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# ELECTRON MICROSCOPIC STUDY OF CALCULUS ATTACHMENT TO SMOOTH SURFACES*

*by*

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The mechanism of formation of calculus on teeth is a problem for all those concerned with oral hygiene and periodontal disease. Many of the factors promoting the attachment of deposits to teeth remain unknown.

In studies involving the attachment of dental calculus, a cuticular layer was occasionally observed between the tooth surface and the bacterial matrix (*Zander,* 1953; *Shroff,* 1955; *Mandel & Levy,* 1957; *Voreadis & Zander,* 1958). The incidence of cuticular attachment was much higher when the calculus was located on the enamel than on the cementum (*Voreadis & Zander,* 1958). Comparable cuticular structures formed on plastic strips attached to tooth surfaces (*Voreadis & Zander,* 1958; *Mandel et al.,* 1957; *Hazen & Zander,* 1959; *Hazen,* 1960; *Turesky et al.,* 1961).

Previous investigations have been conducted using optical microscopy. This study, conducted with the aid of the electron microscope, reveals the presence of a cuticle interposed between any deposit and a Mylar strip attached to teeth.

## MATERIAL AND METHODS

Pieces of .001 inch thick Mylar film**) were fastened on the lingual surfaces of the lower anterior teeth in 18 patients known to form calculus. The plastic strips extended to the gingival mar-

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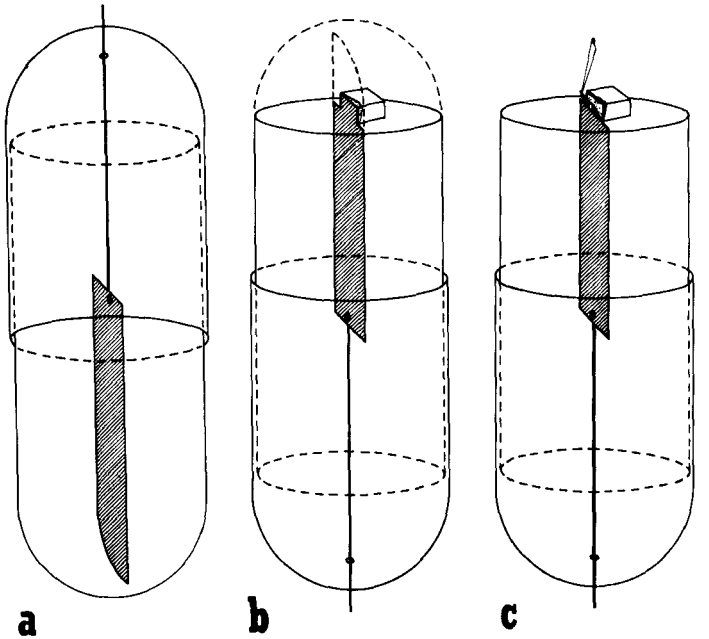



*Figure 1. a.* — Mylar strip is suspended gingival end down from top of gelatin capsule filled with prepolymerized methacrylate. *b.* — Following final polymerization, capsules are turned upside down and trimmed so that strip forms one side of block. *c.* — Before sectioning, the strip is removed and the attached deposit forms edge of section.

gins or slightly into the gingival crevices. At intervals of 1, 2, 9, 14, and 28 days, the strips were detached from the teeth and divided vertically into two halves. These were fixed immediately in buffered osmium tetroxide for four hours, washed in water, dehydrated in alcohol, and infiltrated with an 8: 2 mixture of butyl and methyl methacrylate. With the aid of a pin inserted through the top of the capsule the strips were suspended vertically, gingival end down, in gelatin capsules containing prepolymerized methacrylate (Fig. 1 a). Final polymerization was carried out at 40° C overnight.

Initial attempts to cut thin sections of Mylar films were invariably unsuccessful. The strips and frequently portions of the deposit were torn away from the sections. In order to circumvent this difficulty, the Mylar was removed prior to cutting. This was

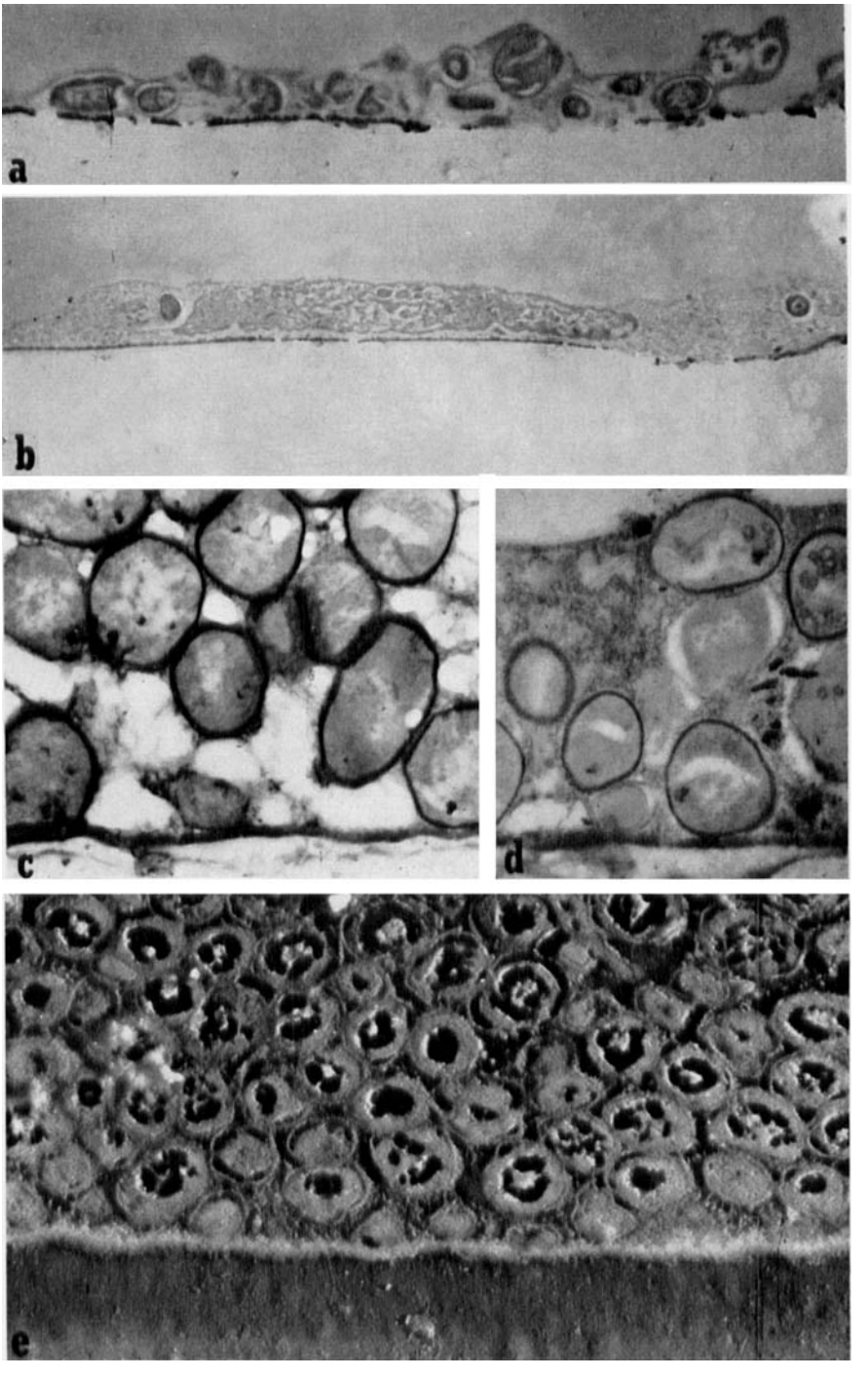
accomplished by trimming the blocks in such a way that the strips formed one of the lateral surfaces (Fig. 1 b), and by subsequently peeling the Mylar film from the embedding (Fig. 1 c). The blocks were mounted in a Porter-Blum microtome so that the deposit appeared along one edge of the section ribbon which was produced. The ribbons were flattened with chloroform vapor and mounted on grids covered with carbon substrates. While most of the sections were examined directly in an RCA-EMU 2 electron microscope, some were immersed in amylacetate for one hour and were subsequently shadowed with tungsten oxide. Other sections were stained in 5 % phosphotungstic acid. Limited area electron diffraction was carried out routinely.

## RESULTS

Deposits were present on all strips at the end of the experimental period. They were generally located at the gingival edges. Most deposits consisted of microorganisms in an intermicrobial matrix (Figs. 2 a, c, d, e). Some deposits, originally in contact with the free gingivae, were composed predominately of epithelial cells with a remainder of microorganisms (Fig. 2 b). Observations of filamentous microorganisms, as determined by morphology, showed that these organisms were not predominant, and they were often absent in 1 and 2 day deposits. When present, they did not seem to be directly associated with attachment of the deposit to the smooth strip.

An electron-dense membrane of variable thickness was observed irrespective of the presence or absence of other deposits. The thickness of the cuticular membrane ranged from 0.05 to 0.4 $\mu$ and appeared to be directly related to the age of the sample (Figs. 2, 3 a, b).

The contour of the cuticular surfaces varied, depending upon the nature of the adjacent material. The surface contiguous to the deposit was serrated, while that in contact with the Mylar was smooth (Fig. 3 a). Occasionally, interruptions in the continuity of the cuticle were noted. At these sites the margins appeared notched with the spaces devoid of embedding medium. In a few instances, the cuticle was located at a distance from the


a
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e

edge of the section and microorganisms were found on both sides.

Mineralization of the calculus matrix was noted occasionally in the 2 day old specimen and always in the 9 day or older samples. In all cases, the first crystals were seen in areas which contained microorganisms rather than epithelial cells. The cuticle usually remained free of crystals until mineralization of the bacterial deposit was well advanced (Fig. 3 c), whereupon it mineralized also (Fig. 3 d).

## DISCUSSION

The attachment of the microorganisms to the smooth surface of the Mylar strip appeared to be mediated by a cuticular layer which separated the cellular elements from the strip even at the earliest stages.

Organic coverings on natural tooth surfaces as well as on artificial hard structures inserted in the oral cavity have been described (*Vallotton,* 1945 a, b, c; *Turner,* 1958 a, b; *Klees & Klees,* 1958; *Meckel,* 1961; *Wertheimer & Fullmer,* 1962; *Schüle,* 1961, 1962). A comparison of the electron dense membrane seen in the present study with those previously mentioned is difficult because of the different methods used for obtaining and demonstrating them. The cuticle studied herein must be classed as an acquired film, however, since it forms supragingivally on an artificial surface.

The acquired cuticles have been presumed to be composed of

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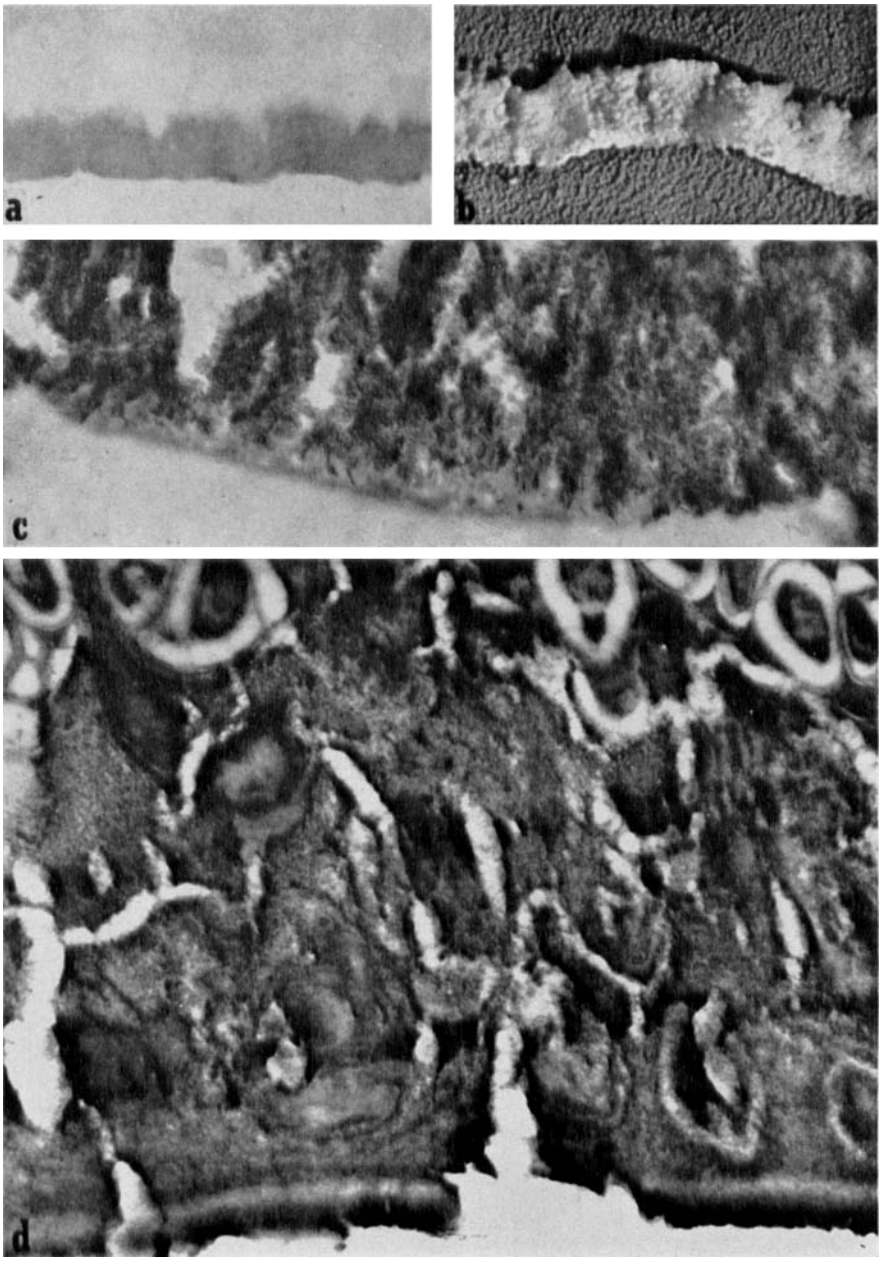
*Figure 2 a.* Section from a 1 day specimen. A thin electron-dense membrane lines the space previously occupied by the strip. A few microorganisms in an intermicrobial matrix are attached to the membrane. × 8,000.

All the illustrations in Figs. 2 and 3 are oriented in such a way that the portion of the deposit which was against the strip is facing downwards.

*Figure 2 b.* Section from a 1 day specimen showing an epithelial cell with microorganisms on either side. A thin electron-dense membrane is seen beneath the entire deposit. × 4,000.

*Figure 2 c and d.* Two day old specimens stained with phosphotungstic acid. The cuticular membrane beneath the deposit stains intensely as do the cell walls of most of the microorganisms. × 28,000.

*Figure 2 e.* Shadowed section from a 9 day old specimen. Numerous closely packed microorganisms in an intermicrobial matrix appear attached to a thin electron-dense membrane. × 16,000.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mucins from saliva (*Vallotton,* 1945 c; *Campaigne & Fosdick,* 1938; *Meckel,* 1961), products of specific epithelial cell activity (*Voreadis & Zander,* 1958), or the result of bacterial activity (*Chase,* 1926). The present experiment did not elicit information regarding the origin of the acquired cuticle. However, if the cuticle originates from one and the same source irrespective of whether it is the only deposit present or it is covered by cellular material, saliva seems to be the most likely contributor.

## SUMMARY

An electron microscopic study was conducted on deposits which formed on plastic strips that had been placed around the lingual surfaces of lower anterior teeth. Deposits were examined after they had formed for 1, 2, 9, 14, and 28 days. Usually, the deposits consisted of microorganisms in an intermicrobial matrix, although desquamated epithelial cells were sometimes found in sites which contacted the gingival margin during formation of the deposits. An electron-dense cuticle that ranged in thickness from 0.05 to 0.4 $\mu$ was interposed between the Mylar strip and any deposit. The omnipresent cuticle at this location suggests that it fosters or mediates the attachment of deposits.

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*Figure 3 a.* Section from a 14 day specimen showing a much thicker membrane free from microorganisms. The surface of the electron-dense membrane which was toward the strip (bottom) is smooth, that facing the oral cavity (top) is more or less undulated. × 40,000.

*Figure 3 b.* Shadowed section from specimen similar to that seen in Figure 3 a. The membrane appears homogenous or slightly granular. × 47,000.

*Figure 3 c.* Section from a 28 day old specimen. The bacterial plaque has undergone extensive calcification. A few needle-like crystals are found in the underlying membrane which is otherwise uncalcified. × 40,000.

*Figure 3 d.* Section from a 28 day old specimen demonstrating mineralization of the bacterial plaque as well as of the cuticular membrane. × 25,000.

RÉSUMÉ

ÉTUDE AU MICROSCOPE ÉLECTRONIQUE SUR LA FIXATION DU TARTRE SUR LES SURFACES LISSES

Une étude au microscope électronique a été effectuée sur les dépôts qui se formaient sur des bandes de plastique placées autour des faces linguales des incisives inférieures. Les dépôts ont été examinés au premier, deuxième, neuvième, quatorzième et vingt-huitième jour de leur formation. En général, les dépôts consistaient en micro-organismes placés dans une matrice intermicrobienne, bien que des cellules épithéliales exfoliées aient parfois été trouvées dans des zones en contact avec le bord gingival pendant la formation des dépôts. Une cuticule dense à l'égard des électrons, d'une épaisseur allant de 0,05 à 0,4 $\mu$ était interposée entre la bande de plastique et tout dépôt. La présence constante de cette cuticule à ce niveau semble indiquer qu'elle favorise ou sert de lien à la fixation des dépôts.

ZUSAMMENFASSUNG

ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN VON ZAHNSTEIN-BEFESTIGUNG AN GLATTEN OBERFLÄCHEN

Beläge, die auf Mylar-Folien gebildet waren, welche auf den Lingualflächen der unteren Frontzähne angebracht waren, wurden elektronenmikroskopisch untersucht. Die Beläge wurden 1, 2, 9, 14 und 28 Tage nach Anfang der Bildung untersucht.

Normalerweise bestanden die Beläge aus Mikroorganismen in einer intermikrobiellen Matrix, obschon desquamierte Epithelienzellen mitunter an Stellen, die während der Bildung der Beläge in direktem Kontakt mit der Margo gingivae war, gefunden wurden.

Eine elektronendichte Kuticula, welche in Stärke von 0,05 bis 0,4 $\mu$ variierte, war zwischen dem Mylar-Folium und jedem Belag eingeschaltet. Die immer anwesende Kuticula an dieser Stelle deutet an, dass sie die Befestigung der Beläge vermittelt.

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