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ANALYSES OF DENTAL PULP IN RESTORED TEETH

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Abstract

Restored teeth were extracted from test animals at four time intervals (1 hour, 1 day, 1 week, and 3 months) following amalgam insertion. Extracted teeth were frozen in liquid nitrogen, cryo-fractured so as to expose the pulps and then freeze-dried. Pulps were analyzed for mercury content by energy dispersive spectrometry (EDS) and atomic absorption spectrophotometry (AAS). Mercury levels appeared below the detection limits of EDS but could be detected by AAS which showed the highest readings seven days after amalgam insertion.

Key Words: Scanning electron microscopy, energy dispersive and atomic absorption spectroscopies, mercury, amalgam, dental pulp, freeze-dried.

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Introduction

During the past two decades, energy dispersive spectrometry (EDS) has been an important analytical tool of dental research in studies concerning amalgam phase distribution (Herzog et al. 1982; Partschefeld et al. 1983; Mahler and Adey 1977, 1984; Mahler et al. 1973, 1976; Malhotra and Asgar 1977; Edie et al. 1978), amalgam corrosion products (Otani et al. 1973; Silness et al. 1979), the movements of metallic ions from implants into soft tissue (Eley 1980, 1982; Eley and Garrett 1983; Harrison et al. 1977), and the penetration of metallic ions from amalgam cavity preparations into dentinal tubules (van der Linden and van Aken 1973; Domagala et al. 1968; Halse 1975; Wei and Ingram 1969; Kurosaki and Fusayama 1970, 1973; Kato 1969). Results of the amalgam metal ion penetration studies vary, but most agree that zinc and tin ions will move from amalgam restorations into dentinal tubules. At least two studies (Kato 1969; Massler and Barber 1953) conclude, however, that all amalgam elements, viz., mercury, silver, copper, tin, and zinc, will penetrate into dentin, at least to some degree. The underlying assumption of these studies appears to be that if amalgam elements penetrate into the dentinal tubules, they will also penetrate into the pulp since the odontoblastic processes extend from the pulp into the dentinal tubules. This assumption may or may not be valid. In any case, it is uncertain whether or not the amalgam elements that can penetrate only short distances into the dentin will make their way into the pulp.

Of the five elements found in amalgam, mercury has been of particular concern. It is known to be a toxic substance and has the potential of causing direct toxic effects, sensitization, neuropathies, and allergic reactions. Studies investigating mercury exposure from dental amalgam have shown that mercury is released during the insertion of amalgam (Frykholm 1957; Mayer 1980), during the removal of amalgam (Cutright et al. 1973; Cooley and Barkmeier 1978; Brune et al. 1980; Reinhardt et al. 1983a, 1983b; Richards and Warren 1985), and during the life of an amalgam (Jolly et al. 1986; Svare et al. 1981; Abraham et al. 1984; Ott et al. 1984; Patterson et al. 1985; Smales

and Gerke 1986). Most of the current research, however, relies on methods that measure the mercury content of blood, urine, and exhaled air without focusing on one of the main points of contact, the pulp chamber. Although some investigators (Mjor et al. 1977; Wennberg et al. 1983; Skogedal and Mjor 1979; Swerdlow and Standley 1962; Mjor and Tronstad 1972), have reported the general reaction of dental pulp to cavity preparations using histological or clinical evaluations, they have not reported the penetration of elements from amalgam, e.g., mercury, into the pulp. However, this has been reported by two studies (Soremark et al. 1968; Moller 1978). Accordingly, it is the purpose of this study to determine the mercury content of pulp tissue in teeth restored with amalgam using EDS and compare results to those obtained with atomic absorption spectrophotometry (AAS), which was previously used in the Moller (1978) study.

Materials and Methods

Eight young, adult, mongrel, long-nosed dogs were obtained from random sources, immunized, and quarantined for 30 days until they attained a stable condition. Dogs were chosen for this study because they have a dentition and overall mouth size suitable for dental research (Navia 1977). Tooth anatomy is similar enough so that dogs may serve as appropriate models for humans (Forssell-Ahlberg et al. 1975). Furthermore, many dental materials research projects have used dogs as experimental animals including the classic amalgam study by Frykholm (1957).

Prior to amalgam insertion, test animals were given subcutaneous injections of atropine sulfate (0.1 mg/kg), as an anticholinergic, preanesthetic medication. Sodium pentabarbitol (30 mg/kg) was administered intravenously to anesthetize the animals. During tooth preparation and restoration, test animals were intubated and their mouths were held open with metal restraining clamps.

Dispersalloy dental amalgam was condensed in twelve healthy teeth of each of the four dogs. Class V restorations were deeply placed (within 1 mm of the pulp) in one molar, one premolar and one incisor in each quadrant of each dog's mouth. All animals were given two to three doses of analgesic, viz., pentazocine (3.3 mg/kg), following each operation.

Teeth were removed from animals one hour, 24 hours, one week, and three months after amalgam insertion. The three month samples were designed to show long term effects, since this is generally considered an adequate time period in pulp studies (Mjor 1980). Teeth were extracted one quadrant at a time and unaffected teeth adjacent to teeth with fillings were extracted during each operation to act as controls. Dogs were anesthetized according to the above procedures except that prior to the first extraction, each was given an IM injection of acetyl promazine (1 mg/kg) to keep them sedated if they began to recover from the anesthesia given one hour previously.

Freshly extracted teeth were immersed in liquid nitrogen and kept frozen in a Dewar flask until operatory procedures were completed. Frozen tooth samples were removed from the liquid nitrogen then cryo-fractured using a chilled microtome blade and mallet. Teeth were quite brittle when frozen and cracked longitudinally with only moderate tapping. Figure 1 shows the amalgam, dentin and pulp of a fractured tooth. The cryo-fractured teeth were placed immediately in a Virtis 10-010 lyophilizer for freeze-drying. Samples warmed slowly to -30°C and remained at that temperature, under vacuum for 48 hours.

Samples were attached to carbon stubs with colloidal graphite paste then coated with 30 nm of carbon in a Denton DV-515 vacuum evaporator. This was done to reduce the anticipated charging problems without interfering with the signal reception from the x-ray detector. Teeth were mounted so that the fractured surfaces were facing upward, parallel to the plane of the stub surface. Samples were inserted in the chamber of a JEOL JSM-840II SEM and exposed to an electron beam of 25 kV. X-rays excited from the surface were collected with an EDAX Econ IV detector and analyzed in the quantitative mode with an EDAX PV9100 analyzer. Mercuric sulfide was used as a standard for mercury. Absorbed current was measured at the beginning of each analysis to determine the beam current factor which was entered into the analyzer as one of the parameters for analysis. Three EDS readings of 200 live seconds each were recorded from all pulp samples. Mercury was analyzed using the $L\alpha_1$ line at 9.987 keV; an isolated peak without overlaps.

After EDS analysis, pulps were cut from teeth using sterile scalpel blades and weighed. Following this, pulps were digested in a solution of nitric acid (sp. gr. 1.42) and stannous chloride (10%) in 0.5 N sulfuric acid to prepare for atomic absorption spectrophotometry. This is similar to the technique described by Gaffin and Hornung (1977). The largest pulps were selected from a tooth in each quadrant for AAS analysis. In this way, results of the two analytical techniques could be compared.

Results

The results of the EDS analysis are shown in Table 1. Of the 180 EDS readings taken from freeze-dried pulps of the first four animals, only 12 indicated the presence of mercury. Statistical errors accompanying the 12 EDS mercury readings ranged from 25.85% to 654.95%. In general, these large errors indicate that the readings are not in the range of acceptability.

When the same teeth (four from each group) were analyzed using AAS, however, minute quantities of mercury were detected. If these data are compared using the Pearson product-moment correlation coefficient (r), a value of -0.0361 is obtained. Accordingly, there appears to be no correlation between the EDS results and the results of the more sensitive technique, AAS. Furthermore, many of the AAS readings were lower than the theoretical EDS concentration detection

Table 1. Mercury concentrations (μmg/g) of pulp in teeth restored with amalgam analyzed by two methods. Each figure in the EDS column is the average of three readings. The results of the AAS analysis on the same pulp is shown in the right column of each section. Only pulps weighing more than 5 μmg (dry weight) were analyzed by AAS. Controls were from intact teeth adjacent to restored teeth. No correlation was found between results of EDS and AAS analyses.

1 hour		24 hours		7 days		3 months		Control	
EDS	AAS	EDS	AAS	EDS	AAS	EDS	AAS	EDS	AAS
0	51.0	0	16.0	0	33.0	0	8.3	1800	19.4
0	3.1	0	13.0	0	40.0	0	9.0	0	5.0
0	2.1	0	56.0	0	40.8	0	5.0	0	5.0
200	49.0	0	13.0	0	167.0	0	10.8	0	4.2
0		0		0		0		0	
1400		4300		0		0		0	
0		0		0		170		0	
0		0		0		0		0	
0		0		0		0		0	
0		300		0		0		0	
0		0		1700		0		0	
0		0		0		0		0	
$\bar{X}_e$ 133	$\bar{X}_a$ 30.7	$\bar{X}_e$ 383	$\bar{X}_a$ 24.5	$\bar{X}_e$ 141	$\bar{X}_a$ 70.2	$\bar{X}_e$ 14.2	$\bar{X}_a$ 8.3	$\bar{X}_e$ 150	$\bar{X}_a$ 8.4
σ_e 403	σ_a 23.3	σ_e 1236	σ_a 21.0	σ_e 490	σ_a 64.5	σ_e 49.0	σ_a 2.4	σ_e 519	σ_a 7.3
n:12	n:4	n:12	n:4	n:12	n:4	n:12	n:4	n:12	n:4

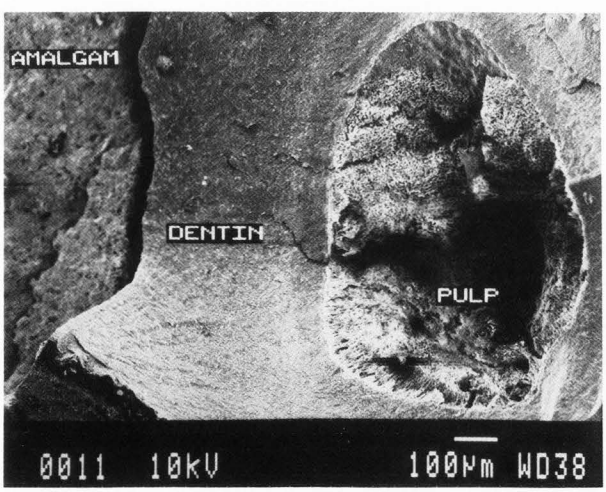

Figure 1. Scanning electron micrograph (secondary electron image) of a cryo-fractured tooth showing the amalgam restoration, dentin, and exposed pulp. Sample was freeze-dried and coated with 40 nm of gold.

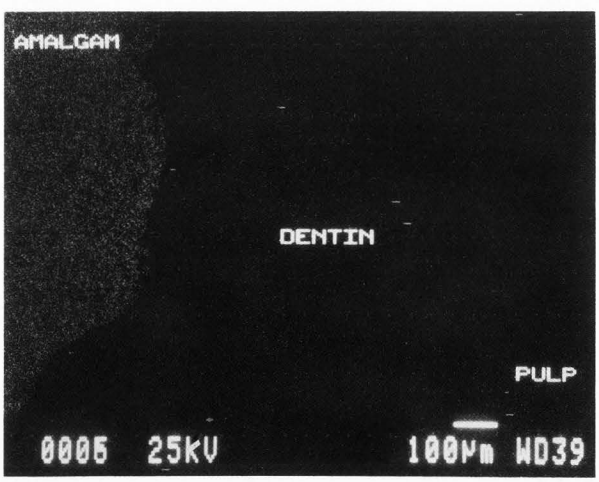

Figure 2. Area map with the window set to illuminate areas with mercury content. The amalgam area is well-defined but the dentin and pulp regions are not. From the same location as Figure 1. Sample was freeze-dried and coated with 20 nm of carbon.

limit (C_{DL}) for mercury, 9.0 μmg/g. The detection limit was calculated using the Ziebold equation with the Ryder correction (Barbi 1980) as follows:

$$C_{DL} = 2\left[\frac{3.29a}{\sqrt{n\tau I_o R}}\right] \quad (1)$$

where

- a = factor relating composition and intensity from the Ziebold-Ogilvie (1964) expression, equal to 1 in this case since interelement effects were minimal.
- n = number of measurements, 3 in this case
- τ = analysis time, 200 live seconds
- I_o = net count rate on a pure element standard, 106.209 CPS
- R = peak-to-background ratio on a pure element standard (net peak counts/background), 8.734

I_o and R were adjusted to account for the fact that the standard was not pure (88.22% Hg). The calculated C_{DL} is theoretical and the actual detection limit may be higher. EDS area maps set with a window on the $L\alpha_1$ mercury peak (Figure 2) also failed to reveal any pulpal mercury content.

Since it became obvious that the EDS analysis was not providing useful data, the study involving eight test animals was completed with AAS only. AAS analysis of pulps in teeth restored with amalgam showed a significant difference ($p<0.01$) in pulpal mercury concentrations among restored teeth and controls (Table 2). A single classification Anova with multiple treatments was used to analyze the data. Results show that mercury levels from pulps in restored teeth were significantly higher ($p<0.01$) after seven days than among control groups. These results are similar to those of Moller (1978) involving AAS analyses of pulps from human teeth restored with amalgam except that in the previous study, mercury levels were higher than controls in both the seven day and 24 hour samples, as well. In the present study, the mercury mean shown in Table 2 for the 24 hour samples was three times that of the control group but due to the small sample size and standard deviation, these results could not be shown to be significantly different.

Conclusions

From the above results it appears that small amounts of mercury will penetrate the pulp of unlined cavities restored with amalgam. It does so at levels that are best detected using cold cathode atomic absorption spectrophotometry. The reader should note that no liners were used in this study and mercury is less likely to penetrate into the pulp in clinical situations where liners are employed. Also, the dentin below carious lesions is often less permeable than healthy dentin due to the formation of sclerotic and secondary dentin as a result of caries. However, more study is recommended to determine if mercury from amalgam restorations will penetrate to the pulp through cavity liners and traumatized dentin.

Table 2. Mercury concentrations (umg/g) determined by AAS in pulp tissue of test animals whose teeth were restored with dental amalgam. The time listed in each case represents the amount of time from amalgam insertion to tooth extraction. Controls were intact teeth adjacent to restored teeth.

TIME	N	Mean	S.D.	RANGE
1 Hr.	8	24.99	33.00	1.0 - 100.0
24 Hrs.	8	26.25	32.51	2.3 - 95.0
7 Days	8	92.47	67.69	33.0 - 216.0
3 Mos.	8	8.93	1.90	5.0 - 11.0
Control	8	7.81	6.80	1.1 - 19.4

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Discussion with Reviewers

B. M. Eley: How do you think that mercury is released from amalgam during the first 24 hours? Why does the mercury concentration, albeit small, rise during the first 7 days and then fall to control levels by 3 months?
Authors: The amalgamation reaction takes several days before it approaches equilibrium. After about seven days following trituration, the mercury is bound up in amalgam crystals but, until this occurs free mercury can be absorbed by surrounding tissues, in this case the dentinal tubules. The movement of mercury through the dentin has been described herein as a diffusion gradient, with the maximum amount of mercury reaching the pulp seven days after amalgam insertion. After seven days essentially all of the free mercury will have been incorporated into the amalgam crystalline structure or absorbed by the body. This is why the three month readings show mercury levels equivalent to those of the controls.

B. M. Eley: Since mercury can be released from amalgam by electrolytic corrosion during its functional life and the overall loss of mercury from amalgam over a long period may be significant do you think longer time periods of 6,12,24,36 and 48 months might be useful in the future?
Authors: Although it is true that mercury can be released from amalgam by electrolytic corrosion, amounts released would be small in comparison to the amount bound in the amalgam product. Once it has set, dental amalgam is a very stable material and corrosion rates are relatively low. To measure mercury release from amalgam as a result of electrolytic corrosion, it would be important to take readings over long periods of time, as you suggest, in carefully controlled studies with large sample sizes. The amounts detected, however, would not be as great as those recorded from tissue shortly after amalgam insertion.

B. M. Eley: As the corrosion behaviour between conventional and high copper amalgams differ would a comparative study of the two types be useful?
Authors: Corrosion rates will vary with amalgam composition, but in terms of mercury exposure to pulp tissue, a far greater amount occurs shortly after insertion than during subsequent corrosion episodes.

B. M. Eley: In the clinical situation cavities are cut in carious teeth. Caries results in the formation of sclerotic and secondary dentine which is less permeable. Also all cavities are lined. For these reasons should the effect of mercury release from amalgam on the pulp be compared on lined and unlined cavities?
Authors: The experiment described in this paper represents an extreme case where amalgam restorations were deeply placed without liners or bases. It seems clear that mercury will penetrate into the pulp under these circumstances. Further study to test the ameliorating effects of liners on mercury penetration is highly recommended.

B. M. Eley: Mercury is a highly volatile element. Do you think that exposure of the pulp tissue to the electron beam during EDS prior to mercury measurement by AAS may have reduced the amount detected?
Authors: It seems likely that exposure to the electron beam would have reduced the mercury content of the samples in this experiment. Care was taken to analyze several small areas of each pulp with as little beam exposure as possible, thereby minimizing this problem. However, future studies should be conducted with AAS alone.

B. M. Eley: EDS is a good technique for detecting elements in deposits in biological tissues which are visible by electron microscopy because the electron beam can be accurately focused on the deposit to reduce background effects. In this study no deposits were visible in the pulp and no mercury was revealed by X-ray mapping. In view of this do you think that EDS was a reliable technique to use?
Authors: Although EDS has been employed in several previous studies to detect mercury in dentin below amalgam fillings, the results of this study show that it is not a reliable technique for measuring mercury from amalgam in pulp tissue.

B. M. Eley: Quantification in EDS is difficult due to multiple compensations having to be made for peak overlaps, peak interferences and background noise. Following this study do you now feel that EDS is an unreliable technique to quantify small amounts of elements in biological tissues?
Authors: While EDS may be useful in some biological applications, its usefulness in detecting minute amounts of selected elements in soft tissues appears to be limited.

S. H. Ashrafi: What is the impact of your study on clinical dentistry?
Authors: The results of this study have shown that mercury will penetrate into the pulp of teeth restored with amalgam. Mercury has known cytotoxic effects and clearly this is an undesirable situation. The findings underscore the need for dentists to provide protective barriers against pulpal exposure to mercury. Currently used liners and bases may provide this protection but not all of these materials have been thoroughly tested for their mercury containment capabilities.