether MS levels could be sufficiently reduced in mothers so that transmission of MS to the infant might be delayed or prevented. These treatments were administered to mothers just after the emergence of their infants' first tooth, but prior to detection of initial acquisition of MS (mean age of the infants at time of mothers' antimicrobial treatments was 8.9 months). The study population included both active- and placebo-treated mothers and their infants, because treatments exerted only a short-term effect on MS levels of mothers and did not affect the infants' acquisition of MS (Dasanayake et al., 1992).

Two subsets, consisting of the mothers and their children with evidence of caries by three years of age (n = 9) or children in whom MS could not be detected (n = 8), were available for further study. Comprehensive medical and dental histories of both mother and child from birth to age three were obtained via a survey of both mothers and their physicians or health clinics. Information on date of illness, medications, chief complaint or illness, and name of physician or health center was obtained from the mothers. Medical records were then reviewed for confirmation of information given by the mother. Compliance with the taking of prescribed medications was assumed, but not documented.

History of caretakers involved with caring for the children during the first three years was also obtained retrospectively via questionnaire and reviewed again with mothers in the same two cohorts. Interviews were conducted by a trained health-care professional who had no involvement or knowledge as to the subgroup being interviewed. This measure was instituted to minimize any information bias. Principal and secondary caretakers were recorded for each year of the infant's life. The principal caretaker was defined as that individual or group (e.g., daycare center, relatives, babysitters) having eight hours or more of contact time per day during five to seven days per week. The total hours per day for contact was defined as being 14 h; the period from 10 p.m. to 8 a.m.—during which time, presumably, the child was asleep—was not included. Secondary caretakers included all individuals or institutions having less than eight hours of contact time with the child per day during a five-day week (Monday to Friday).

Sampling and bacteriological methods.—Oral bacterial samples were obtained from mothers and infants at three-month intervals. Samples of unstimulated whole saliva were collected as described previously (Caufield and Gibbons, 1979; Wright et al., 1992). Briefly, mothers refrained from swallowing for 30 s to allow saliva to pool in their mouths, then gently expectorated saliva into a sterile container. A 0.1-mL aliquot was placed into transport fluid (RTF; Syed and Loesche, 1972), then held on ice until processed. Bacteriological samples were obtained in three different ways: (1) Saliva samples from infants were collected by placement of a sterile cotton-tipped applicator sublingually until saturated [Previous investigation showed these swabs to absorb 0.12 mL of saliva (Toney et al., unpublished data.)]; (2) the teeth or maxillary and mandibular ridges of deciduous and dentate infants were sampled by sterile cotton swab, then streaked directly onto MSB medium selective for MS described below; or (3) plaque samples were collected from dentate children by means of a sterile toothpick passed into interproximal regions and along the cervical margins of all teeth present. For the eight children without MS, three years of age and older, pooled plaque samples were also collected from all approximal surfaces by means of floss on a sterile holder (Caufield and Gibbons, 1979). All samples were placed immediately in 1 mL of RTF and processed within four h of collection; most samples were processed within 30 min of collection.

Samples were sonicated for 30 s (amplitude 8; Branson Sonifier, Danbury, CT) so that aggregates would be dispersed prior to being plated with a Spiral plater (Spiral Plater Systems, Inc., Cincinnati, OH; Loesche and Straffon, 1979). Samples, directly from RTF and dilutions, were plated onto mitis salivarius agar supplemented with bacitracin and sucrose (MSB; Gold et al., 1973) for cultivation of MS, mitis salivarius medium (Difco Laboratories, Detroit, MI) containing 0.001% tellurite (MST) for enumeration of total streptococci, Rogosa medium (Difco Laboratories, Detroit, MI) for lactobacilli, and MM10-sucrose medium for total cultivable bacteria (Loesche et al., 1975). The method used was capable of minimally detecting MS at 2 x 10^6 CFU per mL saliva. All plates were incubated anaerobically (Coy Laboratories, Ann Arbor, MI) at 37°C for 48 h. Presumptive colonies of MS from MSB medium were picked at random and subcultured to obtain pure cultures. Each isolate was then confirmed biochemically (Shklair and Keene, 1974). At various intervals, isolates of MS were collected from both mother and child, then frozen at -70°C in Todd-Hewitt glycerin medium as part of another study.

The age at which an infant was defined as having acquired MS was assigned after two consecutive positive cultures for MS from either plaque, oral swab, or saliva. Following the initial detection of MS, a second confirmatory sample was obtained, usually within two weeks of the first sample.

Statistical analyses.—Bacterial counts were transformed to log_{10} (Caufield and Gibbons, 1979), and both parametric and non-parametric tests were used in the analysis of data. Student's t test was used to test the differences between two groups having continuous variables, including the age of subjects at different phases, bacterial counts, number of decayed, missing, and filled tooth surfaces (DMFS), number of decayed, missing, and filled teeth (DMFT), gestational age, and birth weight. Chi-square tests for homogeneity and the Fisher's exact test were used for examination of racial differences and caretaker histories. The median test was used in comparison of antibiotic episodes of children and their ages at first tooth emergence. Analysis of variance was used for examination of differences in mothers' salivary levels of various
bacteria as they related to the study phase. The Kappa statistic was used for testing the reliability of detecting MS from saliva compared with detecting MS in plaque samples (Cohen, 1960). For all statistical analyses, the probability of type I error less than or equal to 0.05 was considered as statistically significant.

Results.

Thirty-eight of the 46 infants initially acquired MS at a median age of 26 months (Fig. 1). These infants came from a population predicted to be at high risk for acquiring MS based on their mothers' high levels of MS in saliva as well as past dental experience. MS was detected in 25% of these 38 infants by 19 months of age and in 75% by 31 months of age.

Eight children remained free of MS for a period greater than 56 months. This finding was based upon repeated negative samples for MS taken at three-month intervals. The non-MS-colonized children were mainly black (seven of 8; 87.5%) compared with the MS-colonized cohort (20 of 38; 52.6%) (p = 0.07). Neither gender nor mother's past dental history (DMFS or DMFT indices) was significantly different between the MS-colonized and non-MS-colonized cohorts (Table 1). The first primary tooth emerged approximately one month earlier in the MS-colonized infants than in the non-MS-colonized infants (6.8 vs. 7.7 mo), but this difference was not statistically significant (Table 1).

Reliability of detecting MS in infants.—The criteria for defining initial MS acquisition was based upon two con-

TABLE 1
COMPARISONS OF DEMOGRAPHIC AND DENTAL STATUS OF CHILDREN WITH AND WITHOUT MS AND THEIR MOTHERS

<table>
<thead>
<tr>
<th>Variable</th>
<th>MS-colonized (n = 38)</th>
<th>MS-absent (n = 8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race - black (white)</td>
<td>20 (18)</td>
<td>7 (1)</td>
<td>0.07*</td>
</tr>
<tr>
<td>Gender - male (female)</td>
<td>19 (19)</td>
<td>4 (4)</td>
<td>1.0*</td>
</tr>
<tr>
<td>Age at initial MS acquisition (months), mean ± SD</td>
<td>25.0 ± 8.3</td>
<td>&gt;56 ± 5.2b</td>
<td>0.0001c</td>
</tr>
<tr>
<td>Median, 25% and 75%</td>
<td>26, 19, 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>9 - 44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emergence of first tooth, mean age (months) ± SD</td>
<td>6.8 ± 1.4</td>
<td>7.7 ± 1.8</td>
<td>0.1c</td>
</tr>
<tr>
<td>Median</td>
<td>7.1</td>
<td>8.4</td>
<td>0.7d</td>
</tr>
<tr>
<td>Time from emergence of first tooth to detection of MS infection; mean age (months) ± SD</td>
<td>18.4 ± 8.3</td>
<td>&gt;48 ± 6.7b</td>
<td>0.0001c</td>
</tr>
<tr>
<td>Mother:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age ± SD (yr)*</td>
<td>23.1 ± 3.7</td>
<td>24.0 ± 4.0</td>
<td>0.6c</td>
</tr>
<tr>
<td>Decayed, missing, filled surfaces (DMFS); mean ± SD*</td>
<td>35.0 ± 18.0</td>
<td>29.5 ± 18.8</td>
<td>0.4e</td>
</tr>
<tr>
<td>Decayed, missing, filled teeth (DMFT); mean ± SD*</td>
<td>16.4 ± 6.8</td>
<td>13.5 ± 7.2</td>
<td>0.3e</td>
</tr>
</tbody>
</table>

* Fisher Exact test.

b Age of child at last sample in which MS remained undetected.

c Student t test.

d Median test.

* At entry of study.

MS-Colonized Infants

<table>
<thead>
<tr>
<th>Median age (months)</th>
<th>&quot;window of infectivity&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.8 ± 1.4</td>
<td>19</td>
</tr>
<tr>
<td>tooth emergence</td>
<td>31</td>
</tr>
</tbody>
</table>

age (months)

Fig. 1—The window of infectivity. The median time to initial acquisition of mutans streptococci for 38 infants is depicted as a function of infant age in months. The average age and standard deviation at emergence of the first primary tooth are also shown for these 38 infants.
secutively positive samples for MS in either saliva, swab, or plaque. Subsequent saliva or plaque samples were tested following the two positive samples for determination of whether the subsequent tests contradicted the positive designation. In the infants with MS, based on the criteria above, on average, 68% of the subsequent saliva samples and 85% of the subsequent plaque samples were positive for MS. (Oral swabbing was discontinued after a child acquired MS.) At initial acquisition in which two consecutive samples were positive for MS, plaque samples were positive 75% of the time, while the oral swab and saliva were positive 66% and 45% of the time, respectively. The Kappa statistic for agreement in detecting MS from saliva and plaque was unimpressive ($\kappa = 0.042; p = 0.14$), indicating that these two methods failed to attain significant agreement as to the MS status of the infant. These findings indicate that analysis of a plaque sample is more likely to detect early acquisition of MS than analysis of either saliva or an oral swab.

Another concern was whether the sampling method failed to detect MS in children designated as free of MS. In those children deemed to be without MS beyond three years of age, pooled plaque samples were gathered as described in “Materials and methods”; repeated sampling failed to detect MS in any of those eight children, although it is possible that our sampling method lacked sensitivity for detection of very low levels of MS in plaque or saliva.

Influence of mothers’ salivary levels of cariogenic bacteria at different intervals of infant development.—Because mothers are thought to constitute the major source of MS to their infants, mothers’ levels of MS and other oral bacteria in saliva were monitored at approximately three-month intervals throughout the study. Mean levels of MS, lactobacilli, total streptococci, and total cultivable bacteria per mL of mothers’ saliva were calculated for each measurement. Four phases of the study were defined as follows: screening-phase samples obtained when mothers first entered the study; the interval from infant’s birth to infant’s first tooth; the interval from first tooth to initial infant acquisition of MS; and the interval from acquisition to the infant’s third birthday (Figs. 2a, b). For the purpose of comparison, the time interval comparable with the time interval of initial MS colonization for the non-MS-colonized infants was computed as 25.0 months of age, the mean age of acquisition for MS-colonized children (Table 1). Comparisons between mothers’ salivary levels of MS and lactobacilli during each of the four phases are shown in Figs. 2a and b, respectively. Mothers of non-MS-colonized children consistently exhibited lower levels of both MS and lactobacilli per mL saliva. Even though a two-way analysis of variance revealed an overall significant difference in MS levels among the four phases ($F_{3,45} = 2.81; p = 0.04$), differences between the two cohorts of mothers were not statistically different ($p = 0.15$). Declines in all levels of salivary bacteria were observed and may be attributed to short-term reductions from interventions, including restorative treatment; these findings are reported elsewhere (Dasanayake et al., 1992; Wright et al., 1992).

Interestingly, the largest difference for both MS and lactobacilli between the two groups was manifested in the interval from initial tooth emergence to initial MS acquisition (E-Infect). Total streptococcal and total cultivable counts per mL of saliva were not significantly different between mothers whose infants had or had not acquired MS (data not shown).

Prevalence and distribution of MS biotypes.—The prevalence of Streptococcus sobrinus as determined from biochemical characterization within the 46 mothers in this study was 29%. All mothers having $S. \ sobrinus$ also harbored $S. \ mutans$. Of the 13 mothers harboring $S. \ sobrinus$, nine were black and four white; the disparity in distribution was not statistically significant. Only one child of the 38 MS-positive cohort harbored $S. \ sobrinus$ at the time of initial acquisition; the mother of this child was also a carrier of this organism.

Influence of perinatal history, antibiotic usage, and caretaker history on infant’s acquisition of MS.—To determine whether environmental factors such as antibiotic or caretaker history influenced the infant’s MS status, we

---

![Fig. 2](https://ja-sajepub.com/)

**Fig. 2**—(A) The mean ± SD of maternal levels of mutans streptococci (MS) per mL saliva as a function of various infant time intervals; mothers of children with MS ■ and without MS ○. Scr = average of samples taken at entry into the study; Pre-E = samples obtained after entry but before infant’s first tooth emerged; E-Infect = samples obtained between the emergence of infant’s first tooth and the time of initial MS acquisition in infant; 3 yr = samples obtained at child’s 3rd birthday (see text). (B) The mean ± SD of maternal levels of lactobacilli per mL saliva for the same mother cohorts; mothers of children with MS ■ and without MS ○.
TABLE 2
COMPARISON OF POSSIBLE RISK FACTORS ASSOCIATED WITH CARIES-ACTIVE,
MS-COLONIZED CHILDREN, AND CARIES-FREE CHILDREN WITHOUT MS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Caries-Active (n = 9)</th>
<th>MS-free (n = 8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decayed surfaces at 3rd year examination;</td>
<td>3.4 ± 4.4</td>
<td>0</td>
<td>0.0007*</td>
</tr>
<tr>
<td>Antibiotic episodes from birth to 3 years of age;</td>
<td>3</td>
<td>4</td>
<td>1.0b</td>
</tr>
<tr>
<td>Median</td>
<td>0 - 36</td>
<td>0 - 9</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>9</td>
<td>4</td>
<td>0.03c</td>
</tr>
<tr>
<td>Mother as primary caretaker of infant from 1 to 2 years of age</td>
<td>3298 ± 552</td>
<td>3128 ± 744</td>
<td>0.6a</td>
</tr>
<tr>
<td>Birth weight (g); mean ± SD</td>
<td>39.9 ± 1.2</td>
<td>38.8 ± 2.5</td>
<td>0.3a</td>
</tr>
</tbody>
</table>

* Student's t test.
  b Median test.
  c Fisher Exact test.

obtained detailed medical histories from two subsets of the study population: (1) children who remained free of MS past 50 months of age (n = 8), and (2) children who had acquired MS and experienced at least one carious tooth (mean decayed surfaces = 3.4) by the third-year dental examination (n = 9; Table 2). Medical histories from birth to age three years revealed that the median numbers of episodes of illness requiring one or more antibiotics were comparable between the two groups.

Examination of the caretaker histories revealed that, between one and two years of infant age, a greater proportion of the children without MS (4/8; 50%) had primary caretakers other than their biological mothers, compared with the caries-active group with MS in which the primary caretaker was exclusively the mother (9/9; 100%); this difference was statistically significant (p = 0.03; Table 2). Birth weights and gestational ages were similar between the two groups (Table 2).

Discussion.

In this study, the acquisition of MS was monitored in "high-risk" infants. The designation of high risk was based primarily on elevated levels of MS in the saliva of mothers; these samples fell within the upper quartile (27%) of the saliva from over 260 mothers who met initial eligibility criteria and from whom multiple saliva samples were obtained. Children of mothers having high levels of MS are more likely to exhibit levels of MS corresponding to their mothers' levels (van Houte et al., 1981; Kohler et al., 1984; Caufield et al., 1988), and often experience a higher caries incidence (Kohler et al., 1984). The mothers in this study also exhibited high caries levels (mean decayed and filled surfaces, DFS > 30) at least two-fold higher than the average DFS index for 20-24-year-old females living in the southeastern United States (NIDR, 1988). Although the study population does not represent the US population as a whole, attempts to control dental caries by alteration of the natural transmission of the bacteria responsible would probably be targeted toward a high-risk population.

The cumulative probability of MS acquisition as a function of age in this child population is shown in Fig. 3. Cumulative prevalences extrapolated from three other

![Cumulative probability vs Age (months)](Downloaded from jdr.sagepub.com by guest on July 24, 2010)

Fig. 3—The cumulative probability of MS acquisition as a function of child's age in months from the present study ▲, and from three other longitudinal studies by Carlsson et al. (1975) ○, Kohler et al., (1988) △ and Masuda et al., (1979) O. (Inset) The cumulative surface area of the primary dentition as a function of infant age in months. The value for surface area was derived for each tooth type relative to the surface area of the primary incisor, which was assigned a value of 1. Compared with the surface area of an incisor, first molars were assigned a value of 3, second molars a value of 4, and canines a value of 1.5. The cumulative surface area at a given time period is based on the teeth emerging multiplied by their assigned surface area. Age of emergence represented the average infant age for emergence of each of the primary teeth (Lunt and Law, 1974).
longitudinal studies are also depicted in Fig. 3 and generally parallel our findings (Carlsson et al., 1975; Masuda et al., 1979; Kohler et al., 1988). For example, the prevalences of MS acquisition in a Swedish child population at four years of age (Kohler et al., 1988) or in a Japanese child population at three and one-half years of age (Masuda et al., 1979) were 74% and 77%, respectively, similar to the rate of 83% in the present study (38/46). The rapid rise in prevalence as shown by the sigmoidal shape of the cumulative prevalence curve common to all three studies supports our contention that infants experience a finite period of maximum susceptibility for acquiring MS.

A major finding of this study was that the initial acquisition of MS in infants occurred during a well-delineated age range we have designated as the window of infectivity. Given the discrete pattern of acquisition, we wondered what biological events, if any, define the window. Findings from the present study as well as previous studies indicate that MS do not colonize the oral cavity prior to the emergence of teeth; this is because MS requires a non-desquamating surface, such as teeth, to be sustained (Berkowitz et al., 1975; Catalanotto et al., 1975; Stiles et al., 1976). Accordingly, the period during which an infant is at risk for acquiring MS is sometime after teeth emerge. In the present study, the average age the first tooth emerged was seven months, while the median age at initial acquisition of MS was 26 months. It would seem logical to assume, however, that initial acquisition of MS in infants occurred prior to our detection. Coincidental to the period of initial acquisition, i.e., between seven and 29 months of age, 20 primary teeth emerge into the oral cavity, including eight primary molar teeth. Thus, the oral cavity of the infant between approximately one and two years of age experiences a rapid increase in new tooth surfaces. We hypothesize that the discrete nature of initial MS acquisition is directly related to the presence of newly emerged teeth.

Both longitudinal and cross-sectional studies (Berkowitz et al., 1975; Catalanotto et al., 1975; Stiles et al., 1976; Masuda et al., 1979; Kohler et al., 1983) show that the prevalence of MS acquisition by infants increases with age or as the number of emerged teeth increases. The primary molars may be particularly critical for initial MS colonization, because they emerge into the oral cavity between 16 and 29 months of age (Lunt and Law, 1974) and possess both fissured occlusal surfaces and concave approximal surfaces. Occlusal fissures are more readily colonized by MS than are smooth surfaces (van Houte and Green, 1974; Svanberg and Loesche, 1977). Whether initial colonization by MS is dependent upon the presence of special ecological sites such as fissures or whether the probability of infection is a function of the increase in total tooth surface area during the 12- to 24-month period of tooth emergence, or both, is not known. When the cumulative surface area of the primary teeth is plotted as a function of age during the time these teeth are emerging, the cumulative probability of MS acquisition follows a sigmoid-shaped curve (inset, Fig. 3). Perhaps newly emerged teeth represent a “virgin” habitat which enables MS to colonize the oral cavity, avoiding competition with other indigenous bacteria already established or better suited to adhering to enamel surfaces. Also consistent with our proposed model that the presence of primary teeth dictates initial acquisition, age at first tooth emergence for children without MS was nearly one month later than those with MS, even though the difference was not statistically significant (p = 0.1). Alternatively, the emergence of primary molars may be coincidental with other unknown events that alter susceptibility to colonization by MS.

Perhaps more convincing, albeit indirect, support for the concept of a window of infectivity as well as the coincidence of infection with the emergence of teeth comes from animal studies. For example, colonization of S. mutans occurs readily in the oral cavities of rats if the bacterium is introduced when the rats are between 18 and 21 days of age (Schuster et al., 1978; Ooshima et al., 1988). Attempts to introduce MS outside of this 18- to 21-day “window” result in markedly less colonization or caries activity. Interestingly, and germane to the present study, first and second molars of rats begin emerging at between 18 and 21 days of age (Ooshima et al., 1988). In this same study, it was also observed that attempts to inoculate with MS ten- to 15-day-old rats having only incisors present were unsuccessful. The similarities between the human and the rat in terms of the presence of a discrete window of infectivity support the contention that tooth emergence and MS acquisition are related events.

The apparent clustering of 38 infants acquiring MS within a discrete window, coupled with the observation that eight children appeared to be free of MS for more than 25 months past the median time of acquisition, suggests that a child’s susceptibility toward acquiring MS may be limited, at least within the first five years of life. While it remains possible that we were unable to detect the presence of MS in these eight infants due to sampling error, repeated sampling failed to show evidence of MS. Our present hypothesis, which links temporally the emergence of primary teeth with the acquisition of MS, may also explain why the window of infectivity apparently closes. The last primary tooth emerges into the oral cavity at around two years of age; no additional teeth appear until around six years of age, when the first permanent molars emerge. If the acquisition of MS depends upon newly exposed tooth surfaces, it seems reasonable to speculate that MS colonizes the mouths of infants only during the period when teeth emerge into the oral cavity, i.e., between seven and 24 months of age. MS may have difficulty becoming established in the oral cavity at a later time, because they would have to compete with other indigenous bacteria already present and established on the tooth surfaces. The difficulty of implanting MS into the oral cavities of adults (Krasse et al., 1987; Edman et al., 1975) supports this contention. Hence, between two and six years of age, the period of time between the emergence of the last primary tooth and the first permanent tooth may represent a period in which the child is less susceptible to acquiring MS. Our model would suggest that children who escape MS acquisition during the initial window period will remain MS-free until at least six years of age, when first molars emerge. This hypothesis remains to be tested, however.

The question then arises whether another window of infectivity for MS will be present when the permanent teeth begin emerging between six and 12 years of age. If a second window period exists, the eight children in whom we were unable to detect MS may acquire MS at this time. It may also be possible that children, who have already
acquired MS, acquire additional strains of MS during this
"second window". Support for the concept of the second
window comes from two observations. First, cross-
sectional prevalence surveys demonstrate that greater than
90% of teenagers are colonized with MS (Klock and Krass, 1977). In adults, Kohler et al. (1983) found that only 3% of
the 249 mothers screened for their study had no detect-
able levels of MS. Second, studies using DNA fingerprint-
ing (Caufield and Walker, 1989; Hagan et al., 1989;
Kulkarni et al., 1989) and bacteriocin typing (Davey and
Rogers, 1984) indicate that adults harbor a greater diver-
sity of genotypes of MS than do their children, suggesting,
among other possibilities, that children acquire addi-
tional strains of MS as they get older and new teeth
emerge. Alternatively, infants may acquire their entire
repertoire of MS strains during the proposed window of
infectivity, but some or all of these different genotypes
remain undetected because they are present in low num-
bers.

Our data suggest that the mother's level of MS in saliva
may be related to the acquisition of MS in their children,
a finding reported in the study of Kohler et al. (1983). Fig.
2a suggests that mothers' levels of MS, preceding the time
to infection, are lower in the MS-free children compared
with the MS-colonized cohorts. Consistent with the re-
results of a study examining the variation in MS levels in
saliva (Bentley et al., 1988), the variation from sample to
sample within the same individual in the present study
exceeded 1 log (data not shown). Unfortunately, quantifi-
cation of MS in saliva remains imprecise, making defi-
nitive correlation between mothers' levels and their infants'
acquisition difficult, at least in the size of the population
studied here.

The fact that most teenagers and adults eventually
acquire MS (Klock and Krass, 1977), at least according to
cross-sectional surveys, indicates that transmission oc-
curs readily in human populations; our data suggest that
children acquire MS at around two years of age. Several
studies indicate that the mutants streptococci are trans-
mitted vertically, along familial lines, from mother to
cild (Berkowitz and Jordan, 1975; Caufield et al., 1982,
1988; Davey and Rogers, 1984; Masuda et al., 1985;
Caufield and Walker, 1989; Hagan et al., 1989; Kulkarni
et al., 1989). Intuitively, a mother colonized by MS, and
who often has the most contact with the child in terms of
feeding and caring for the child, would also be the most
logical source of transmitting MS to the child. This would
not rule out as potential sources other individuals colo-
nized by MS, such as siblings, grandparents, and non-
family caretakers who share responsibility for caring for
the child; however, previously published results on this
same study population failed to show genotypes other
than those of the mothers in infants at the time of initial
MS colonization (Caufield and Walker, 1989; Hagan et al.,
1989). As mentioned previously, several studies show a
relation between the levels of S. mutans in mothers
and their children. These studies taken as a whole suggest
that the mother not only constitutes the principal source
of MS to her infant, but also that a mother's level of MS
can dictate the extent of colonization in her infant. The
studies by Kohler et al. (1983, 1984) strongly support
the role of the mother in the colonization status and disease
outcome in her child.

We surveyed mothers of both caries-active and MS-free
children as to the post-natal contact of the infants with
other caretakers, since this exposure could conceivably be
a risk factor in terms of acquiring MS infection. Interest-
ingly, infants free of MS were significantly more likely
to have been cared for by a caretaker other than the mother
between one and two years of age than were the caries-
active infants. In spite of the small numbers, it seems
reasonable that reduction in exposure or contact hours
between the infant and the principal source of MS, i.e.,
the mother, would result in a decreased likelihood of MS
acquisition. In addition, observations from the present
study population showed that children acquire MS almost
exclusively from their mothers and not from other indi-
viduals (Caufield and Walker, 1989; Hagan et al., 1989).
This suggests, among other possibilities, that the child's
immune system may be capable of selecting which strains
are permitted to colonize, with preference toward mater-
nally-derived strains.

Other potential influences upon MS acquisition or
disease status in infants were also examined, including
birth weight and gestational age, and antibiotic experi-
ence within the caries-active and the MS-free children. No
significant differences in terms of any of these potential
influences were found between these two cohorts, how-
ever. It would be reasonable to speculate that children
receiving frequent antibiotics during infancy may be less
likely to acquire MS or develop caries. We did not observe
this pattern; in fact, we observed the opposite trend. There
was a positive correlation between overall illness experi-
ence and antibiotic usage and dental caries experience.
These findings should be viewed with caution, however,
given the small numbers involved.

The question as to whether other biological events
occurring in infants around 26 months of age, besides the
emergence of primary molars, influence MS acquisition
remains open for speculation. For example, the role of
the immune system, if any, in initial colonization of MS in
the infant remains unknown. By 12 months of age, the infant
produces 60%, 75%, and 20% of its total adult level of
serum IgG, IgM, and IgA, respectively (Roitt et al., 1989).
Antibodies acquired transplacentally from the mother are
credited with protecting infants, during the first year or so
of life, from many infectious diseases, but the role these
antibodies play in the acquisition of indigenous biota,
such as MS, is not known. Also not known is the role, if
any, of immunoglobulins in colostrum in the colonization
of MS in the oral cavity; none of the mothers in this study
breast-fed her infant.

Another aspect which may affect colonization by MS is
that infants undergo changes in their diet, in terms of
content, consistency, and frequency, during the first few
years of life. In fact, a recent study in Norway showed that
the consumption of sugar-containing food increased in
children between ten to 24 months of age (Rossov et al.,
1989). Dietary histories were not included in this study,
however, so we do not know the influence of diet on MS
acquisition. Clearly, the association between MS levels
and diet high in fermentable carbohydrates cannot be
ignored as a possible influence in initial colonization of
MS. The association between the intake of fermentable
carbohydrates and MS acquisition in infants has been
amply demonstrated in cases of early-onset nursing-bottle
caries (van Houte et al., 1982).

The observation that acquisition of MS in humans is
discrete, within a well circumscribed window, suggests several therapeutic implications beyond altering mother's risk toward transmitting as mentioned above. For example, proponents of replacement therapy might want to introduce genetically attenuated effector strains or strains antagonistic to MS colonization during the window period around two years of age when the infant is probably most susceptible to acquiring MS. Likewise, vaccination regimens may be more efficacious if administered just prior to acquisition and limited in duration to just the window period. In any case, the universality of the window of infectivity for MS in human populations is yet to be determined outside the select population studied here. Additional studies of the natural history of these indigenous organisms may reveal not only specific information about the prevention of dental caries, but also information as to the natural history of indigenous biota in general.

Acknowledgments.

We acknowledge the technical assistance of Peggy Still and Charles Kellum in obtaining and processing bacterial samples. Lisa Ballard obtained and reviewed medical records. Dot Bradley helped with the entry and analysis of data. H.M. Stiles contributed valuable insight into study design and the interpretation of results. Tim Wright and Brad Rodu managed the dental health of mother participants. Donna Rice and Donna Hughes helped prepare the manuscript.

REFERENCES

Ooshima T, Sum N, Izumitani A, Sobue S (1986). Maternal trans-


