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SHORT COMMUNICATIONS

APPLICATION OF THE CAMPHENE TECHNIQUE IN THE EXAMINATION OF TEETH BY SCANNING ELECTRON MICROSCOPY

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Summary—A modification of a camphene sublimation procedure was developed to examine plaque in relation to tooth surfaces. The method is relatively simple and produces most acceptable results. Examples of plaque in the region of pits and fissures illustrated the type of result to be expected from such a technique.

WITH increasing interest in scanning electron microscopy of biologic tissues, several specimen preparation techniques have been developed. These have been the subject of reviews by BOYDE and WOOD (1969) and BOYDE (1971). Freeze drying, while the most common procedure for preparation of wet specimens, is subject to two principal disadvantages, ice crystal formation and precipitation of surface exudate. Although these can be minimized, and while some techniques are designed specifically to eliminate them (ANDERSON, 1951), the methods are somewhat complex and best suited to small tissue samples.

The introduction of simple sublimation procedures (BUCK, 1958; WATTERS and BUCK, 1971) have shown considerable promise, particularly with large tissue samples. Since teeth and associated bacterial plaque fall into this category, the camphene sublimation technique (WATTERS and BUCK, 1971) was evaluated for its usefulness in preparing whole tooth samples for examination by scanning electron microscopy.

The principal advantage of the camphene procedure, other than simplicity, is that camphene is a solid at 45°C which readily sublimates at room temperature, leaving no fluid interface. This significantly reduces surface tissue damage. The steps in the procedure are as follows:

(1) After extraction, teeth are fixed in 10 per cent neutral formalin.
(2) The specimens are transferred to 5·4 per cent sucrose in 0·1 M phosphate buffer overnight under refrigeration.

Subsequent steps involve constant gentle agitation.

(3) Post-fixation in 1 per cent osmium tetroxide in phosphate buffer for 2 hr.
(4) Two washes in demineralized water, each for 15 min, then through three changes of 30 per cent ethanol, each for 30 min.

(5) Dehydration by a series of 30 min changes in 50, 70, 90 and 95 per cent ethanol and 100 per cent acetone.
(6) Lipids are removed by transferring specimens to benzene for 30 min followed by equal parts of benzene and propylene oxide and finally to propylene oxide for a similar time.
(7) Specimens, are then immersed in a 50 per cent solution of camphene (practical grade, m.p. 35–45°C, J. T. Baker Chemical Co., Phillipsburg, N.J.) in propylene oxide for 20 min at 45°C, then to melted camphene at 45°C for 30 min.

The specimens are then allowed to cool to room temperature after which they are placed under vacuum for 24 hr. Following complete sublimation of the camphene, the specimens are shadowed with gold–palladium for examination by scanning electron microscopy. Since a tooth represents a reasonable bulk of tissue, an empirical experiment was conducted to determine the minimum time necessary to achieve complete camphene sublimation.

Figures 1 and 2 illustrate typical results to be expected after applying the procedure. Both show examples of bacteria and bacterial plaques on enamel in the region of the tooth fissures. A desirable minimum time to complete sublimation for a molar tooth is approximately 16 hr. For shorter periods, sublimation is incomplete and artifacts are introduced. Common examples are smearing effects (Fig. 3) leading to masking of structures and localized "pools" (Fig. 4). The gold–palladium coating frequently cracks and residue appears to leach out of such coated "pools". Residue may arise not only from incomplete camphene sublimation but also from any of its impurities and any residual volatile agents used in the method. These may collect and disperse at the surface by constant replenishment through diffusion from within the specimen. A gold–palladium coating serves to trap any residue giving rise to artifacts. Clearly, adequate time must be allowed for complete sublimation and volatilization.

This technique is now routinely used in our investigations of plaque, and other accumulations associated with the pits and fissures of teeth. While our results can be correlated with those of KALBERER *et al.* (1971) and HUXLEY (1971), using transmission and scanning electron microscopy respectively, we have not observed any "corn cob" phenomena as reported by JONES (1972). Coccoid forms are readily demonstrated as well as rod and filamentous organisms. It may be conjectured that the "corn cob" appearance relates to the presence of lipids. These would be removed in the camphene technique but would persist in the liquid nitrogen method used by JONES (1972).

Résumé—Une modification d'un procédé de sublimation du camphène a été développé pour examiner la plaque, à l'égard des surfaces des dents. La méthode est relativement simple et donne des résultats des plus satisfaisants. Les exemples des plaques dans la région des cavités et des fissures ont démontré le genre de résultat qu'on peut attaendre d'une telle technique.

Zusammenfassung—Es wurde eine Modifikation eines Sublimationsverfahrens für Camphen entwickelt, um Plaque in Bezug auf Zahnflächen zu untersuchen. Das Verfahren ist verhältnißmäßig einfach und ergibt äußerst annehmbare Resultate. Beispiele von Plaque in der Gegend von Grübchen und Fissuren illustrierte die Art des Ergebnisses, welches man mit einem solchen Verfahren erwarten kann.

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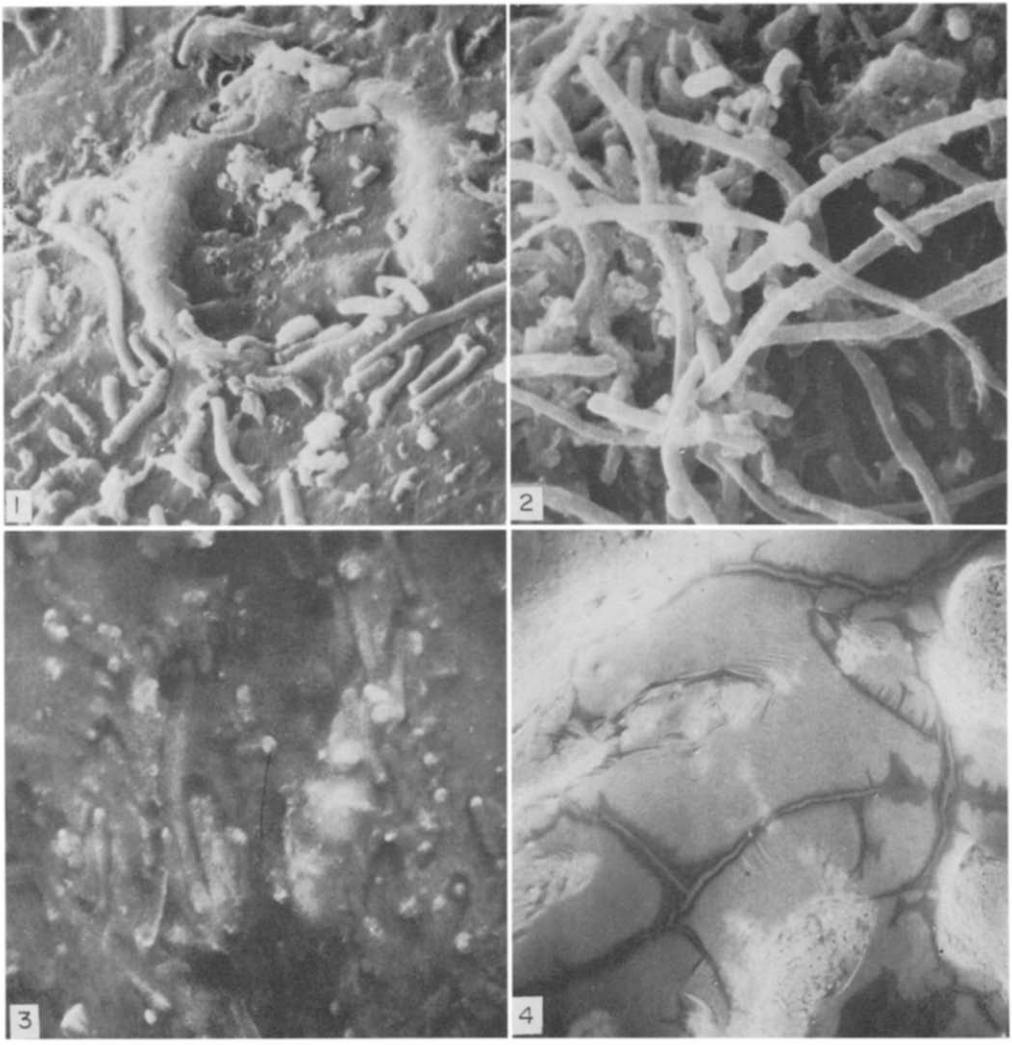

FIGS. 1 and 2. Scanning electron photomicrographs showing a number of different microorganisms located in the fissure region of a human molar tooth. ×5000

FIG. 3. Scanning electron photomicrograph in which bacterial structures have been masked because of faulty camphene drying technique. ×5000

FIG. 4. Scanning electron photomicrograph showing "pools" on enamel and associated cracking of the gold–palladium coat. Small ripples can be seen within the "pools". ×100

PLATE 1

A.O.B. f.p. 928