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THE BORDER ZONE TOOTH-ENAMEL AND EPITHELIUM AFTER PERIODONTAL TREATMENT

AN EXPERIMENTAL ELECTRON MICROSCOPIC STUDY IN THE CAT

by

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INTRODUCTION

In 1921 *Gottlieb* published a study, in which he contested the earlier opinion (*i.e. Black*, 1915) that the bottom of the gingival pocket was located at the cemento-enamel junction. According to this opinion there existed a true epithelial attachment and this view of Gottlieb's was generally supported until 1952. In that year *Waerhaug*, backed by different experimental procedures, stated that the epithelium formed a cuff around the tooth and that there was no organic connection between these two tissues. Since then many works have attempted to throw more light upon this question and many different techniques have been applied, comprehensive surveys of which are to be found in modern text-books.

The electron microscopic appearance of the zone between enamel, cementum and epithelium has been studied by *Stern* (1962, 1963), *Listgarten* (1966a, b, 1967), *Selvig* (1966) and *Ito, Enomoto & Kobayashi* (1967). In broad outline these authors have found that the border zone consists of hemidesmosomes, a cementing layer and one or two types of cuticles.

No report has, however, dealt with the fine structure of this border zone after conservative periodontal treatment *i.e.* if there exist a real re-establishment from a structural point of view. The purpose of this study was therefore to investigate this problem experimentally in the cat.

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MATERIAL AND METHODS

The material consisted of three cats. Young animals were used in order to avoid advanced destruction of the periodontal tissues. When the experiment started one month before the specimens were taken all cats had marked gingivitis. They were fed a hard diet consisting of raw unprepared whole bovine tracheas. Two to three times a week the teeth were carefully scaled and polished. The gingivitis gradually disappeared and one week before the specimens were taken no inflammatory changes in the gingiva could be observed. During this last week no scaling or polishing was carried out.

After anaesthetizing the cats with Pentothal sodium by intraperitoneal injection, parts of the upper and lower jaws were removed (Fig. 1) and the animals were killed by an overdose of Pentothal sodium. During the surgical procedure the fixative used (glutaraldehyd, see below) was dropped on the buccal part of the thin marginal gingiva. After removal the specimens were immediately immersed into ice-cold buffered glutaraldehyd fixative for 2 hours. The concentration of the glutaraldehyd was 4 per cent and a phosphate buffer was used. The specimens were divided into smaller pieces in the fixative and were then transferred to ice-cold 1 per cent osmium tetroxide buffered at pH 7.6 with phosphate buffer. The post fixation time was 1–2 hours. A total of 60 specimens (=teeth) were used in this study.

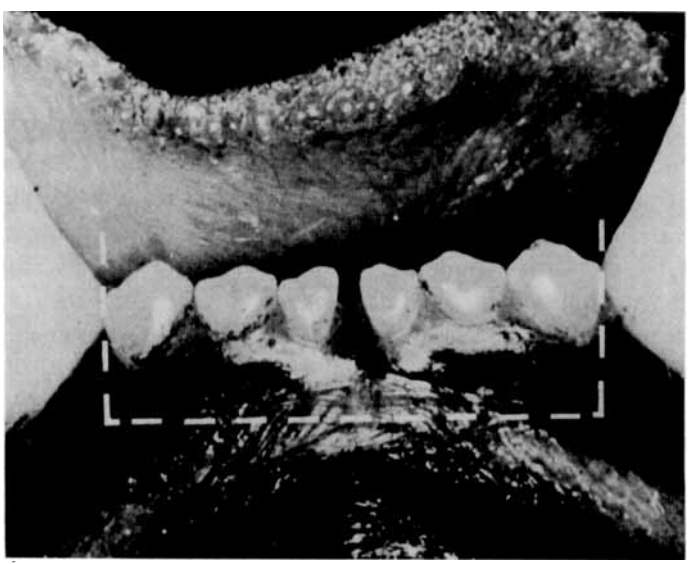

Fig. 1. The size of the specimens from the different regions were primarely rather large. The figure shows the front region in the lower jaw and exemplifies that the specimens include rather much of the gingiva in order not to interfere traumatically with the structure in the border zone epithelium-enamel.

One third of the specimens were then dehydrated in graded ethanols and without any demineralization embedded in Epon. The remaining two thirds of the specimens were first demineralized in EDTA at pH 7.4 and 37°C, one third for 2 days and the other for 4 days before the embedding in Epon. The Epon mixture used consisted of equal parts of Epon 812 and Nadic methylic anhydride in order to make the Epon as hard as possible.

The specimens were trimmed with a diamond stone in a dental engine under a water spray coolant. The location of the trimmed areas is shown in Fig. 2. Sections for orientation were obtained by cutting 1—2 micron thick sections in an ultramicrotome. These sections were placed on ordinary glass slides and were stained in toluidin blue. After final trimming according to the above-mentioned method the specimens were sectioned in an LKB ultrotome using a diamond knife (inclination 47°). The thin sections were stained with uranyl acetate or double stained with uranyl acetate and lead citrate. The sections were examined in a Philips electron microscope EM 300.*

RESULTS

At the time for the removal of the specimens the marginal gingiva of the cats was pink and firm and with no clinical signs of inflammation. Light microscopic investigation revealed, however, the normal feature of a slight inflammatory reaction, especially in the most marginal part (Fig. 2).

The tooth enamel and the epithelium was separated by a 400—600 Å thick osmiophilic zone. This zone consisted of a finely granular or occasionally filamentous substance. Between the pocket epithelium and this osmiophilic zone there was a less electron dense zone of about 500—600 Å in width. Here hemidesmosomes are visible with the typical lamellated appearance. The structural composition of this border zone was quite similar to that between epithelium and connective tissue. The electron dense zone corresponds to the ordinary basement membrane of the lamina densa and the less electron dense zone to the lamina lucida. The same nomenclature will therefore be used in the following.

The hemidesmosomes were long and seemed to be a rather continuous structure, interrupted only by invaginations in the cell membranes and by the orifices of the intercellular spaces (Fig. 3). The invaginations were filled or partly filled with a substance morphologically similar to that of the lamina lucida. Often filamentous structures seemed to extend from the peripheral density of the hemidesmosomes to the basement membrane.

*) The electron microscope was kindly put at the authors' disposal by Dr. G. Bloom, the Department of Histology, University of Umeå.

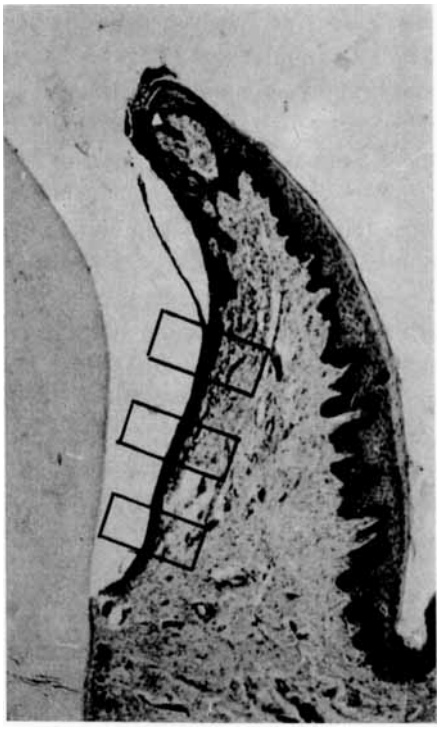

Fig. 2. Marginal gingiva. A slight inflammatory reaction is still evident in the connective tissue. The three framed areas exemplify the locations of investigated border regions. Hematoxylin and eosin.

In no instances was it possible to observe a cuticle or cuticlelike structure between the basement lamina and the enamel, the basement lamina was in direct apposition to the enamel.

A comparison was made between the border zone enamel epithelium and the zone epithelium-connective tissue on the opposite side of the pocket epithelium in the same specimen (Fig. 4). As mentioned above, the corresponding structures of these two border zones (basement lamina, lamina lucida and hemidesmosomes) seemed to be similar. Measurements that were taken verified this impression, the only difference seeming to be the length of the hemidesmosomes: they were longer and had a more continuous course on the enamel side.

No differences in the structural organization of this border zone could be discerned between different specimens or between lower and higher areas of this contact zone enamel- epithelium.

Fig. 3. Electron micrograph of the border zone epithelium-enamel. No cuticula-like structure is visible. The lamina densa is distinct and the hemidesmosomes show a different length. e.r. = enamel remnants. ep. = epithelium. X 72.000

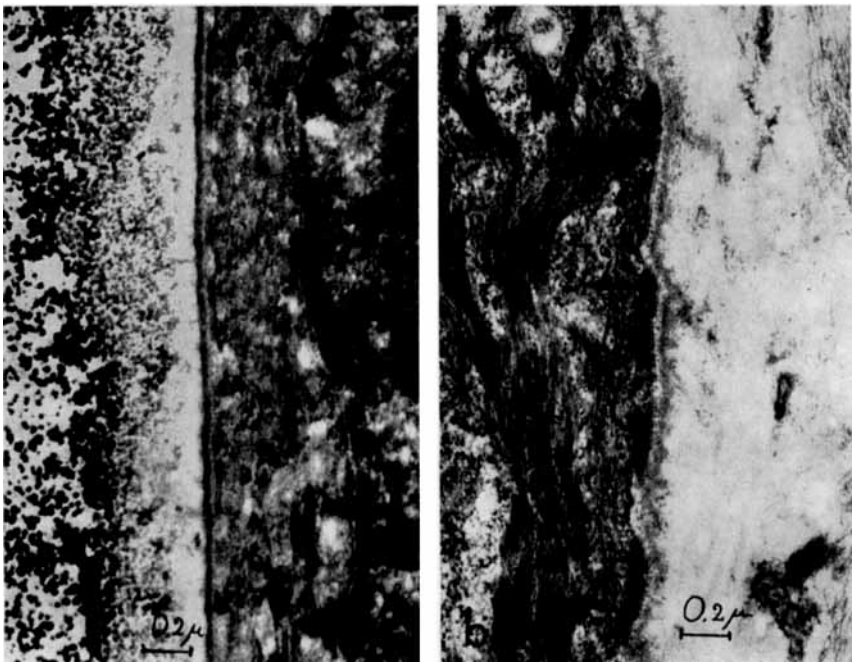

Fig. 4. Electron micrographs. 4a shows the border zone epithelium-enamel and 4b the border zone epithelium-connective tissue in the same specimen. The two border zones show a similar structure what the lamina densa, the lamina lucida and the hemidesmosomes are concerned. e.r. = enamel remnants. ep = epithelium. c.t. = connective tissue. X 40.000

DISCUSSION

In a light microscope investigation *Waerhaug* (1955) found that after a thorough scaling of teeth in humans, the epithelium can rejoin the tooth-surface in areas covered early by calculus in the same way as a normal epithelial cuff joins the tooth-surface. The results of the present investigation confirm this observation and extend it to the ultrastructural level with the findings of a re-establishment of hemidesmosomes and the lamina densa in this border zone after proper periodontal treatment.

No kinds of cuticles between the enamel and the epithelium has been observed in this material as previously has been reported in works by *Listgarten* (1966 a) and *Ito et al.* (1967). There is some uncertainty about the presence and origin of these cuticles. The type B cuticle of Listgarten may correspond to the so-called secondary or dental cuticle. Most authors have considered it to be a product of the epithelium, but during the last ten years other origins have been suggested as the saliva (*Turner*, 1958) and erythrocytes, which have compacted (*Hodson*, 1966). The theory of an epithelial

origin may, however, be questioned as no such cuticle has been found in unerupted teeth (*Listgarten*, 1966 b). The type A cuticle of Listgarten seems to be of cemental origin judging from its structure and its location in close relationship to the cemento-enamel junction. As mentioned above no kinds of cuticles were observed in the present material and there may be several possible explanations for this. One is that the scaling procedures had removed these structures. Another explanation is that there have primarily been no cuticles in the regions studied. It is possible that the type B cuticle or the secondary cuticle is an artificial product and that the type A cuticle only is connected with the presence of cementum, a region which not has been investigated here.

The border zone between enamel-epithelium and the opposite border epithelium-connective tissue showed here a similar structural organization in the form of a lamina densa, a lamina lucida and hemidesmosomes. Measurements taken showed, too, that the above-mentioned structures had the same dimensions in the two locations. This structural arrangements seems therefore to be the usual mechanism for epithelial contact. This view is further supported by the observations of *Flaxman, Lutzner & vanScott* (1968). They found namely a similar arrangement as contact mechanism between epidermal basal cells and polymerized nitrocellulose in vitro. Not much is known about the origin and developement of the lamina densa. The results of recent studies, in which different techniques have been used, suggest, however, the lamina densa to be a product of epithelial origin (*Kurtz & Feldman*, 1962; *Stallard, Diab & Zander*, 1965; *Blümcke, Rode & Niedorf*, 1969). The findings in this study, where a lamina densa was observed between the epithelium and the enamel, are in line with this theory of an epithelial origin.

This contact mechanism between epithelium and enamel must be of a dynamic nature. Beside the fact that there was an re-establishment after the scaling procedures in this material, the dynamic nature of this contact mechanism is stressed by tre fact that there is a continuous replacement of epithelial cells in the epithelial cuff. Further investigations may throw light upon the time necessary for a reestablishment after an injury and the substances which may affect this contact mechanism between epithelium and enamel.

SUMMARY

An electron microscope investigation has been carried out of the border zone epithelium-enamel in the cat after periodontal treatment. The material con-

sisted of 60 specimens (= teeth with intact surrounding gingiva) from 3 cats. The results showed that there was a reestablishment from a structural point of view in the form of hemidemosomes, lamina lucida and lamina densa. No cuticles could be observed between the lamina densa and the enamel. A comparison between this border zone and the zone epithelium-connective tissue on the opposite side of the pocket epithelium was also made. Measurements showed the same dimensions of the correspondent structures in these two zones. (lamina densa, lamina lucida and hemidesmosomes).

RÉSUMÉ

LA ZONE LIMITE ÉPITHÉLIUM-ÉMAIL DENTAIRE APRÈS TRAITEMENT PARODONTAL. ÉTUDE EXPÉRIMENTALE AU MICROSCOPE ÉLECTRONIQUE CHEZ LE CHAT

Les auteurs ont effectué une étude au microscope électronique portant sur la zone limite épithélium-émail chez le chat après traitement parodontal. Le matériel se composait de 60 spécimens (= dents avec la gencive environnante) provenant de 3 chats. Les résultats ont montré qu'il se faisait du point de vue de la structure une reconstitution dans la forme des hémi-desmosomes, de la lamina lucida et de la lamina densa. On ne pouvait observer aucune cuticule entre la lamina densa et l'émail. Une comparaison a aussi été faite entre cette zone limite et la zone épithélium-tissu conjonctif du côté opposé de l'épithélium du cul-de-sac. Les mesures ont montré que les structures correspondantes avaient les mêmes dimensions dans ces deux zones (lamina densa, lamina lucida et hémi-desmosomes).

ZUSAMMENFASSUNG

DIE GRENZZONE ZWISCHEN ZAHNSCHMELZ UN EPITHEL NACH PERIODONTALER BEHANDLUNG

EINE EXPERIMENTELLE ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNG AN KATZEN

Nach periodontaler Behandlung von Katzen machte man eine elektronenmikroskopische Untersuchung der Grenzzone zwischen dem Epithel und dem Emáil. Das Material bestand aus 60 Präparaten von 3 Katzen. (Zähne mit intakt anschliessender Gingiva.) Gesehen von der strukturellen Seite fand man eine Reetablierung der Hemidesmosomen, der Lamina lucida und der Lamina densa. Keine Cuticilae konnten beobachtet werden zwischen der Lamina densa und dem Emáil. Man machte auch vergleichende Untersuchungen zwischen dieser Grenzzone und der Zone der anderen Seite, die das

Epithel und Bindegewebe beinhaltet. Die Messungen zeigten diselben Dimensionen der korespondierenden Strukturen in den zwei Zonen (die Lamina densa, die Lamina lucida und die Hemidesmosomen).

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