Abnormal reaction to subgingivally placed dental amalgam studied by transmission electron microscopy and microprobe analysis. Case report

Graham Ellender, BDS, MDSc, PhD, LDSRCS(Eng)*

Abstract

An abnormal clinical response to dental amalgam is reported. The patient presented with a generalized burning sensation having a focus of pain in a region approximating a subgingivally placed dental amalgam restoration. Replacement of the amalgam restoration with glass ionomer cement relieved the pain and burning. Gingival tissue associated with the amalgam, excised to render the new restoration supragingivally, displayed ultrastructural changes in both the epithelium and connective tissue, with mercury demonstrated in the connective tissue and the epithelium by electron microprobe analysis. The significance of the presence of mercury and absence of the other constituents of dental amalgam are discussed.

(Received for publication October 1985. Accepted June 1986.)

Introduction

The following case of chronic pain and burning is reported as an example of an unusual response to dental amalgam. Tissue which was surgically removed from around a restoration at the focus of the pain was examined by conventional transmission electron microscopy and by energy dispersive X-ray analysis in the transmission mode.

Clinical history

A male Caucasian, 71 years old, was referred to the author by a specialist periodontist with the provisional diagnosis of galvanism arising from a subgingivally placed dental amalgam. The patient presented with generalized pain and burning in the mouth with a focus in the lower-right third molar region. The pain and burning which had been present for eighteen months had started approximately two months after the placement of some dental amalgam restorations. Since the onset of the pain the patient had been dentally examined on several occasions. Pulpal and periodontal causes were discounted. During the course of one of these examinations referral for chronic pain counselling was proposed but was rejected by the patient. The patient was referred in due course to the Periodontics Clinic for routine scaling, and during this appointment he broached the subject of his discomfort with the attending Periodontal Specialist. Medical history of the case revealed no relevant information.

On examination by the author, several heavily restored teeth were present and some of these had subgingival extensions. Of note was a distal amalgam restoration in 48, which extended 4 mm subgingivally and had a gross overhang. This tooth was vital and did not respond unduly to dry ice. No pulpal abnormality was suspected. The gingival tissue associated with this distal restoration was hyperplastic and could be easily displaced without eliciting bleeding to reveal the pitted surface of the amalgam restoration. At the first visit, the surface of the restoration was coated with a thin coat of varnish.† This relieved the symptoms for a few days. At an appointment two weeks later the tissue forming the distal wall of the pocket was excised to render the restoration margins supragingival.

*Senior Lecturer, Department of Preventive and Community Dentistry, University of Melbourne.

†**Fuji varnish.** G-C Dental Industrial Corporation, Tokyo, Japan.

The existing restoration was then replaced with a contoured zinc oxide eugenol temporary restoration. Regional block anaesthesia was employed throughout.

Materials and methods

The excised tissue was immediately divided into representative block of 1 mm slices. Some blocks were fixed in 2.5 per cent glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.3) at room temperature; and others were quenched in liquid nitrogen without cryoprotection.

Tissue fixed in glutaraldehyde was processed for conventional TEM using established routines (with post fixation in osmium tetroxide and infiltration with Spurr's resin). Thick sections (1 μm) were obtained and stained with toluidine blue for optical examination and for block orientation. Ultra-thin sections (~80 nm) of tissue were mounted on copper grids and stained with lead and uranium.

Tissue which had been quenched in liquid nitrogen was freeze substituted with Spurr's resin and semi-thin sections (0.25 μm) cut with a diamond knife onto a trough of ethylene glycol. Sections were collected on pioloform coated beryllium grids and vacuum coated with spectrographically pure graphite.

Section for TEM and microprobe analysis were examined in a **Philips EM300**‡ equipped with a goniometer, minilens, **Edax detector** and **Edax 9100** hardware.§ Those for TEM were examined by a conventional mode, and those for microprobe analysis were examined using standardized lens conditions and tilt angle (42-45°) with the minilens activated. Raw spectra were obtained with a count of 200 seconds live time and at count rates in the order of 1000 cps. Spectra were stored on floppy discs for subsequent study and processing.

Results

1. Clinical

Two weeks after the excision and placement of the temporary restoration the focus of pain in the lower-right third molar region had disappeared. Slight discomfort and burning was then apparent in the upper left first molar region associated with another subgingivally placed dental amalgam restoration. In this instance replacement of the restoration with zinc oxide and eugenol cement without surgical pocket elimination was clinically indicated. At review examination two weeks later all pain had gone. Subsequently the temporary restorations were replaced with glass ionomer cement. At follow-up, six months, 18 and 30 months later, there had been no recurrence of pain, burning or discomfort.

2. Biopsy

Optical microscopy of the tissue removed at biopsy indicated that the pocket was lined with epithelium. Ultrastructural examination revealed changes both in the gingival epithelium and the underlying connective tissue. The epithelium displayed an increase in the width of the intercellular spaces. The normally close contact between cells of the sulcular epithelium was increased (Fig. 1). Slight keratinization was present. In the prickle cell layer there was an increase in the number of free ribosomes, and in the germinal layer some nuclear membranes displayed complex invaginations (Fig. 2). In a few instances nuclear vacuolation was present (Fig. 3). The epithelial basement membrane was often separated from the germinal layer, and appeared incomplete (Fig. 4) and lacked anchoring filaments. Connective tissue elements were perturbed in the superficial layer of connective tissue. Collagen in this region tended to be disordered (Fig. 5). Fibroblasts showed signs of cellular toxicity characterized by dilated and poorly organized rough endoplasmic reticulum (Fig. 6) and scant irregular ribosomes. Mitochondria exhibited swelling and enlargement, and abnormal structural forms. Few inflammatory cells were identified in the survey and ultrathin sections.

Microprobe analysis showed the tissue to contain mercury in components of both the connective tissue and the epithelium. The connective tissue matrix consistently and clearly displayed X-ray spectral peaks of mercury. Mercury was characterized in the raw data by distinct Hg L-α and Hg L-β peaks at 9.99 keV and 11.82 keV respectively (Fig. 7) and displayed significant peak to background ratios which contrasted with the background support film. Often the Hg M-α (2.31 keV) was displayed compounded with the S K-α peak (2.31 keV (Fig. 8). Silver as Ag L-α and tin as Sn L-α were not obvious. Cells identified as fibroblasts contained distinct and diffuse mercury (Fig. 9), but no peaks for silver or tin. Regions of extracellular space were noteworthy in their absence of strong peaks. However, traces of sulphur, chlorine, calcium were all identified and occasionally tin as Sn L-α (Fig. 10). Silver was never detected. Epithelium was variable in its content of mercury.

‡Philips, Eindhoven, The Netherlands.
§EDAX, Prairie View, Illinois, USA.

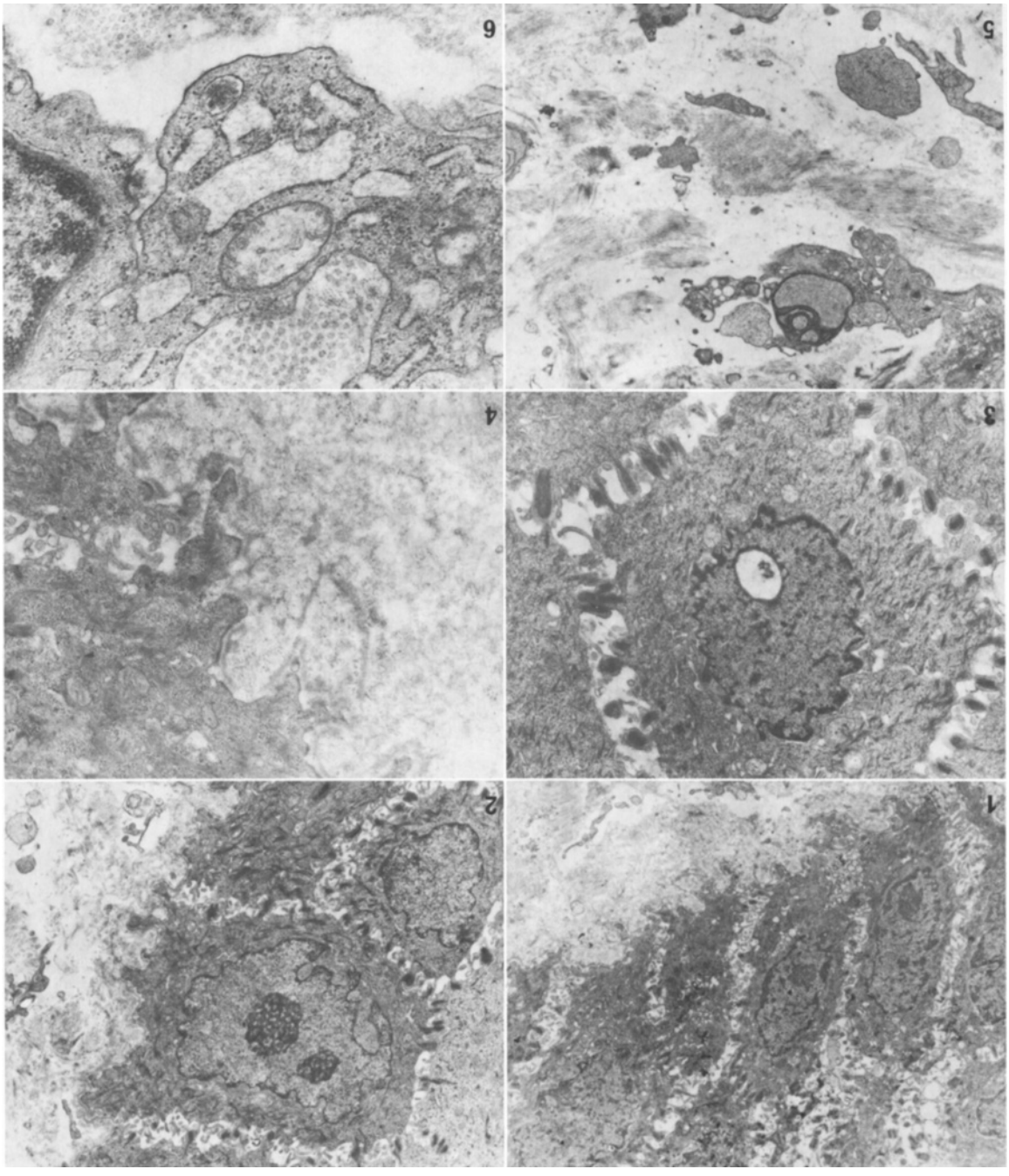

Fig. 1. – Epithelial cells were disrupted (× 5,500).

Fig. 2. – Nuclei of the germinal layer displayed complex invaginations (× 7,400).

Fig. 3. – Nuclear vacuolation in the germinal layer (× 11,000).

Fig. 4. – Basement membrane was incomplete (× 15,000).

Fig. 5. – Collagen was scant and disordered (× 8,700).

Fig. 6. – The organization of fibroblastic rough endoplasmic reticulum was irregular (× 30,000).

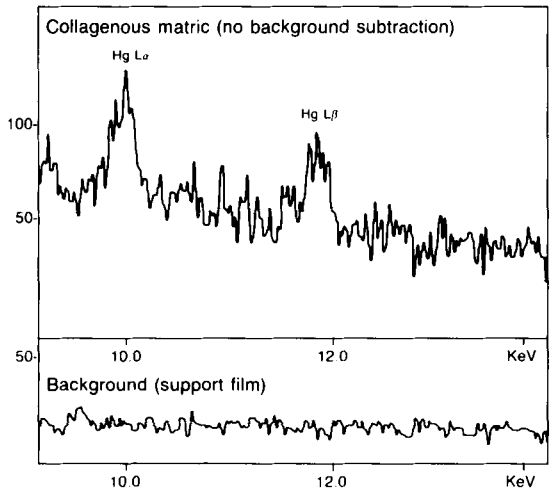

Fig. 7. – X-ray spectrum of representative area of the connective tissue matrix showing mercury L-α and β peaks.

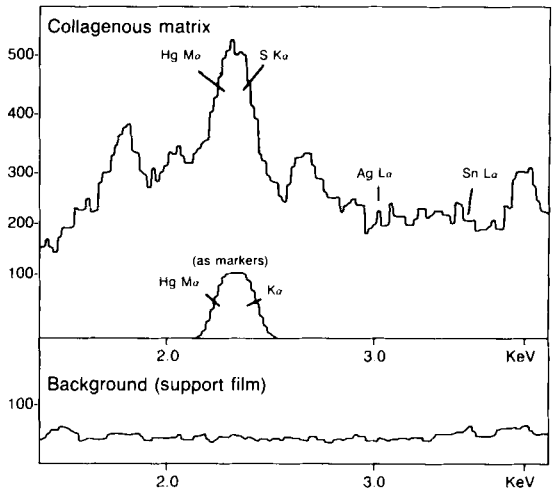

Fig. 8. – Corresponding X-ray spectrum of the mercury M-α peak compound with sulphur K-α. Silver and tin if present would be identified as L-α lines at 2.98 and 3.43 keV, respectively.

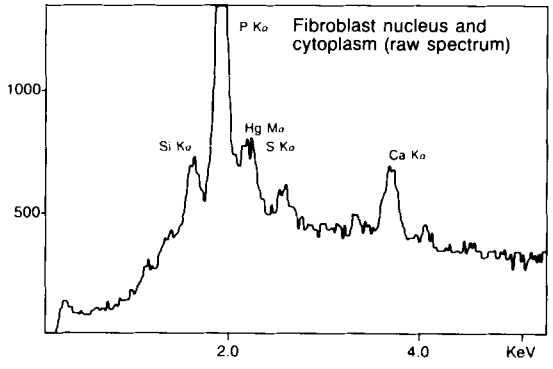

Fig. 9. – Fibroblasts displayed X-ray peaks for mercury. Neither tin nor silver were identified.

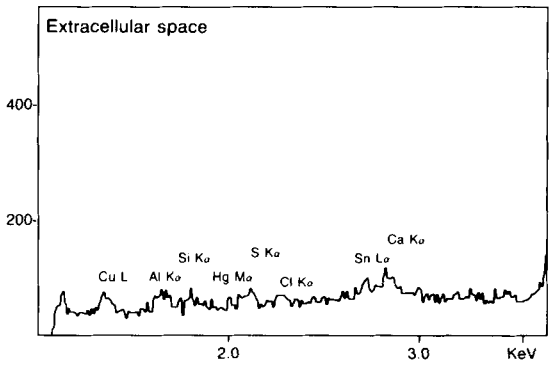

Fig. 10. – Extracellular space was devoid of strong X-ray peaks.

Discussion

Subgingival amalgam overhangs are a relatively common clinical finding, and are responsible for direct gingival irritation with ultimate alveolar crest destruction. Hyperplastic lesions associated with overhangs are generally pain free unless complicated by infection. This case represented a rare instance of pain which responded to the removal of the offending amalgam restoration, elimination of the subgingival pocket and insertion of a non-metallic restoration. Despite the small block of tissue available for examination, a range of tissue response was observed. Epithelium was characterized by distortion of the cellular contacts[1] and an increase in free ribosomes.[2] The underlying gingival connective tissue reflected many features of cellular and matrix disruption which could be attributed to metallic influences as well as to plaque. The tissue response in epithelium and basement membranes appeared to be somewhat uneven. This uneveness could feasibly be related to areas of greater anodic activity as observed in amalgam implants.[3] No features consistent with overt allergy were observed. Electron dense aggregations were neither observed extracellularly nor intracellularly, in this case, unlike those observed when dental amalgam was directly implanted into connective tissues as discs,[4] or as rods or powder.[5] The complex environment of the epithelial-lined gingival pocket cannot be related to that of the uncomplicated environment utilized in an experimental connective tissue site.

In the oral environment plaque enhances corrosion and reduces the oxidation-reduction

potential in deep pockets[6] to levels below that at which all phases of dental amalgam corrode. Mercury and tin are released from the γ_2-phase and silver and tin from the unreacted γ-phase. The γ_1-phase, although the least susceptible to corrosion, would release silver and mercury into the environment of a periodontal pocket. The presence of characteristic mercury peaks with significant peak-to-background ratios in the raw spectra without characteristic peaks for either tin or silver was not anticipated. The absence of all three constituents would be considered a likely finding in a case of an epithelial lined pocket, reflecting the effective barrier which epithelium presents to inorganic intoxicants.

Of the three major constituent metals of conventional dental amalgam, tin would be expected to form tin ions more readily from the various phases of dental amalgam than Ag(I) and Hg(II) ions and that Sn (II) compounds would be the favoured corrosion products in areas of low oxygen tension.[7] Various Sn (II) compounds of significance exist as insoluble oxides and sulphides and soluble sulphates, chlorides and tartrates and these salts form more stable complexes with sulphydryl compounds than Sn (IV) compounds.[8]

Silver cations released into pockets would likely form insoluble sulphides, chlorides, oxides or protein complexes. Because silver has an avidity for sulphydryl radicals[9] any silver entering epithelium would likely become bound to epithelial proteins and subsequently be eliminated from the pocket. By this mechanism silver penetration through the epithelial lined pocket into the gingival connective tissue in large amounts would be inhibited. Any silver gaining access would be retained for long periods in association with connective tissue elements as occurs with deliberately introduced silver.[10]

Mercury parallels silver in many of its reactions and affinities[11] especially with respect to sulphydryl radicals and sulphide formation. Epithelium is reported to be an effective barrier and only permits soluble inorganic mercury to transverse as far as the stratum corneum.[12] Cationic radiomercury [^{203} Hg] in connective tissue is very labile and is rapidly cleared from tissue [Ellender G. Unpulbished observation], and would be expected to be below EDAX detection limits in the connective tissue. A previous report of mercury in gingival tissue[13] based on digests of whole gingival tissue did not distinguish between the concentration in the epithelium and that in the connective tissue.

The presence of mercury in the connective tissue may indicate modification in the form of the available mercury, possibly resulting from a biotransformation by micro-organisms in the pocket environment. Such transformation occurs in nature[14] and may also be produced by certain oral micro-organisms resident in the mouth.[15] The tissue retention of Ch_3Hg^+ is greater than that of Hg^{2+}; rats fed mercury as CH_3HgCl and $HgCl_2$ retained far higher concentrations of the former than the latter.[16]

Conclusions

This article reports an unusual case of pain and a burning sensation related to subginigivally placed dental amalgam. Pain was not considered to be a result of pulpal pathology.

The ultrastructure of the gingival biopsy displayed features of disordered gingival metabolism, particularly in the connective tissue elements. Electron microprobe analysis demonstrated the presence of mercury and the absence of silver and tin in some tissue components. Mercury was associated with the cellular components of the epithelium and connective tissue – cellular elements and the collagenous matrix. The presence of mercury, with the absence of silver and tin suggests a selective absorption or modification in the normal barrier to diffusion by the tissues or modification of this corrosion product to a more reactive or readily absorbed form. Biotransformation of mercury by oral micro-organisms in the gingival pocket might provide such a mechanism. The reactions presented in this case were neither considered to be attributable to allergy nor mercury vapour, but to galvanism generated by a concentration cell superimposed on local gingival pathology. The presence of mercury, although not the cause of pain and discomfort, is potentially significant. The ultrastructural findings and elemental analyses highlight the need to understand the behaviour of dental materials in the extreme rigours of the oral environment by means of laboratory and clinical studies.

In the clinical situation, it is essential that dentists minimize the predisposing factors which enhance the corrosion process. This case illustrates the need to avoid the placement of restorations into subgingival sites, to adequately finish all restorations and to ensure effective home care. In this case the use of conventional and analytical electron microscopy revealed a cause of pain not related only to simple galvanism.

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Address for reprints:
Department of Preventive and Community Dentistry,
University of Melbourne,
711 Elizabeth Street,
Melbourne, Vic., 3000.