

## *STREPTOCOCCUS MUTANS*, EARLY CHILDHOOD CARIES AND NEW OPPORTUNITIES

**S**cientific progress is a funny thing. We learn more every day; our understanding of human health grows exponentially. We have understood—and essentially conquered—diseases such as polio, smallpox, diphtheria and tuberculosis. Yet the prevalence of dental caries in secondary teeth—even in industrial countries like the United States, Japan and Western Europe—remains at nearly 50 percent, despite water fluoridation, dental sealants, mouthwashes, multiple oral hygiene products and a campaign for regular preventive oral health care.

It's even more serious than this. Caries of the primary teeth—early childhood caries, or ECC—is among the most prevalent health problems of infants and toddlers. It can be considered an epidemic in lower-income families and in underdeveloped parts of the world. ECC is one of the major causes of hospitalization in young children, who often must receive general anesthetic for extraction or tooth restoration.

A major obstacle to understanding ECC is defining the disease, which has been described by many other terms: baby-bottle tooth decay, caries of the incisors, rampant caries,

labial caries and nursing-bottle caries. A recent meta-analysis of more than 90 studies found that the studies varied widely in the name used to identify childhood caries, the diagnostic criteria and case definitions. As an example, 27 studies defined the disease as the presence of at least one maxillary incisor that was decayed, missing or filled, or dmf; 23 studies used two dmf maxillary incisors as the cutoff, and nine studies used three.<sup>1</sup>

No matter how it is defined, our professional problem is widespread, and it is at epidemic proportions among lower-income children. The prevalence of caries in preschool Head Start children may be as high as 90 percent in some subpopulations. A national oral health survey, conducted in 1996, found that 38 percent of children aged 2 through 9 years had caries in their primary teeth. Among children aged 2 through 4 years, 32 percent of Mexican-American children were affected, compared with 22 percent of black children and 13 percent of white children.<sup>2</sup> A recent study conducted in the Commonwealth of the Northern Mariana Islands found that in children aged 24 months through 36 months, 73 percent had white-spot lesions and 65

percent had enamel cavitation.<sup>3</sup> The U.S. Department of Health and Human Services stated in its "Healthy People 2000 Review 1997" that 50 percent of children 6 through 8 years old and two-thirds of all 15-year-olds had dental caries.<sup>4</sup>

Though the common refrain lays the blame for ECC on inappropriate feeding practices—particularly the use of sugar-containing beverages in baby bottles—the condition results from a complicated combination of factors (Box, "Factors Determining a Child's Susceptibility to Dental Caries"). To understand ECC, it is necessary to understand human and microbial genomics; microbial ecology; and the complexities of pathogen, host and environment interactions.

### THE MICROBIAL ECOLOGY OF CARIES

In a single human mouth, there often are more microorganisms than there are people in the entire world. The growth of these microbes in an infant's mouth follows a pattern of microbial ecological succession, analogous to the succession that occurs in forests, grasslands and other ecosystems. First, pioneer species move in and settle. They alter the environment,

creating a habitat that is friendly to other species, which move in and begin to grow. By the time an infant reaches adolescence, the mouth will harbor more than 400 microbial species (mostly bacteria), each with its own preferred habitat within a biofilm.

In any ecosystem, species have the ability to mutate and to alter the environment. Some changes come at a species' own expense. For example, alder trees have nitrogen-fixing fungi in nodules on their roots. When alders colonized glacial moraines in Glacier Bay, Alaska, there was little or no nitrogen in the soil. After a century of nitrogen-fixing by the alders, there was enough nitrogen in the soil to support conifers, which moved in and displaced the alders.

Less is known about the microbial succession that occurs in the mouth during the first year or two of life. Within the mouth, microbial biofilms form; they are composed of many commensal bacterial species and usually exist in equilibrium with host defenses. But when the composition of the biofilm and the metabolic activities within it are disturbed, the proportion of disease-causing bacteria increases, usually at the expense of other biofilm inhabitants.

Our understanding of caries development will continue to evolve, especially in light of human and microbial genomics, host mucosal immunity and the microbial ecology of biofilms. Significant insights into the architecture of oral biofilms will be gained from the application of advanced imaging and probe technologies that now are available. Newer areas of investiga-

tion, such as biofilm-related gene expression and cell/cell communication among oral bacteria, will progress only on a solid foundation of information gained from studies of microbial genomics, immunology, biochemistry and physiology, coupled with research in human behavioral and public health sciences. The National Institute of Dental and Craniofacial Research, or NIDCR, has made biofilm research a major priority.

#### THE BACTERIAL CULPRIT

*Streptococcus mutans* is the bacterium most pointed to as being partly responsible for causing ECC, although it is not in itself sufficient for caries development. Caries occurs when the bacteria break down sugars for energy, producing acid and reducing the mouth's pH. This reduction leads to the removal of mineral ions from the enamel surfaces. Between demineralization episodes, the ions return via the saliva. If demineralization exceeds remineralization, a carious lesion is initiated.

*S. mutans* is a gram-positive bacterium that adheres to tooth surfaces for colonization. Significantly, there is thought to be a window of infectivity from 19 months to 31 months of age. Still, this microbial species has been found in children as young as 10 months of age, in 60 percent of 15-month-olds,<sup>5</sup> and in even younger, predentate children—one study found *S. mutans* in 53 percent of 6- to 12-month-old children.<sup>3</sup> This suggests that the window of infectivity might be larger than previously considered.

*S. mutans* has developed defense systems to establish,

and sometimes dominate, the microbial ecosystem. First, this species is acidophilic, flourishing at very low pH values. Below a pH level of 4.5, not only is the competitiveness of cariogenic bacteria enhanced, but also the growth and metabolism of non-caries-associated species are inhibited.<sup>6</sup> Within three months of this pattern's being established, virulent bacteria can account for 75 percent of the oral flora.

Besides its role in microbial succession, *S. mutans* seems to behave differently depending on where in the mouth it is found. In one study, four *S. mutans* bacterial isolates—from caries-free adults, from adults with active caries, from root caries and from a laboratory strain—each were incubated with neutrophils.<sup>7</sup> The caries-free strain displayed the highest level of neutrophil activation, followed by the strains from the adults with active caries and from the laboratory. The root caries strain exhibited the lowest neutrophil activation; that is, it was the most successful in evading recognition by the human immune system.<sup>7</sup> Natural selection may be working to delete the *S. mutans* strains that are recognized and destroyed by our immune system, leaving us with opportunistic infectious bacteria that can survive long enough to induce caries.<sup>7</sup>

And as if all of this isn't enough, *S. mutans*, like many gram-positive bacteria, produces its own antibiotics, called mutacins, which inhibit the growth of other streptococci and many other gram-positive microorganisms. Recent research has deduced that mutacins work by a completely new antibiotic mechanism: stop-

ping essential enzyme functions and preventing the generation of adenosine triphosphate.<sup>8</sup> The production of mutacins varies among isolates of *S. mutans*. Last year, a study of mothers and children examined the relationship between transmission of *S. mutans* and the inhibitory activity of mutacins. Of 20 sets of mothers and their children, transmission of *S. mutans* was probable in nine cases. Bacterial strains shared between mother and child produced mutacins that were active against a broader spectrum of oral flora than did non-transmitted strains.<sup>9</sup>

Many children are infected with *S. mutans* by their mothers, and current genetic techniques allow researchers to examine this phenomenon in detail. DNA fingerprinting can distinguish strains of bacteria from one another. When comparing the genetic material of two bacteria of the same species, researchers who find restriction fragment-length polymorphisms—differences in the patterns of DNA fragments cut by specific restriction enzymes—know that the two bacteria are from different strains. A recent Swedish study used DNA fingerprinting to investigate the similarities of a group of bacterial species called mutans streptococci, or MS.<sup>10</sup> This group includes *S. mutans*, as well as several other species. Among 11 families in whom MS bacteria were found in mother, father and child, six children showed MS genotypes identical to their mothers'. Five children harbored MS genotypes different from both parents'. No mother-father pairs had matched MS strains.<sup>10</sup>

However, a study of Chinese

families found that among 11 families, there were three father-child matches, four mother-child matches and two mother-father-child matches (in which all family members had matching genotypes).<sup>11</sup> In one family, each person's genotype was unique, and in another family, the parents had matching genotypes but the child's genotype was unique.<sup>11</sup>

#### CARIES SUSCEPTIBILITY

Of course, not all children colonized with cariogenic bacteria have ECC. This indicates that susceptibility to ECC, as with

#### **Population-based studies do not support a definitive link between prolonged breast-feeding and caries.**

susceptibility to any disease, probably has a genetic component. A recent animal study found that caries susceptibility in mice was linked to a specific region of chromosome 17.<sup>12</sup> The Human Genome Project—as well as an NIDCR-supported project designed to sequence microbial genomes, including that of *S. mutans*—will provide us with a much-needed knowledge base. Teasing out the separate effects of genes, the transmission of specific strains of *S. mutans* from mother to child and the family diet will be a complex task. For now, we appreciate that caries susceptibility runs in families, owing to some combination of factors, and children from caries-susceptible families should be identified as being at risk.

Besides bacteria, diet and

oral hygiene are major factors in ECC. Breast-feeding long has been implicated in the disease; as late as last year, research abstracts claimed that prolonged nursing—particularly at night—was a risk factor for this disease. However, population-based studies do not support a definitive link between prolonged breast-feeding and caries.

Many children develop decay unrelated to breast-feeding, and human breast milk does not cause a significant drop in plaque pH, nor does it cause enamel decalcification. In fact, in a study using enamel powder, calcium and phosphate were deposited onto the enamel when breast milk was present. However, when supplemented with 10 percent sucrose, breast milk caused caries in 3.2 weeks.<sup>13</sup>

However, Swedish researchers found that children still being breast-fed at 18 months of age were more likely to have caries, compared with those no longer breast-fed. But the children with caries also had significantly more cariogenic bacteria in their mouths and ate significantly more cariogenic foods.<sup>14</sup> As with most complex conditions, ascribing a cause to only one factor is oversimplifying the issue, and usually is short-sighted.

The role of the nursing bottle has received considerable attention. The culprit here is a bottle filled with a sugar-containing beverage, often given to a child overnight or during naps. The nursing solution bathes the teeth in simple sugars and increases the risk of caries. One study found a fourfold increased risk of caries development in children who used nursing bot-

## FACTORS DETERMINING A CHILD'S SUSCEPTIBILITY TO DENTAL CARIES.

- The child's own genetics
- Genetics of the child's parents
- Genetics of infectious microorganisms
- Transmission of infectious microbes and age of infant or child
- Mucosal immunity
- Diet and nutrition
- Biofilms and microbial ecology
- Relative susceptibility of incisor and molar teeth
- Prevention measures
- Early detection of dental caries

tles this way.<sup>15</sup> These children's mouths were also more likely to be colonized by cariogenic bacteria than were the mouths of children who consumed only milk in their bottles.

Diet is critical to ECC. Children who begin eating salty meals early in life apparently increase their risk of developing ECC.<sup>16</sup> Early dietary habits often are continued into adulthood, so high consumption of salty and sugary snack foods can lead to lifelong dental problems.

Malnutrition can delay the eruption and composition of primary teeth and bone and thus can increase caries prevalence. Recent evidence shows that salivary gland function is impaired by iron deficiency and by prenatal exposure to lead, the latter being a serious problem in many low-income inner-city families.<sup>17</sup> Although ECC is a particular problem among lower-income children, factors such as lead exposure have been largely overlooked. Supplementing milk with vitamins during the first several years of life reduces the prevalence of linear enamel hypoplasia, a caries-associated condition common in lower-income populations that can increase

the risk of caries as much as tenfold.<sup>3,18</sup>

## PREVENTION

**Antimicrobial therapy.** The transmission of *S. mutans* is a key step toward childhood caries, and one that may be arrested by preventive methods. Antimicrobial therapy in young children may help reduce the risk of ECC. An application of 10 percent povidone-iodine every two months reduced the risk of ECC in low-income Puerto Rican children aged 12 months through 19 months.<sup>19</sup> But direct antimicrobial therapy for young children is not the only possible solution. One study showed that when women in their seventh month of pregnancy rinsed daily with sodium fluoride and chlorhexidine, bacterial colonization in their children's mouths was delayed by an average of four months.<sup>20</sup>

Specific immune defense against cariogenic bacteria is provided by salivary secretory immunoglobulin A, or IgA, antibodies, which are generated by the mucosal immune system. Newborns develop salivary IgA antibodies as their mouths become colonized by oral microorganisms. These antibodies interfere with the attach-

ment of bacteria to tooth surfaces and may inhibit the bacteria's metabolic activities.

Strategies of mucosal immunization include the use of surface adhesins and glucosyltransferase as key antigens, which are being incorporated into novel mucosal vaccine delivery systems. Animal studies have shown that rats receiving intranasal vaccines using these antigens against *S. mutans* had significantly higher IgA responses and fewer surface enamel lesions than nonimmunized animals had.<sup>21,22</sup>

The oral application of genetically engineered antibodies also offers new prospects for passive immunization against dental caries. Oral passive administration of a monoclonal antibody to *S. mutans* prevented recolonization of the species in human volunteers who had been treated with chlorhexidine first. Protection against *S. mutans* colonization lasted up to two years, although the monoclonal antibody was applied over a period of only three weeks.<sup>23</sup>

"Edible immunotherapy" is another option. Plant-based production of biologically active recombinant proteins facilitates conventional oral delivery through the consumption of plant tissues. Potatoes, bananas and tomatoes all are being investigated for vaccine production. Recently, a monoclonal antibody for an *S. mutans* adhesion protein was made in a tobacco plant and applied topically to the mouths of healthy human volunteers. The immunization reduced *S. mutans* colonization for up to four months.<sup>24</sup> A secretory antibody derived from transgenic plants, when compared with its "par-

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ent" (a murine immunoglobulin G antibody), had a higher affinity and survived three times as long. The plant-derived antibody afforded protection in humans for at least four months.<sup>24</sup> The company associated with the vaccine hopes it will be available by 2002.

**Fluoridation.** Possibly the most effective strategy known to prevent dental caries—at least in older children and adults—is water fluoridation. Currently, more than 60 percent of people served by community water systems have optimally fluoridated water. Although new water systems have initiated fluoridation, the proportion of people receiving optimally fluoridated water remains virtually unchanged from the 1989 baseline of 61 percent.

Also, the advent of bottled water may reduce fluoride consumption. Recently, the American Academy of Pediatric Dentistry, or AAPD, warned parents about giving bottled, nonfluoridated water to infants and young children. Currently, the AAPD recommends that a child's first dental appointment occur at the age of 12 months. For many children, an earlier appointment—even if the child has few teeth—may help educate parents and make the health care provider aware of potential problems. In fact, a recent study found that dental visits every six months beginning sometime before birth were helpful. Data collected through pregnancy and until children reached 4 years of age showed that these visits resulted in reduced caries incidence and *S. mutans* colonization in children, as well as significant improvements in oral health for the mothers.<sup>25</sup> However, access to care may be difficult for lower-income families, where attention is most needed.

**CONCLUSION**

In the case of ECC, there still is much progress to be made. On the research side, researchers continue to tease out associations among strains of *S. mutans*, parent-child transmission and susceptibility to ECC. The development of vaccines against *S. mutans* is receiving renewed effort, as are studies of biofilms and microbial ecology.

On the education side, it is evident that educational interventions, combined with regular preventive care, can go a long way in helping prevent ECC—and, possibly, subsequent dental problems. Informing our patients about what makes for



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good oral health care is a crucial part of the fight against ECC. ■

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