Inhibition of In Vitro Plaque Formation by L-Lysine

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Accumulation of dental plaque was inhibited by L-lysine, but not by L-arginine or L-histidine. The monohydrochloride form was twice as effective as free amino acid.

The organism Streptococcus mutans is known to possess the potential for forming dental plaque (2, 6), and primary plaque formation which is closely associated with dental caries and periodontal disease can be produced by S. mutans in an in vitro system containing sucrose (9). Though the optimal pH for plaque formation has been reported to be 4.5 (10) on one hand and 8.5 (3) on the other, it is agreed that certain polysaccharides which are elaborated by streptococci are also important constituents of dental plaque (5, 7). Bladen et al. (1) also observed secondary in vitro plaque formation with several strains of Veillonella when an initial diphtheroidal plaque deposit was present. Such in vitro systems are particularly amenable to the testing of numerous agents exhibiting potential in retarding bacterial depositions.

Low-molecular-weight dextran added to the diet effectively prevented formation of plaque on the coronal surfaces of teeth of hamsters (4). The polymer did not destroy the oral streptococcal flora of these animals but inhibited the ability of the organisms to form an organized, gelatinous deposit on the surfaces of teeth. Whereas high-molecular-weight dextrans effect agglutination of cells over a wide pH range (3), high concentrations of levan, glucose, and dextran with low molecular weight (20,000) inhibit agglutination. Certain polyphosphates have recently been shown to inhibit streptococcal plaque growth through both bacteriostatic and bactericidal activities (11).

S. mutans 6715, a streptomycin-resistant mutant, was generously supplied by P. H. Keyes (National Institutes of Health, Bethesda, Md.). Plaque was grown on nichrome steel wires, some of which were coated with medical grade silicones (Clay-Adams, Inc., N.Y.). The inoculum was grown for 48 hr in thioglycolate medium (Difco) which was then used to seed the medium of Jordan et al. (8) with 5% sucrose added. The model system of McCabe et al. (9) was employed for in vitro plaque formation. Amino acids (Sigma Chemical Co., St. Louis, Mo.) were filter-sterilized and added to tubes containing sterile medium.

Figure 1 shows that an initial coating (center tube) with silicone of the nichrome steel wires, or coatings prior to each transfer (tube on right) of wires by the method of McCabe et al. (9), did not limit aggregation of cells when compared to wires not treated with silicone (tube on left). It
should also be noted that in addition to adhering to the sides of the tubes, large aggregations of cells accumulated at the bottom of actively growing cultures.

The effectiveness of L-lysine in inhibiting plaque formation is illustrated in Fig. 2. In $5.4 \times 10^{-2}$ M L-lysine-hydrochloride, there was no plaque formation on the wires, but the organisms did grow and accumulate at the bottoms of the tubes. In the absence of lysine, there was massive aggregation of cells on wires and moderate accumulation of the same on the walls and the bottoms of tubes. Thus, the monohydrochloride of the L-amino acid at a concentration of $5.4 \times 10^{-2}$ M is capable of inhibiting in vitro plaque formation without a cidal effect. It was also noted that the concentration of free amino acid had to be doubled to achieve the same effect.

Concentrations of L-arginine-hydrochloride up to $4.8 \times 10^{-2}$ M did not inhibit in vitro plaque formation (Fig. 3). In fact, when cells were grown in the presence of this amino acid, there was increased adherence of cells on the side of the tube. Results were similar for both the monohydrochloride and free amino acid. L-histidine ($5.2 \times 10^{-2}$ M) also was unable to inhibit plaque formation.

These studies indicate that L-lysine is capable of inhibiting in vitro plaque formation without inhibiting the growth of *S. mutans*. Other basic amino acids, arginine and histidine, do not possess this potential. The mechanism of inhibiting accumulations of bacterial mass by lysine warrants further investigation.

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LITERATURE CITED