# Antibacterial properties of and element release from some dental amalgams

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Nine commercial dental amalgams were tested for antibacterial properties in vitro. A bactericidal test on salivary bacteria, a growth inhibition test on *Streptococcus mutans* OMZ 176, and a time-dependent bactericidal test on *Strep. mutans* were used. All amalgams displayed some antibacterial properties. Dispersalloy and Revalloy were strongly antibacterial in all tests; ANA 2000 and Sybraloy killed *Strep. mutans* but were less potent in the salivary test and in the growth inhibition experiments. The copper amalgams, Neo-Silbrin and Cupromuc, were the most active in the salivary test but less inhibitory in the growth curve experiments. Spheraloy, Indiloy, and Amalcap showed intermediate activity in the salivary bactericidal test but were relatively weak in the growth inhibition studies. Analysis of Hg, Ag, and Cu in media from the growth inhibition studies showed release of Hg from the copper amalgams and, particularly, from Revalloy; Indiloy gave off Ag, whereas Neo-Silbrin, Cupromuc, Sybraloy, and ANA 2000 released more Cu than the other brands. □ *Dental materials; microbiology;* Streptococcus mutans

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Dental caries is a microbial disease caused by the metabolic activities of plaque-associated bacteria, of which *Streptococcus mutans* is considered the most cariogenic (1). Conventional treatment of caries entails removal of carious dental hard tissue and insertion of a filling that in posterior teeth is most frequently made of amalgam. Amalgam fillings require replacement because of the recurrence of disease at the tooth/filling interface, and a high proportion of such fillings must be replaced within 10 years (2).

Owing to the content of bacteriotoxic elements (such as Hg, Ag, and Cu) in dental amalgams, one might surmise that the corrosive processes releasing these elements might inhibit bacterial activity and thereby prevent recurrence of caries. Indeed, antibacterial activity of copper amalgam was demonstrated by Miller as early as 1890 (3), and copper amalgams were recognized as having a cariostatic effect clinically. With the introduction of silver amalgams containing large amounts of copper and having various corrosive properties, the question may be posed whether some of these amalgams may have enhanced antibacterial properties.

The present study assessed the in vitro antibacterial properties of several conventional and copper-rich amalgams, with emphasis on their action on *Strep. mutans*. Three different test methods were used: this was done to assess and compare different methods in evaluations of dental amalgams' antibacterial activity. Moreover, an attempt was made to relate antibacterial activity to the release of mercury, copper, and silver.

## Materials and methods

### *Dental amalgams*

Table I lists the amalgams tested, their manufacturers, batch numbers (when available), and the elemental composition of the alloys. ANA 2000, Dispersalloy, Revalloy, and Sybraloy were included in all tests. The amalgams were prepared in accordance with the manufacturers' instructions, and test specimens of 0.2 ml volume and an approximate surface area of 200 $mm^2$ were used in all experiments. The codes listed in Table I will be used as abbreviations.

Table 1. Dental amalgams tested

| Brand | Manufacturer | Lot no. | Alloy composition, percentage | | | | | | | Ref. | Alloy type | Phase | Code |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ag | Sn | Cu | Zn | In | Cd | Hg | | | | |
| ANA 2000 | Nordiska Affineriet | 7-128 81.04 | 41.3 | 29.5 | 25.4 | 0.17 | | | 1.5 | 15 | Lathe-cut | 'Non-$\gamma_2$'* | ANA |
| Dispersalloy | Johnson & Johnson | 2093 | 69.6 | 17.7 | 11.8 | 0.7 | | | | 17 | Mixed | 'Non-$\gamma_2$' | DIS |
| Revalloy | SS White | 78 80 30 | 69.6 | 26.9 | 2.8 | 0.96 | | | | 15 | Lathe-cut | $\gamma_2$ | REV |
| Sybraloy | Kerr | BSO 3827014 | 39.9 | 30.2 | 29.9 | | | | | 17 | Spherical | 'Non-$\gamma_2$' | SYB |
| Amalcap | Ivoclar/ Vivadent | 19 12 78 1029 | 69.9 | 17.0 | 12.9 | | | | | 17 | Mixed | 'Non-$\gamma_2$' | AMA |
| Indiloy | Shofu | 217601 | 59.1 | 23.9 | 12.6 | | 4.3 | | | 17 | Spherical | 'Non-$\gamma_2$' | IND |
| Spheraloy | Kerr | 05 20 76 2049 | 70.0 | 25.6 | 4.2 | 0.24 | | | | 13 | Spherical | $\gamma_2$ | SPH |
| Cupromuc | Merz & Co. | 90301/1 | 0.4 | | 30.0 | 0.3 | | 0.6 | Bal† | 18‡ | Pre-mixed tablets | | CUP |
| Neo-Silbrin | Siccu-Gesellschaft | None given | 0.5 | | 3.13 | | | 1.5 | Bal† | 13 | Pre-mixed tablets | | NEO |

* 'Non-$\gamma_2$' is placed in quotation marks in recognition of the fact that small amounts of this phase may be present.
† Balance to 100%.
‡ Includes manufacturer's information.

*Bacteria*

*Strep. mutans* OMZ 176, originally from Dr. B. Guggenheim, was subcultured on blood agar plates every 4th week and kept at 4°C between transfers.

In some experiments, bacteria from whole saliva were used. Five milliliters of saliva, stimulated through chewing on Parafilm, was collected from one person.

*Bactericidal effect on salivary bacteria*

A modification of the procedure described by Gilbert et al. (4) was used: specimens of freshly mixed amalgam were added one of each to 1 ml of Todd–Hewitt broth (THB) (Oxoid) and incubated at 37°C for 24h. The broth was then decanted and filter-sterilized (0.45 µm, Millipore); this broth would contain the material's extract and will be referred to as THB-E. As diluent (DIL) in incubation mixtures and for dilution series, 0.1% yeast extract (Oxoid) in 0.5% sodium chloride in water was used. Human saliva was obtained through stimulation from chewing on Parafilm by one donor; 5 ml of this saliva was centrifuged (10,000 *g*, 15 min) and then filter-sterilized before use (SAL). Similarly collected whole saliva, diluted 1:5 in diluent, constituted the target bacterial suspension (WSD).

Reaction mixtures were set up as follows:

| | Control | Extract | Extract and saliva |
|---|---|---|---|
| WSD | 20 µl | 20 µl | 20 µl |
| DIL | 40 µl | 40 µl | |
| THB | 40 µl | | |
| THB-E | | 40 µl | 40 µl |
| SAL | | | 40 µl |

The reaction mixtures were incubated aerobically for 1 h at 37°C. Serial tenfold dilutions were then made and plated in triplicate on blood agar plates, which were incubated for 24 h at 37°C in a $CO_2$ atmosphere (BBL). The number of viable bacteria was counted and expressed as percentage of living organisms in the controls. For each material extract, the procedure was repeated at least three times.

*Growth inhibition of* Strep. mutans

*Strep. mutans* OMZ 176 was grown overnight in the partially defined medium (PDM) of Shiota (5). To each of several tubes with 4.5 ml of fresh PDM was added 0.5 ml of this culture and one specimen of freshly mixed (5 to 10 min after trituration) amalgam. Control mixtures were without amalgam. During incubation at 37°C in $CO_2$, optical density measurements at 540 nm were performed at 0, 4, and 24 h. The results were analyzed graphically (time versus $\log_{10} OD_{540}$); also, the percentage growth inhibition at 24 h was calculated to the nearest 5% for comparisons with analyses of released elements (see below).

*Analysis of Hg, Ag, and Cu content in growth medium*

On termination of the growth inhibition experiment at 24 h, 2 ml of each incubation mixture of PDM with suspended bacteria were removed and frozen at −20°C. After collection of all samples, they were solubilized in 10% nitric acid and analyzed for concentrations of Cu, Hg, and Ag by flameless atomic absorption spectrometry (conducted by Dr. P. E. Paus, Central Institute of Industrial Research, Oslo).

*Time-dependent bactericidal effect on* Strep. mutans

An overnight culture of *Strep. mutans* OMZ 176 was inoculated (0.5 ml to 4.5 ml) into fresh PDM and incubated for 3 h at 37°C in $CO_2$. Ten microliters of this actively growing culture was then added to 5 ml of a salt solution (SL) containing 0.1 g $CaCl_2$, 0.1 g $MgSO_4$, 0.5 g $K_2HPO_4$, 0.5 g $KH_2PO_4$, and 1.0 g NaCl to 1 l distilled water (6) autoclaved at 120°C for 20 min before use. After thorough mixing, 100 µl of this diluted bacterial suspension were transferred to either 10 ml of SL (control) or to 10 ml SL containing an extract of amalgam obtained by either of two means. In one series, specimens of freshly mixed (5 to 10 min after trituration) amalgam were placed directly into 10 ml of SL and incubated at 37°C for 24 h. The 10-ml supernatants from this series thus

contained extracted material from freshly made specimens, and each received 100 µl of diluted bacterial suspension. In the other series, the amalgam specimens were allowed to set at 37°C for 7 days before a 24-h extraction in 10 ml SL, the supernatant from which was incubated with the diluted bacterial suspension.

The diluted bacterial suspensions were incubated at room temperature. Aliquots were withdrawn at 0, 15, 30, 60, 90, and 120 min, plated in quadruplicate on blood agar plates, and incubated for 24 h at 37°C. The number of surviving bacteria in extract-containing mixtures was then counted and related graphically to the controls. Linear regression analyses of time curves versus $\log_{10}$ number of surviving cells were performed, and the slopes thus obtained were used for comparative purposes; the higher the slope value, the more potent the extract.

## Results

### *Bactericidal effect on salivary bacteria*

The amalgam extracts in THB showed a wide variation in their bactericidal effect on mixed salivary bacteria (Fig. 1). Compared with control values for bacterial survival, the bactericidal effect ranged from 2% (ANA) to 95.3% (CUP). The bactericidal potency of the extracts did not appear to be directly related to the Cu content of the amalgams, inasmuch as the two most Cu-rich silver amalgams (ANA and SYB) showed the least inhibition. Of the silver amalgams, however,

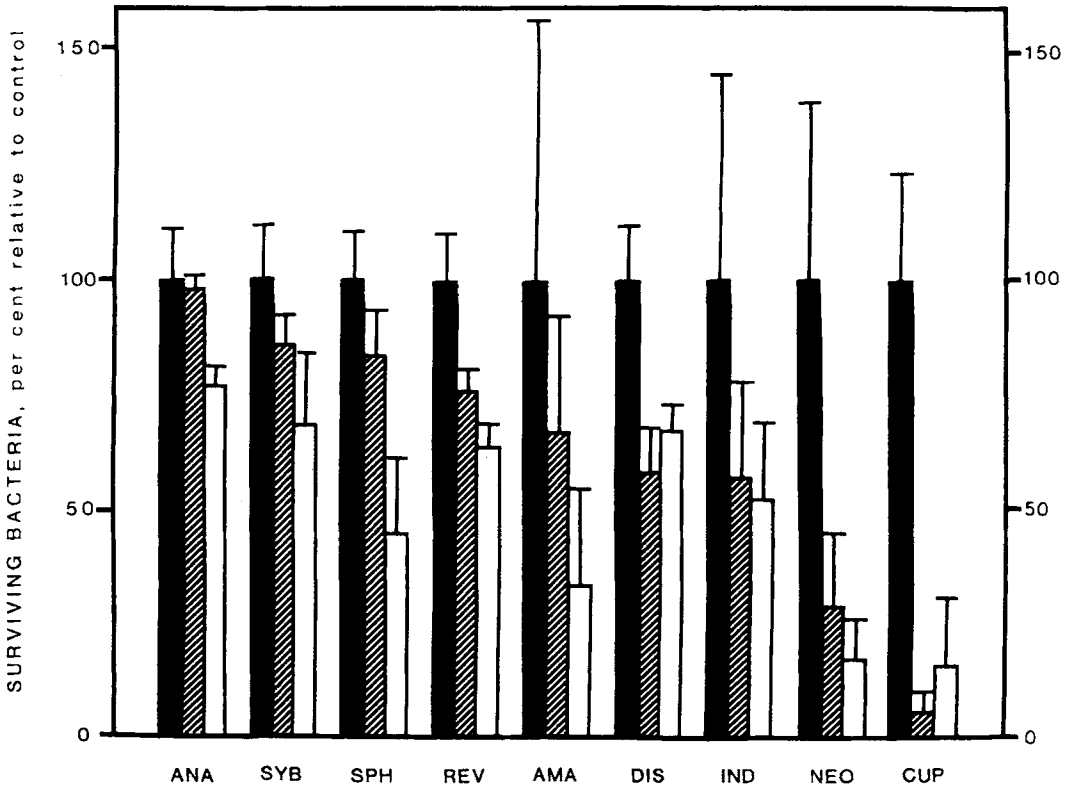


Fig. 1. Survival of salivary bacteria in the presence or absence of amalgam extract in Todd-Hewitt broth. Filled bars: controls with absence of extract; hatched bars: extract present; open bars: extract and saliva present. See text for details.

the intermediate Cu-containing (11–13%) brands (AMA, DIS and IND) showed the most pronounced effect, and the copper amalgams NEO and CUP were markedly the most bactericidal of all.

In most cases, the presence of diluted saliva filtrate potentiated the killing effect. In contrast, incubation with bacteria and saliva filtrate alone increased viable counts after 1 h incubation by 10.2% (data not illustrated).

*Growth inhibition*

Typical results from growth curve experiments with *Strep. mutans* in PDM in the presence of amalgam are illustrated in Fig. 2. The pattern of antibacterial potency differed somewhat from the effects observed in the killing experiments with salivary bacteria. The amalgams could be placed in three groups depending on the mode and extent of antibacterial activity: total inhibition of growth was observed with REV and DIS; delayed growth was observed with CUP, NEO, and, to a lesser extent, with ANA and IND; whereas AMA, SYB, and SPH showed only a weak inhibition over the time range tested.

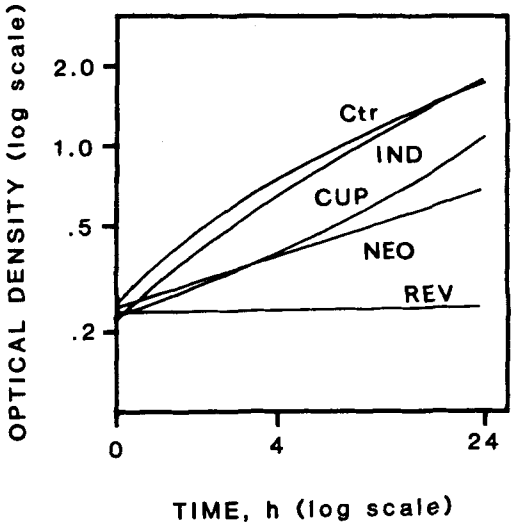


Fig. 2. Growth curves of *Streptococcus mutans* OMZ 176 in the absence (Ctr) or presence of amalgam test specimens.

*Element analysis*

The results from atomic absorption measurements of mixtures from the end of the growth curve experiments are shown in Table 2. IND released markedly more Ag to the medium than other brands; the copper amalgams, especially NEO, gave off more Cu, but SYB and ANA also released Cu to the medium. Mercury was found in highest amounts in media with REV. CUP, NEO, and ANA followed, but all amalgams released more Hg than the other two elements.

The Hg-releasing amalgams REV, CUP, and NEO also showed growth inhibition; however, in the case of DIS, growth inhibition was complete even in the absence of conspicuous amounts of any of the three elements in the media.

*Time-dependent bactericidal effect on* Strep. mutans

The presence of amalgam extract in salt solutions always increased the rate of killing of *Strep. mutans* relative to control incubations in salt solutions alone. An example of the regression plots obtained with this method is illustrated in Fig. 3. Table 3 lists the slopes of regression curves and the correlation coefficients for the individual plots for each material extract. In terms of rate of kill, most extracts killed 90% of the bacteria in 23–47 min. Extracts from 1-week-old specimens appeared as effective as extracts from fresh specimens. The data obtained were not indicative of great differences among the materials in their antibacterial activity in this system.

## Discussion

All amalgams tested displayed some antibacterial activity. This could be expected from previous studies of this phenomenon (7–11). Depending on the test system used, the brands showed various extents of bacteriostatic/-cidal activity, but with some trends indicating systematic differences among the amalgams. Dispersalloy and

Table 2. Element analysis of medium and growth inhibition of *Strep. mutans* after 24 h in partially defined medium

| Amalgam | Experiment no. | Metal content of PDM, μg/ml | | | Growth inhibition, (%) at 24 h |
|---|---|---|---|---|---|
| | | Ag | Cu | Hg | |
| ANA | 1 | 0.09 | 1.03 | 100 | 15 |
| DIS | 1 | 0.07 | 0.53 | 77 | 100 |
| | 2 | 0.21 | 0.61 | 42 | 100 |
| REV | 1 | 0.04 | 0.32 | 410 | 100 |
| | 2 | 0.03 | 0.17 | 139 | 100 |
| SYB | 1 | 0.15 | 3.11 | 21 | 10 |
| | 2 | 0.18 | 2.45 | 18 | 5 |
| SPH | 1 | 0.05 | 0.43 | 13 | 20 |
| IND | 1 | 2.00 | 0.74 | 59 | 5 |
| AMA | 1 | 0.07 | 0.76 | 19 | 15 |
| NEO | 1 | 0.03 | 13.84 | 190 | 60 |
| | 2 | 0.02 | 13.49 | 23 | 10 |
| CUP | 1 | 0.03 | 2.31 | 114 | 40 |
| | 2 | 0.03 | 1.41 | 23 | 40 |
| Control | $n = 3$ | 0.03 ± 0.00 | 0.40 ± 0.08 | <1 | 0 |

Revalloy were strongly antibacterial in all three test systems; ANA 2000 and Sybraloy showed marked bactericidal activity in the bactericidal test on *Strep. mutans* but were clearly less toxic in the salivary killing test and in the growth inhibition experiments.

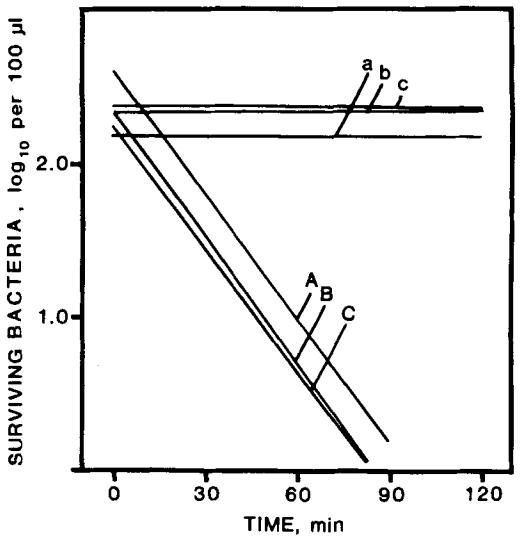


Fig. 3. Regression plots of *Streptococcus mutans* OMZ 176 surviving in the presence or absence of amalgam extract in salt solution. Revalloy. A = extract from freshly made specimens; B and C = extracts from 1-week-old specimens; a, b, and c = corresponding controls.

The copper amalgams, Neo-Silbrin and Cupromuc, were the most bactericidal in the bactericidal test on salivary bacteria but were less potent in growth inhibition. Spheraloy, Indiloy, and Amalcap showed intermediate activity in the bactericidal test on salivary bacteria but were relatively weak in the growth inhibition studies.

It was a conspicuous feature of the present results that they were strongly dependent on the test method used. Theoretically, the growth inhibition experiment may be the most complex and least analyzable of the methods, combining bacteriostatic and bactericidal properties and a continuous and uncontrolled degradation/dissolution of potentially inhibitory components from the test specimens. On the other hand, such is the situation in the mouth. The salivary bactericidal test makes use of mixed salivary bacteria as target organisms; these are difficult to standardize and will vary in susceptibility to a given antibacterial compound. However, this test is reproducible, as judged by relatively small standard deviations in parallel experiments, and it pinpoints bactericidal properties of the material tested. Moreover, it makes use of material extracts that are defined in terms of method of preparation. So does the time-dependent bactericidal test of *Strep. mutans*, which is

Table 3. Time versus surviving numbers of *Strep. mutans* in the presence of amalgam extracts: slopes of regression curves and time to kill 90% of cells

| Brand | Extraction period | Correlation coefficient* | Slope | Time to kill 90%, min |
|---|---|---|---|---|
| ANA | 1 day | −0.96 | $3 \times 10^{-2}$ | 33 |
| | 1 day | −0.96 | $4 \times 10^{-2}$ | 23 |
| | 1 week | −0.90 | $1 \times 10^{-2}$ | 85 |
| | 1 week | −0.94 | $2 \times 10^{-2}$ | 31 |
| DIS | 1 day | −0.92 | $3 \times 10^{-2}$ | 39 |
| | 1 day | −0.97 | $0.7 \times 10^{-2}$ | 152 |
| | 1 week | −0.94 | $2 \times 10^{-2}$ | 41 |
| | 1 week | −0.94 | $2 \times 10^{-2}$ | 47 |
| REV | 1 day | −0.96 | $3 \times 10^{-2}$ | 37 |
| | 1 week | −0.96 | $3 \times 10^{-2}$ | 38 |
| | 1 week | −0.95 | $3 \times 10^{-2}$ | 37 |
| SYB | 1 day | −0.98 | $3 \times 10^{-2}$ | 31 |
| | 1 day | −0.90 | $4 \times 10^{-2}$ | 29 |
| | 1 week | −0.83 | $3 \times 10^{-2}$ | 35 |
| | 1 week | −0.90 | $0.7 \times 10^{-2}$ | 134 |
| Control ($n$ = 15) | | −0.92 to +0.66 | $1 \times 10^{-3}$ to $4 \times 10^{-5}$ | |

* The number of individual points used in each calculation ranged from 4 to 6.

well standardized in terms of material extract, bacterial target, and antibacterial property assessed.

The main difficulties in assessing (bacterio)toxic properties of dental amalgams lie in the method of extraction of active ingredients and in the assessment of the form in which the active components reach the target cell. In the present work, extraction occurred a) in Todd–Hewitt broth, a complex bacteriological medium; b) in a low-molecular, partially defined bacteriological medium, and c) in a salt solution of known composition. The resultant extract may of course vary in composition and chemical form among these media. Thus, results from different antibacterial tests on amalgams are hardly comparable unless the methods of extraction are identical.

Furthermore, the conditions for incubation during actual antibacterial action may influence the results. For instance, it might appear that the salivary killing test and the time-dependent *Strep. mutans* killing test are similar. However, in the case of ANA 2000 and Sybraloy, they were strongly bactericidal in the *Strep. mutans* test but showed very little activity in the salivary killing test. The influence of the media was indicated by the experiments with saliva filtrate, which indicated a potentiating effect on the antibacterial activity by the saliva. The present experiments do not, however, furnish enough data to distinguish among the experimental variables that may have caused this differential behavior of the materials.

It would seem natural to ascribe the antibacterial activity of amalgams to release of metals with toxic properties, which is why the element analysis was carried out in the growth inhibition study. The release of Hg from the specimens was substantial for all materials. The large amounts of Hg given off by Revalloy were somewhat surprising, inasmuch as a previous study (12) did not indicate exceptionally high release values of Hg from this amalgam. Moreover, reported high values of Hg from Sybraloy (12) were not matched in the present study. Marked differences in conditions for extraction (bacteriological medium versus artificial saliva) may account for the observed differences.

On the other hand, a large release of Cu from Sybraloy was evident in both studies; moreover, Cu release patterns from Dispersalloy, Sybraloy, and Neo-Silbrin matched those in a study by Gjerdet & Berge (13), in which physiological salt solution was used for extraction. However, these authors observed large release of Cu from Indiloy in their system; this was not the case in the present study. Indiloy, on the other hand, gave off Ag to a much larger extent than the other amalgams in the present study. It should be noted that the results varied considerably in duplicate experiments. Although this might have been controlled by increasing the number of parallels, it testifies to the observed variability in element release from dental amalgams (e.g. 12, 13).

Attempts to relate antibacterial activity to phase structure, content, or release of metals meet with some difficulties. The two most potent amalgams, Revalloy and Dispersalloy, are of widely different composition and chemistry. Neither releases Ag or Cu to any great extent, but Revalloy may apparently give off much Hg (as shown in this study), whereas Dispersalloy may release Zn, which also acts antibacterially (13, 14). Thus, the strong activity of these two materials may depend on two different mechanisms.

The large amounts of Cu released from Neo-Silbrin and Cupromuc may account for the strong activity of these two materials; however, release of Cd (13) might also be considered.

At the other extreme, the copper-rich alloys ANA 2000 and Sybraloy also released some Cu from their amalgams. This was apparently not sufficient for substantial activity in the growth inhibition assay or in the salivary killing test, but may have caused the effect of these materials in the possibly more sensitive *Strep. mutans* killing test.

Why Spheraloy should show poorer activity than the compositionally similar Revalloy is difficult to explain, but the present observation of differences in Hg release and possible differences in Zn release may account for this phenomenon. Indiloy and Amalcap, which have compositions similar to that of Dispersalloy, may owe their poorer antibacterial activity to the relative absence of Zn in these alloys (15). Tin may be another element to consider as a source of variation among amalgams in their antibacterial properties, but Leirskar (16) could not detect Sn in culture media from incubations with a conventional silver amalgam.

Some tentative conclusions may be drawn from the present work. While results varied depending on the method, some consistency was observed. The bactericidal test on *Strep. mutans* appeared sensitive in detecting antibacterial properties of Disperaslloy and Revalloy, alloys which were potent in all tests. Because the bactericidal test is the best defined and best controlled of the tests applied, it may be considered in future assessments of amalgams' antibacterial properties. Moreover, the use of *Strep. mutans* as target organism makes this test a natural corollary to in vivo (17, 18) and in vitro (19) tests of plaque formation with this organism.

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