

# Influence of Amalgam, Alloy, and Hg on the In Vitro Growth of *Streptococcus mutans*: III. Effect of Specimen Age and Composition

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*In vitro* growth inhibition of *Streptococcus mutans* is lost with sample age at different rates for amalgams prepared from a spherical, fine cut, and dispersion alloy. Varying the Hg composition from 48 to 52% has little effect on growth inhibition.

A procedure was presented that allowed estimation of the effect of amalgams and alloys on the in vitro growth of *Streptococcus mutans* under controlled experimental conditions.<sup>1,2</sup> Amalgams prepared in the same manner from spherical, fine cut, and dispersion alloys inhibited the growth of *S mutans* in different amounts. In all of these instances the amalgams remained under ultraviolet light approximately 40 minutes after preparation to assure sterilization. They were then introduced into the test medium followed by inoculation with *S mutans*. Thus, the amalgam age before testing was the same for all samples. Aging of the amalgam is now considered as a variable and its effect on the growth of *S mutans* in vitro is the purpose of this study. The Hg composition of the amalgam is also varied and the effect is evaluated.

## Materials and Methods

A detailed description of the bacterium, medium, and test agent facets of the test were previously described.<sup>1</sup> Initial prepara-

tion of the sample proceeded as previously described.<sup>1</sup> Instead of placing the amalgams under the ultraviolet light immediately after preparation, they were placed in clean covered Petri dishes and allowed to stand at 37 C for the desired time interval. After expiration of the time interval, the amalgam cylinders were placed under the ultraviolet lamp for 20 minutes on each side. They were then introduced into the sterile test medium, inoculated with bacteria, and incubated at 37 C. Spectrophotometric readings were taken in the usual manner.

## Results

The % relative growth of bacteria in contact with amalgams prepared from three different alloys as a function of aging time is given in Table 1. Each %RA<sub>60</sub> value given in the table represents the average of three measurements. It is apparent that at an aging time of two hours the amalgam prepared from the dispersion alloy is inhibiting bacterial growth considerably more than amalgams prepared from the fine cut and spherical alloys. By 24 hours, however, the bacterial inhibition property of the dispersion amalgam is not substantially different from that of the other amalgams, and by 72 hours all three amalgams have about the same effect.

The % relative growth of bacteria in contact with amalgams prepared from the spherical alloy as a function of short aging times is seen in the illustration. Each point plotted in the illustration represents the average of 4 determinations and the standard deviation is so indicated by the vertical spread. It can be seen that the inhibition of bacterial growth seen at aging times of

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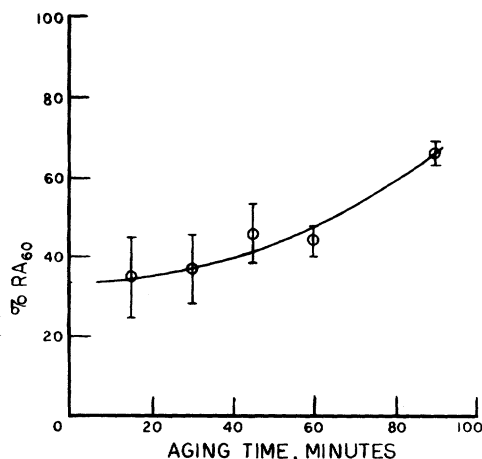
TABLE 1  
% RELATIVE GROWTH AS A FUNCTION OF AGING  
TIME AT LONG AGING TIMES

Aging Time (hr)	Alloy Type		
	Spherical	Fine Cut	Dispersion
2	85 ± 6*	75 ± 3	11 ± 10
24	88 ± 3	82 ± 3	79 ± 5
72	83 ± 4	81 ± 2	81 ± 6

Note: Fifty percent Hg, 1.56 gm/test tube was used.  
\* % RA<sub>60</sub> ± SD.

less than 30 minutes is largely diminished at 90 minutes although some inhibition still takes place (66% growth of control).

To estimate the effect, if any, of mercury composition on the in vitro growth of *S mutans*, fine cut and dispersion alloys were selected on the basis of our opinion that amalgams prepared from those alloys would most likely show some effect. Amalgams of 48 and 52% Hg composition for each type of alloy were therefore tested. After aging times of 1, 24, and 72 hours, the effect on bacterial growth was estimated by means of the %RA<sub>60</sub> values given in Table 2. The values of %RA<sub>60</sub> represent averages of 4 determinations. The effect of bacterial growth inhibition with short aging times is observed again, the effect being more pronounced with the dispersion amalgam. There is no significant difference in bacterial growth effects between 48 and 52% Hg for both types of alloy studied at an aging



Loss of growth inhibition with increased aging at short aging times. Spherical alloy, 50% Hg, 1.56 gm/test tube.

TABLE 2  
% RELATIVE GROWTH AS FUNCTION OF Hg  
COMPOSITION AT VARIOUS AGING TIMES

Aging Time (hr)	Alloy Type Used*			
	Fine Cut		Dispersion	
	48	52	48	52
1	70 ± 6†	76 ± 3	28 ± 10	23 ± 7
24	91 ± 2	88 ± 4	84 ± 6	79 ± 4
72	86 ± 5	86 ± 4	85 ± 5	88 ± 4

\* Composition, % Hg.

† % RA<sub>60</sub> ± SD.

time of 72 hours. At 24- and 1-hour aging times, the small differences in growth inhibition between 52 and 48% Hg amalgams of the dispersion alloy are not significant at the  $\alpha = 0.05$  level. At one-hour aging time, the small difference in growth inhibition between 52 and 48% Hg amalgams of the fine cut material is significant at the  $\alpha = 0.05$  level.

### Discussion

Strength measurements on amalgams suggest that reactions between the Hg and the alloy may continue for long periods of time within the amalgam.<sup>3</sup> The observed effect of growth inhibition associated with freshly prepared amalgams and the subsequent loss of growth inhibition on standing are likely to be associated with change in amalgam phase composition on aging.

It would seem that those properties of the amalgam that are responsible for growth inhibition and change with aging, change most rapidly in the spherical amalgam and least rapidly in the dispersion amalgam, with the fine cut amalgam somewhere in between. The greatest growth inhibition (lowest %RA<sub>60</sub> values) appears at the shortest aging times for all the amalgams studied. The power to inhibit bacterial growth is lost with aging at different rates for the various types of amalgams studied, but even after 72 hours' aging time there is some growth inhibition (81 to 88%) compared to a control growth of 100%.

Varying the Hg composition of fine cut and dispersion alloy amalgams from 48 to 52% Hg seemed to have little if any effect on growth inhibition properties, particularly after aging times of 24 hours had been reached. Growth curves with pure Hg metal in the bottom of the test tubes have been

determined, with the result that Hg as a metal does not inhibit bacterial growth to any appreciable extent under the conditions of the test. Thus, it would seem that amalgamation tends to neutralize the toxicity of some metal components present in the alloy and that this effect is time dependent.

It would seem to be significant that the dispersion alloy amalgam had the highest copper content.<sup>2</sup> Several in vitro studies have demonstrated that copper amalgams exert strong bactericidal effects.<sup>4,5</sup>

Norman et al<sup>6</sup> have demonstrated in vivo that restorations influence plaque composition with respect to time up to one year. They also report a higher incidence of secondary caries associated with amalgams than with resins or silicate restorations. Our results imply a bactericidal action in freshly filled lesions that is dependent on the nature of the amalgam. Whether this effect results in a clinical advantage for the dispersion alloy is not demonstrated here.

### Conclusions

We will now summarize the conclusions from parts I, II and III of this study.

A test procedure has been developed that provides a simple, quick, and nondestructive means of monitoring the in vitro growth of *S mutans* in the presence of amalgams and alloys. The spectrophotometric readings are related in a simple way to growth expressed as dry weight of bacteria and metabolic products.

Results are expressed as growth relative to controls which represent bacteria growing under identical conditions but not in contact with metals.

The %RA<sub>60</sub> value that represents growth after 60 hours relative to controls is used as a measure of growth in the presence of alloys or amalgams.

Spherical, fine cut, and dispersion alloys were studied as well as amalgams prepared from these alloys.

The dispersion alloy inhibits growth less than the spherical alloy which in turn inhibits growth less than the fine cut alloy.

The results for amalgams prepared from the alloys are reversed. At an aging time of two hours, dispersion alloy amalgams inhibit growth more than spherical alloy amalgams and fine cut alloy amalgams.

Aging time of amalgams greatly influences the growth inhibition. Immediately after trituration growth is inhibited, but this inhibition is lost with aging. Different types of amalgams seem to lose growth inhibition at different rates.

Hg composition in the range of 48 to 52% seems to have little effect on growth inhibition.

It remains for clinical studies, which are concerned with the incidence of secondary caries associated with amalgams, to demonstrate that the age and nature of the amalgam are significant.

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