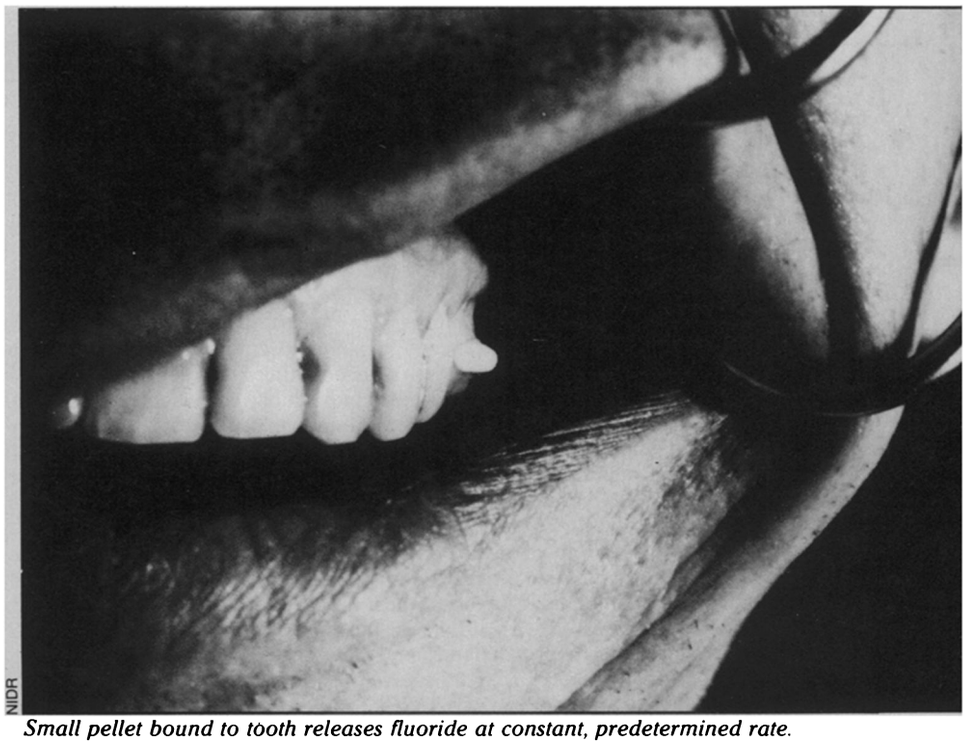
PROTECTING THOSE PEARLY WHITES

Scientists are getting inside the mouth with new defenses against cavity-causing bacteria


NIDR

Small pellet bound to tooth releases fluoride at constant, predetermined rate.

BY JULIE ANN MILLER

We all play host to a collection of microorganisms that frequent the human skin, gut and mouth. Among the most destructive long-term guests are the bacteria that cause tooth decay. They glue themselves to a tooth, consume the host's food and churn out acid that dimineralizes the tooth. Having adapted to withstand the cleansing flow of more than a liter of saliva a day, these bacteria hold their ground tenaciously. Evicting them from the mouth's cozy niche has proved to be a formidable task, but a variety of research forces are bearing down on the harmful bacteria. In addition to progress with antiseptics and vaccines, one novel approach involves replacing destructive bacteria with a laboratory-devised, nonvirulent bacterial strain that may defend the mouth from more destructive varieties.

Since 1924 bacteria have been known to cause tooth decay. Rats raised under germ-free conditions, for instance, do not develop dental caries, or cavities. When the same rats are allowed to consort with normal, germ-infested animals, they quickly pick up the harmful bacteria and became subject to tooth decay.

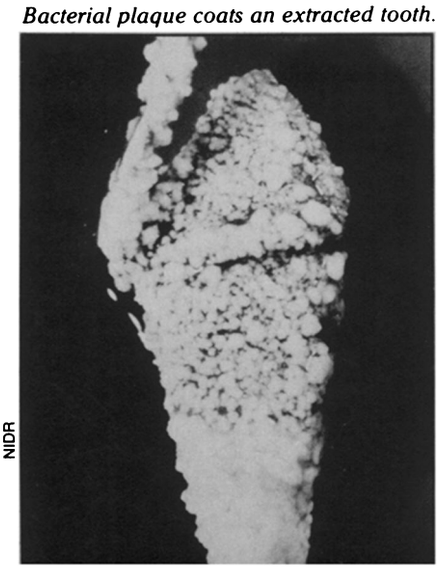
The prime biological culprit in human tooth decay is a bacterium called *Streptococcus mutans*. Although other microorganisms in the mouth produce acid, none cause cavities at the same rate or to the same extent as does *S. mutans*, says William Bowen of the National Institute of Dental Research.

"What is so peculiar about *S. mutans* to be so cariogenic?" Bowen asks. He suggests that much of its virulence comes from its ability to attach and colonize the tooth surface. Once microorganisms adhere to the tooth, they form a coherent layer of transparent or white material called plaque. The plaque also traps bacteria of various species. In samples of plaque, *S. mutans* makes up only 0.1 to 20 percent of the microorganism population. In addition to live bacteria and their sticky products, plaque contains food particles, dead bacteria and other debris.

Plaque also has an electrical charge distribution that further contributes to the damage the bacteria promote. It allows sucrose from food to diffuse into the plaque to nourish the densely packed bacteria, but it prevents outward diffusion of the vast quantities of acid produced. "It amounts to a sponge of acid being supplied to the tooth surface," Bowen says.

Limiting that acid is one approach to avoiding dental caries. Besides being champion at sticking to teeth, *S. mutans* characteristically produce a huge amount of acid from sugar metabolism, says Jeffrey D. Hillman of the Forsyth Dental Center in Boston. Two years ago Hillman isolated a mutant strain of *S. mutans* that seemed to differ from the parent bacteria in only one characteristic: The new strain makes much less acid. Hillman suspected that the mutant bacteria could provide a dental service by occupying the same niche as more harmful bacteria, but doing less damage there.

Bacterial plaque coats an extracted tooth.


NIDR

Animal tests and further characterization of the mutant strain now support that possibility. The mutant is deficient in the enzyme that forms lactic acid, so its metabolism of sugars leads to different end products, some of which are neutral instead of acidic.

When Hillman and collaborators introduced the mutant bacteria into the mouths of germ-free rats and then fed the rats a high sugar diet, virtually no decay resulted. In contrast, rats exposed to the parent *S. mutans* strain suffered rampant caries. Although successful in animals, the method is still only in the experimental phase, Hillman cautions. Rats are generally good models for tooth decay, but rats and humans have significantly different oral practices.

The idea of replacing harmful bacteria with more benign strains is not new in medicine, but Hillman is the first to employ a strain derived using laboratory procedures. In the past a naturally occurring, less virulent strain of *Staphylococcus aureus* was used to prevent serious infections of newborns in hospital nurseries. More recently, bacterial replacement has been used to prevent diarrhea in swine. "This [*S. mutans*] is the first time a laboratory-derived strain has been used for re-

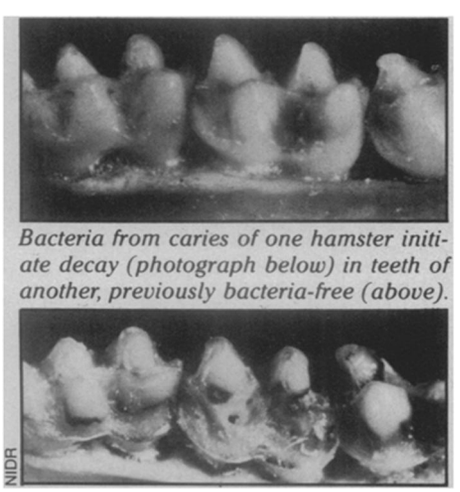
placement therapy," Hillman says. Daniel Green of the American Association for Dental Research comments, "That's exciting stuff — putting good bugs in for bad bugs."

Seven slightly different strains of *S. mutans* have been identified in human mouths. Yet in almost every case, a person hosts only a single strain. A child, after acquiring a tooth or two, probably is infected with a *S. mutans* strain from a parent. Therefore, if many persons become infected with the harmless strain, the protective bacteria should spread naturally to future generations.

Once ensconced in a mouth, they should provide their host life-long protection. "It is hard to displace a strain that occupies colonization sites," Hillman says. "Competing strains have no place to go." So getting rid of a person's endogenous *S. mutans* infection is a major problem if the mouth is to be colonized with the less virulent mutant. However, Hillman says, a potent mouthwash has been developed in Sweden, and clinical tests with the mutant bacteria have begun there.

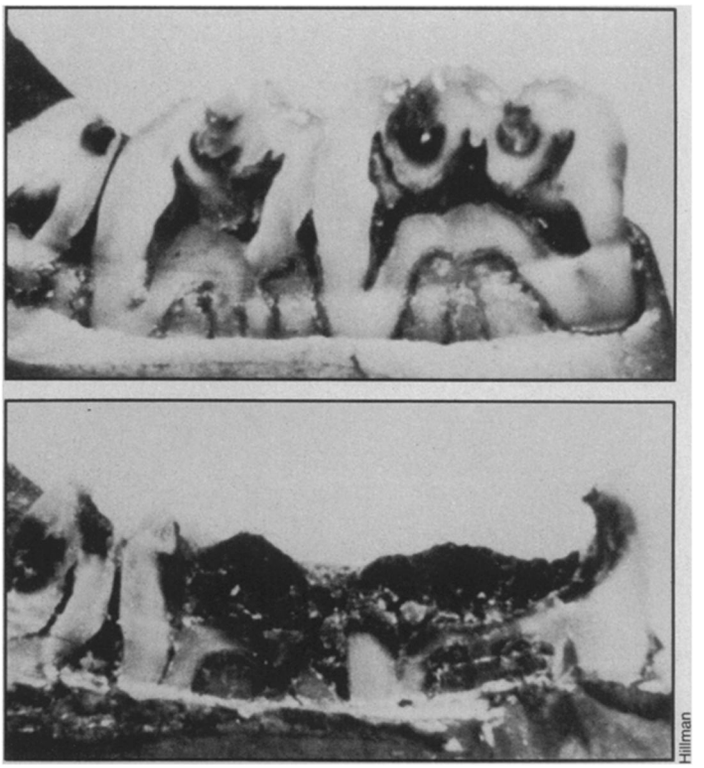
A more traditional goal of dental researchers has been a vaccine against the caries-causing bacteria. Can a simple injection, or more likely a swallow, of bacterial material produce long-lasting protection against dental decay? In medical practice successful vaccines against bacteria (rather than against their toxins) are rare. And creation of a vaccine against *S. mutans* involves special problems. The harmful bacteria are present in the mouths of almost all individuals and the "disease" can last as long as a person has teeth. The tissue at risk is a hard surface, instead of soft tissue, as in most disease. Finally, scientists know much less about the immune system present in saliva than about the immune system of the blood, which fights most other diseases.

The principal protein of the secretory immune system, found in breast milk and tears as well as in saliva, is called immunoglobulin A. "It is uniquely suited to the mucosal environment," says Martin Taubman of the Forsyth Dental Center. It seems to interact directly with bacteria, instead of calling in secondary fighters as do the antibodies of the blood immune system.



Bacteria from caries of one hamster initiate decay (photograph below) in teeth of another, previously bacteria-free (above).

NIDR



Infection with bacteria unable to cause dental decay protects rat from caries (upper photograph). Virulent strain of bacteria creates rampant caries in unprotected animal (lower photograph).

Hillman

Despite its differences from the blood system, the secretory immune system has been enlisted in the fight against caries. "Immunization does seem to be protective," Taubman says. In laboratory experiments, antibodies against *S. mutans* have already protected rodents and primates from dental caries. Young, bacteria-free animals injected with killed *S. mutans* showed significantly less disease than did control animals when each group received a high-sugar diet and was exposed to virulent bacteria.

The best way to administer such a vaccine will probably be oral. Progress on an injectable vaccine was set back when scientists discovered that some of the antibodies to *S. mutans* appear to bind to heart muscle. The investigators do not know whether such binding would be harmful, but for caution's sake use of such a vaccine should probably be avoided. Therefore investigators are favoring an oral vaccine that would stimulate the secretory immune system with little triggering of antibodies in the blood, which comes into contact with heart muscle.

Germ-free rats fed killed *S. mutans* produced saliva and milk antibodies to the bacteria and the rats were protected from infection. An experimental vaccine has been administered to humans in preliminary tests, and there is evidence of antibodies to *S. mutans* in their saliva, although no data yet link the immunization to decreased tooth decay.

Another approach to a safe vaccine involves selecting a single component of the bacteria and stimulating production of antibody to it. The choice is important, because the component selected must be essential to the bacteria's harmful effect. Antibody bound to a bacterium doesn't kill it, but it may interfere with its functioning. Components that have been extensively studied as potential vaccine material are the glucosyltransferase enzymes (GTF) crucial to a bacterium's ability to accumulate on the tooth surface. Glucosyltransferase enzymes are responsible for converting sugar from food to the sticky material by which bacteria adhere to teeth and form plaque.

Antibodies to glucosyltransferase enzymes (GTF) impair the ability of *S. mutans* to stick to a hard surface. When rats and hamsters were injected with GTF they produced the appropriate antibodies, and the number of caries formed was less than that in control animals. Experiments with GTF from several of the seven strains of *S. mutans* indicate that GTF from one type may elicit a protective immune response against infection with many or possibly all the strains, thus simplifying the immunization problem. However, the rat and hamster injections include materials to enhance the immune response that are not suitable for human use.

"Oral immunization studies are necessary to get a form acceptable for human use," says Daniel J. Smith of the Forsyth Dental Center. Working with Taubman, he finds that feeding animals GTF, instead of killed bacteria, produces a good immune response. It also prevents much bacterial colonization of tooth surfaces and reduces the decay. Smith and Taubman hope to soon begin limited clinical trials on GTF oral immunization.

Continued on page 397

. . . Dental

Another traditional approach to cavity control is administration of an antiseptic to limit the number of microorganisms in the mouth. "The big problem with any anti-microbial agent for use in the mouth is that it must be present for sufficient duration to exercise its maximum potential," Bowen says. Many mouthwashes are cleared by saliva within minutes of the rinse, so they never get to do their job.

If an antiseptic does not linger long enough in the mouth, controlled release may be the answer. A small device that can be attached to a back tooth and that releases fluoride is undergoing preclinical tests at NIDR. (Fluoride acts to kill bacteria, as well as to increase a tooth's resistance to decay.) Bowen says that the device has already been tested in dogs and monkeys and the results are "extremely promising." Still, he does not expect it ever to play a general, public health role. Instead it is intended for special cases: persons unusually susceptible to caries and those unable to clean their teeth adequately, for instance persons with handicaps or extensive bridgework or a dental appliance.

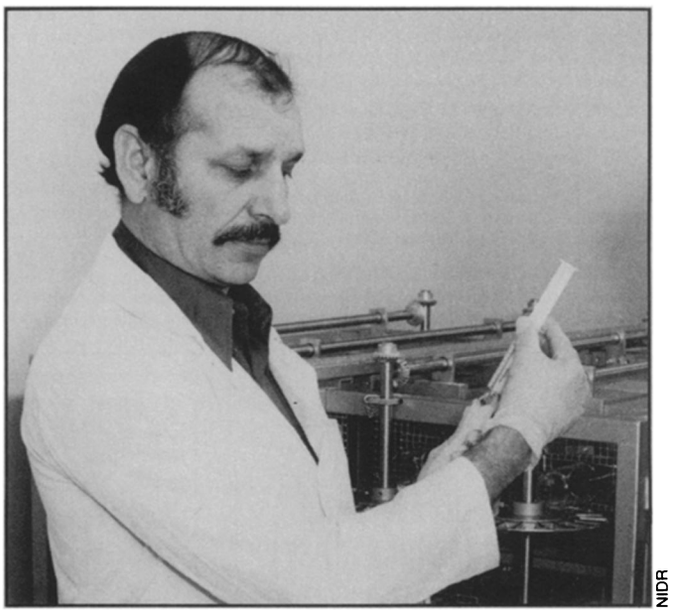
Several possibilities are under consideration for the slow release of other antiseptics, possibly for more general use. Tiny capsules containing antiseptics could bind to the tooth surface and gradually leak their contents. Or an anti-microbial agent might stick to teeth more effectively if it were sprayed on under pressure. Bowen says such approaches may be more practical than the fluoride-releasing device for compounds that, unlike fluoride, do not bear an electrical charge.

Saliva contains a complex mixture of proteins beyond its antibodies. Although the natural functions of most of the saliva components are obscure, they are being eagerly examined as enhancers of the body's defense. Saliva is known to retard tooth decay, because people with impaired saliva flow (xerostomia or "dry mouth") are unusually susceptible to caries.

Sialin is currently one of the most promising saliva factors. This small molecule, made up of just four amino acids, was isolated in 1972 by Israel Kleinberg who is now at State University of New York at Stony Brook. Kleinberg had been studying the ability of saliva to counteract the acid produced when bacteria metabolize sugar. Sialin appears to be taken up by bacteria and converted to compounds that neutralize the acid bacteria produce. Sialin is probably the most important member of a family of saliva compounds that Kleinberg calls arginine peptides.

Kleinberg recently contracted with Warner-Lambert pharmaceutical company to do further studies toward using Sialin in mouthwash and toothpaste and in soft drinks and candy to offset the effects of sugar. Other researchers have focused on different compounds among the more than 50 proteins found in saliva, as well as on the natural buffers in saliva, agents that affect saliva flow and a large peptide called statherin, which controls calcium and phosphate incorporation into tooth and plaque material.

The prospect of antiseptics, vaccines and additives limiting dental caries should not be taken as an alternative to water fluoridation programs, nor should it be considered license for unrestricted consumption of sugars, Bowen says. "There is overwhelming evidence that sugars form an essential part of the pathogenicity of dental caries," he warns. "There is no doubt that people who curb their sugar intake have better dental health than those who do not."



NIDR

A tube into the rat's stomach allows it to be fed without the food touching its teeth. At programed intervals, machine in background presents test food to caged rats for normal chewing and swallowing.

Ways to sweeten the task of cutting back on sugars are being examined at NIDR. In collaboration with industry, the scientists there are trying to identify sweeteners that don't promote tooth decay. Among the promising candidates are derivatives of citrus peel (dihydrochalcones), the dipeptide aspartame, a component of leaves of an African plant (thaumatin) and three other naturally occurring compounds: trichlorosucrose, stevioside and monellin.

Identification of the most tooth-damaging snacks on the market is another task of NIDR investigators. A new approach allows for a rigorous assessment of the decay potential of specific foods. In those tests no food other than the snack being assessed touches the animal's teeth. The procedure involves delivering through a tube to a rat's stomach an essential liquid diet. The test foods are then offered to each of 40 cages by a computer programed feeder, so that a food is available only at a specific time and all the rats are fed identically. "All lesions [caries] can then be ascribed only to the test food," Bowen says.

Another evaluative technique uses a partial denture fitted with electrodes connected to a pH meter. A person wearing the appliance chews the test food while the researcher observes how much acid is produced. Bowen warns, however, that instantaneous acid production is only one aspect of the caries problem. Eating patterns are also important. Each time a person eats a food containing sugar, the bacteria in plaque begin churning out acid. Bowen explains, "if you had a pound of candies and were foolish enough to eat them all at one go, you would probably do more damage to your waist than to your teeth. But if you ate them as 60 snacks during a day, you would have 60 acid attacks on the tooth surface."

Avoiding sweet snacks, promoting oral cleanliness and expanding fluoridation — the current methods for prevention of dental caries — can substantially decrease tooth problems. But these approaches generally are not expected to prevent tooth decay completely.

" . . . until far more effective preventive methods become available, oral diseases will remain a costly public health problem for the nation," says the staff of the Forsyth Dental Center in a background paper for *Healthy People, The Surgeon General's Report on Health Promotion and Disease Prevention.* "The pandemic nature of the major oral disease suggests that the more simple direct approaches, which have been used to overcome many other infectious diseases . . . may not be effective by themselves and therefore, simultaneous implementation of many different preventive techniques may be needed before total eradication of the major oral disease can be achieved. In short, success is attainable but it will be achieved in progressive steps."

Perhaps it will take the equivalent of bouncers, watchdogs, noxious chemicals and crowds of more welcome guests to dislodge the undesirable, uninvited freeloaders from the human mouth. □