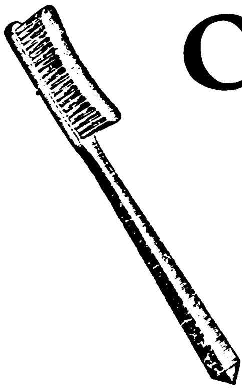
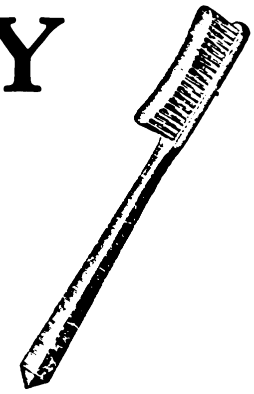


ORAL ECOLOGY



A matter of attachment

By JULIE ANN MILLER

First of two articles

A complex ecosystem, with more than 1,000 different types of organisms, sits between your lips and your throat. Of all parts of the human body, the mouth appears to contain the most extensive microbial interrelationships.

Dental problems triggered the original interest in the microorganisms populating the mouth. Bacteria have been charged with causing both tooth cavities (SN:3/29/86,p.203) and periodontal diseases (SN:10/5/85,p.221), in which infections of the soft tissues and bones that support teeth often lead to tooth loss. Periodontal diseases today affect about 94 million people in the United States.

Many dental researchers believe that preventing and curing oral diseases will require a better understanding of the ecology of the healthy mouth and of the conditions that allow harmful microbes to gain a foothold. And information on the oral ecosystem may be applicable to other infectious diseases and other ecological problems, such as microbe-caused corrosion (SN:7/20/85,p.42).

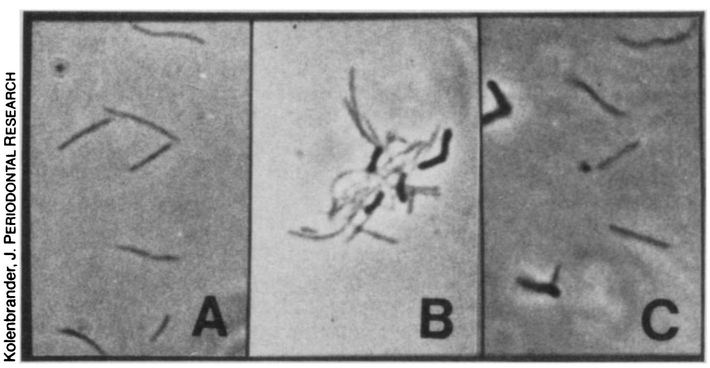
Emphasis on the dynamic interactions between organisms has led an increasing number of scientists to call their research area "oral ecology" instead of dental microbiology. These scientists are applying both the principles and techniques of microbiologists studying communities of organisms in external environments.

"In general, the establishment of the indigenous microbiota in humans proceeds in accordance with the broad principles which govern ecologic processes elsewhere in nature, as, for example, in the development of the fauna and flora on a previously uninhabited island," says Robert J. Fitzgerald, a researcher at the Veterans Administration Medical Center in Miami, Fla.

A tooth freshly cleaned in a dentist's office acquires a bacterial population in an orderly manner. In the first minutes, certain colonizing organisms attach to the saliva-coated tooth. These lead the way for later arrivals, which may adhere hours or even weeks later. The mature community made up of a variety of bacteria is called plaque.

Firm attachment of bacteria is a key element in colonization of the mouth. "A tooth is like a rock in a stream. It is bathed by fluid," says Paul E. Kolenbrander of the National Institute of Dental Research (NIDR) in Bethesda, Md. Saliva, with a flow rate of about 1 liter per day, is replaced faster than the bacteria can multiply.

"If the bacteria don't attach, they are swallowed, and that's the end of them," says Stephan E. Mergenhagen of NIDR.



Kolenbrander, J. PERIODONTAL RESEARCH

Coaggregation: Oral bacteria of one species (Capnocytophaga ochracea) *do not adhere to each other (A), but they form aggregates with* Actinomyces naeslundii *(B). The sugar lactose makes the bacteria separate (C).*

But bacteria in the mouth have a variety of surfaces to which they may attach, and a distinct collection of bacteria adheres to the different surfaces. The microbes of the tooth surface, for example, are distinct from those that coat the tongue. The human mouth contains a variety of ecological niches.

Scientific description of the oral ecosystem is progressing on several fronts: the identification of its inhabitants, the mapping of their relationships and the analysis of their means of recognition and adherence. In these pursuits, researchers now are using computer data-analysis techniques and modern methods of molecular biology, including gene cloning, DNA hybridization and monoclonal antibodies.

To analyze an ecosystem, it is necessary to list the organisms present. In the mouth, microbiologists have only made a small dent in this task. According to Kolenbrander, more than 1,000 different bacterial strains have been detected, but only about 10 percent of these have been sufficiently identified to determine their species. Microbiologists have recently begun to search for segments of DNA, called DNA probes (SN:8/18/84,p.104), that will be useful for rapidly identifying even small samples of organisms.

Even though only a fraction of the mouth bacteria have been identified, scientists are discovering associations among them. "We have a lot of good bacterial taxonomists. Now it is time to take that information and put it to practical application," says Jeffrey D. Hillman of the Forsyth Dental Center in Boston. "We are now using computers to look at [bacterial] associations. Which organisms are found together? Is there protection, aid or antagonism between them?"

Hillman and his colleagues have found evidence that three bacterial species common in the healthy mouth, all of the genus *Streptococcus*, are antagonistic to several bacterial species thought to be involved in periodontal disease.

The researchers analyzed 172 plaque samples taken from below the gum line of 32 human subjects with destructive periodontitis. The more of these *Streptococcus* bacteria a sample contained, the less likely it was to harbor a member of the alleged pathogenic group. From laboratory studies, Hillman suggests that the *Streptococci* produce hydrogen peroxide, which is known to inhibit growth of the alleged pathogens.

Bacteria commonly found together, rather than antagonists, are the focus of several lines of dental research. The natural formation of plaque and its role in oral disease is thought to be based on wave after wave of bacteria adhering either to the tooth or to the bacteria already bound. In what order, scientists ask, do the different organisms attach to the tooth surface and to each other?

"Periodontal plaque is a complex bacterial ecosystem that carries an innate history of colonization, selection and maturation," says Steven Offenbacher of Emory University in Atlanta. "Detailed examination of this balanced environment can reveal developmental sequences and certain interrelationships, much like an archaeological record, that can provide insight in the understanding of plaque formation and maturation."

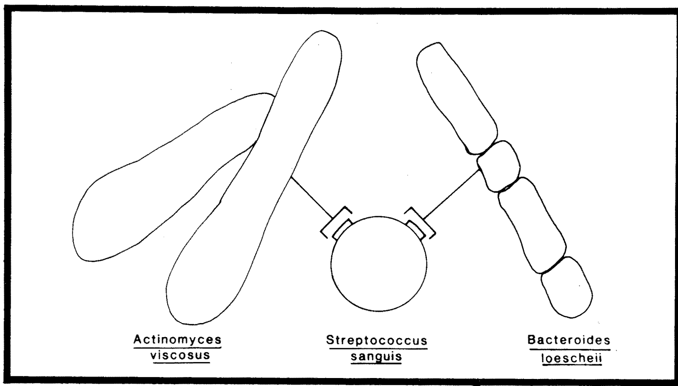
One approach to this question is to analyze the bacteria present in plaque removed from human mouths. Data collected on thousands of samples over the last two decades indicate that the earliest colonizers are *Streptococci* and *Actinomyces*. These two genera fall in the category of gram-positive bacteria, based on their ability to bind a stain commonly used in microscopy.

Later a variety of gram-negative bacteria attach to the developing plaque. These include microbes of such genera as *Bacteroides, Capnocytophaga, Fusobacterium, Actinobacillus* and *Treponema*. Certain of these bacteria are especially prevalent in patients with periodontal diseases, although in most cases a single species has not been clearly shown to "cause" the disease.

Some scientists are using complex statistical methods to determine the natural history of dental plaque. "The fundamental concept is that many organisms which are present in plaque prefer or require a preexisting bacterial milieu," Offenbacher says. For example, the dense matrix of plaque can provide a constant, low-oxygen environment required by some bacterial species.

Offenbacher, T.E. Van Dyke and their colleagues determined which of 10 different categories of bacteria were present in plaque samples from each of 60 patients with periodontal disease. Their data indicate a "temporal interdependent colonization pattern," Offenbacher says, where motile, spiral bacteria called small spirochetes are essential for colonization by medium spirochetes, followed by large spirochetes, then motile rods, spindle-shaped fusiforms and finally nonmotile rods.

Kolenbrander, J. PERIODONTAL RESEARCH



Competition sometimes occurs between bacterial species that coaggregate with the same bacteria. For example, A. viscosus *and* B. loeschii *both adhere to* S. sanguis *and appear to compete for similar binding sites.*

The colonization pattern of oral bacteria appears to be ruled in large part by their binding characteristics. This binding can be mimicked in the laboratory by a phenomenon called coaggregation, which the scientists define as aggregation between bacteria of different genera.

If two species of bacteria — say, a *Streptococcus* and an *Actinomyces* — are mixed in a test tube of solution, visible clumps may form and settle to the bottom of the tube. This test, Kolenbrander says, "is a quick and easy initial determination of cell-to-cell interactions."

"From an ecological viewpoint, cells with the ability to coaggregate with or to attach to plaque bacteria have a great advantage over noncoaggregating cells, which would be removed from the oral environment by salivary flow," Kolenbrander says.

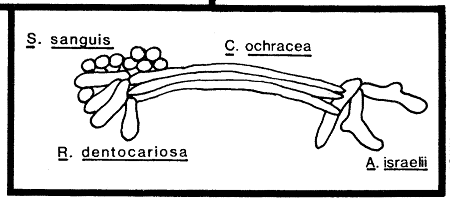
He and his colleagues have observed surprising specificity in the coaggregation reactions among hundreds of bacterial strains. These bacteria have an elaborate network of highly specific cell-to-cell interactions, he says.

Initially the scientists examined strains of two species of *Streptococcus* and of two species of *Actinomyces*. All the bacteria that coaggregate with another tested strain fall into one of six groups for *Streptococcus* or for *Actinomyces*. These groups are characterized by: the strains of the other genus with which it coaggregates; whether the ability to coaggregate is affected by heating the cells; and whether the sugar lactose inhibits the coaggregation.

More recently the biologists have extended this analysis to other oral bacteria. These bacteria, later arrivals to tooth plaque, also coaggregate with specific strains of various genera.

The results of experiments in which two bacterial strains are combined can predict what will happen when several species are mixed together. If A binds to B and B binds to C, then a mixture of A, B and C will form clumps. Kolenbrander refers to the bacterium B that binds to both other species as the "bridge."

He proposes that such coaggregation bridging is critical in the development of plaque, allowing organisms that do not



Kolenbrander, J. INFECTION AND IMMUNITY

C. ochracea *serves as a bridge allowing* A. israelii *to coaggregate with the two other species.*

bind directly to each other to be part of the same microbial community. Kolenbrander also sees evidence of competition, similar to situations in other branches of ecology, among bacteria that coaggregate with the same strains.

In his laboratory studies, Kolenbrander and his colleagues have mimicked plaque formation by adding bacterial strains, one after another, to a solution. He says they have succeeded in adding five strains, each of which binds to the microbe previously added. The early strains tend to have many potential partner strains, the later strains tend to have fewer. They refer to this coaggregation product as "simulated plaque."

The ecological view of the mouth appears to be a promising approach to fighting periodontal diseases. It is difficult to say that a single organism "causes" most periodontal diseases, because various organisms tend to be associated with each disease state. These organisms are also often present in low levels in healthy mouths.

"We are still trying to establish which bacteria are the most important pathogens," says James Carlos of NIDR. "Seven to 10 microbes in plaque have been implicated by at least some evidence."

It is as if ecologists were analyzing a lake overgrown with algae: They would need to determine not only what algal species are abundant, but also what conditions allowed the imbalance to arise.

"Some kind of ecological change has to take place in the mouth," Kolenbrander says. "There must be a progression of events that lead to the position where pathogenic organisms can establish themselves." □

Next: Mechanics of Attachment