

From:
The Department of
Periodontology,
The Royal Dental College,
Aarhus, Denmark

THE THREE-DIMENSIONAL CONCEPT OF THE EPITHELIUM-CONNECTIVE TISSUE BOUNDARY OF GINGIVA

by

T. KARRING

H. LÖE

INTRODUCTION

In histological sections the epithelium-connective tissue boundary of skin and mucous membranes is characterized by epithelial projections into the connective tissue and connective tissue papillae. It is generally accepted, however, that in the third dimension these »rete pegs» are actually epithelial ridges separated by conically shaped connective tissue papillae (*Sicher*, 1966; *Ham & Leeson*, 1961). Studies of human epidermis, after its separation from the dermis by maceration, have demonstrated that the morphology of the epithelium-connective tissue interface differs in various regions (*Hoepke*, 1926; *Greb*, 1940; *Horstman*, 1952, 1957; *Oberste-Lehn*, 1962). Such variations include the presence of a few short and thick connective tissue papillae (the skin of the face) and numerous long and slender papillae arranged in rows (the palm of the hand and the sole of the foot).

Similar variations also occur in the mucous membrane of the oral cavity (*Horstman*, 1954). In man and monkey the inner surface of the epithelium of the free and attached gingiva is flat, pitted at intervals by numerous conical connective tissue papillae. Epithelial pegs are rarely seen. At the

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gingival margin, horizontal epithelial ridges are prominent (*Emslie & Weinmann, 1949; Emslie, 1951; Horstmann, 1954*). Histological studies indicate that variations in the morphology of the epithelium-connective tissue boundary of the gingiva also exist between individuals (*Wentz et al., 1952; Zachinsky, 1954; Shklar, 1966*). The outer surface of the gingiva is normally characterized by multiple depressions in the epithelium (stippling). These depressions seem to coincide with intersections of underlying epithelial ridges (*Emslie, 1951; Owings, 1969*).

The purpose of the present investigation was to study the morphology of the epithelium-connective tissue interface of the gingiva by the use of three-dimensional wax models. An attempt has also been made to determine the relationship between the stippling and the subsurface structures of the epithelium.

MATERIAL AND METHODS

Thirty-nine gingival biopsies from 24 male and 13 female Caucasians between the ages of 20–77 years were used in the study. Seven biopsies were obtained from the labial gingiva of incisors and thirty-two from the buccal aspect of premolars in the upper and lower jaw. In addition five labial and buccal gingival biopsies were taken from the incisors and premolars of three rhesus monkeys.

Prior to biopsy the state of the human gingiva was assessed according to the criteria of the Gingival Index system (*Löe, 1967*). Twenty-nine areas scored $GI = 0$ and ten scored $GI = 1$.

Each biopsy, comprising the tissue from the gingival margin to the alveolar crest, was carefully dissected free and fixed in 10 per cent buffered formalin. Serial sections were cut at 10 microns and stained in haematoxylin and eosin. Separate wax models ($200\times$) of the epithelium and the connective tissue were produced according to a method described elsewhere (*Karring & Löe, 1970*). The models were made from 50 or more consecutive sections. In 11 cases the models included the crevicular epithelium and in 28 cases only the oral aspect of the free and attached gingiva were reproduced.

The height, width and density of the connective tissue papillae and the width of the epithelial ridges were measured in each model. The height was measured from the base to the tip of ten randomly selected connective tissue papillae. The width was measured with a sliding caliper half way between the base and tip of ten randomly selected connective tissue papillae and epithelial ridges. Mean values were calculated for each specimen and expressed according to the original size of the specimen. The papillary den-

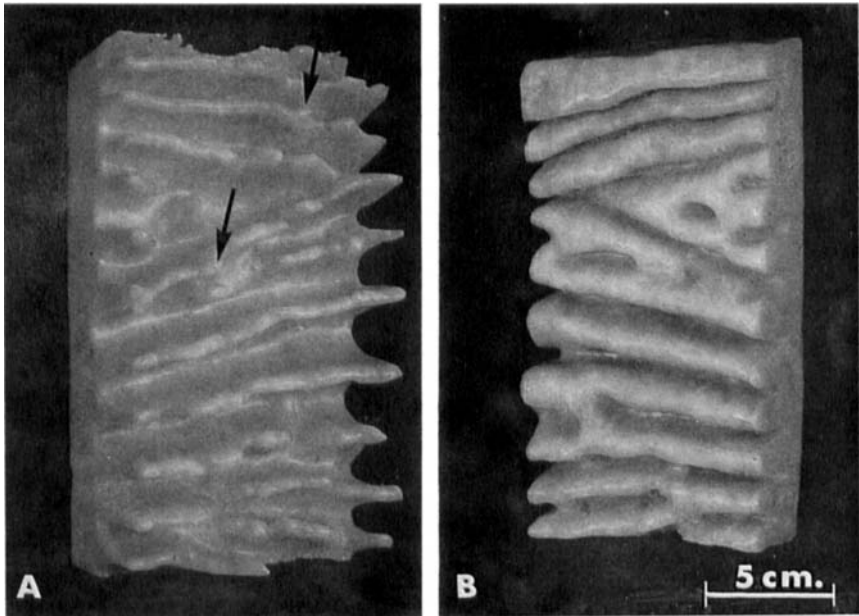


Fig. 1. Models ($200\times$) showing the subsurface of the epithelium (B) and the corresponding connective tissue (A) of the oral aspect of the free and attached human gingiva adjacent to the buccal surface of $\bar{5}$ (24 year old male). The specimen which is characteristic of group I shows connective tissue ridges projecting into the epithelium. Small secondary papillae occur on top of some ridges (A, arrows).

sity was assessed by counting the number of connective tissue papillae within a unit area and expressed as the number of papillae corresponding to 1 mm^2 at the surface of the original specimen. In specimens showing the ridge pattern, the number of ridges was counted and expressed as the number corresponding to 1 mm at the surface of the original specimen.

The measurements were made in order to assess gross variations in the morphology of the epithelium-connective tissue interface.

RESULTS

The wax models of the human gingiva could be divided in two groups on the basis of the morphological characteristics of the epithelium-connective tissue interface:

- I. those showing principally connective tissue ridges (Fig. 1) (13 specimens)
- II. those showing principally connective tissue papillae (Fig. 2) (26 specimens).

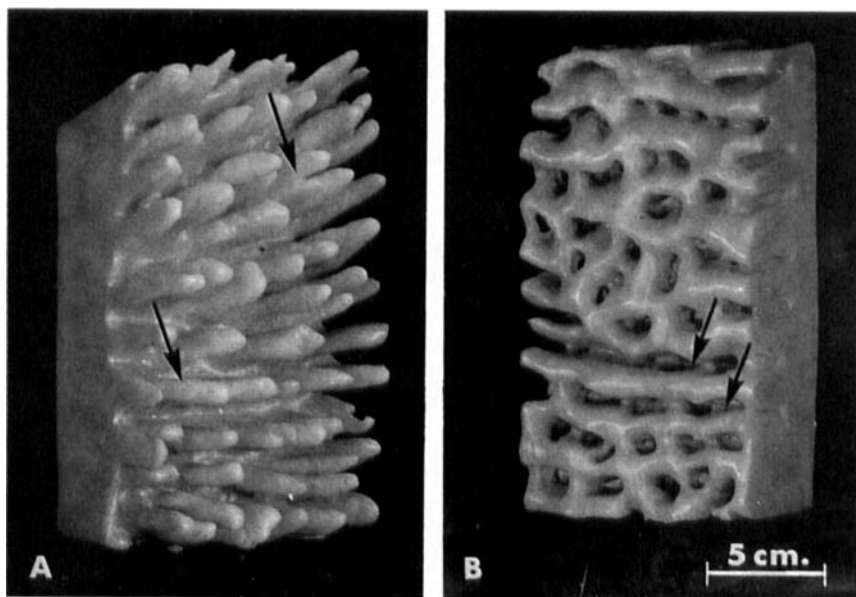


Fig. 2. Models ($200\times$) showing the subsurface of the epithelium (B) and the corresponding connective tissue (A) of the oral aspect of the free and attached human gingiva adjacent to the buccal surface of [5] (22 year old female). The specimen, which is characteristic of group II shows an even and regular distribution of tall conical connective tissue papillae, some of them having a common base (arrows). The subsurface of the epithelium (B) is pitted and some pits are connected by shallow grooves (arrows).

Intermediate stages were present in both groups.

Group I. The connective tissue ridges extended from the connective tissue core at right angles to the surface of the epithelium and had a course parallel to the gingival margin (Fig. 1A). In six of the thirteen specimens the continuity of the ridges was broken at intervals, thereby forming shorter ridges arranged in rows parallel to the gingival margin (Fig. 3A).

From one specimen to another the mean height of ten ridges varied between 120 and 300 μ , and the mean width between 30 and 80 μ . Between 10 and 14 ridges occupied the length corresponding to 1 mm at the gingival surface (Table I).

Small secondary connective tissue papillae were found at intervals on top of some ridges (Fig. 1A, arrows). Towards the gingival margin the connective tissue ridges became smaller, narrower and more closely packed. The subsurface of the corresponding epithelium showed continuous epithelial ridges (Fig. 1B). The mean width of ten ridges varied between 30 and 70 μ from one specimen to another (Table I).

Table I

The height, width and density of connective tissue ridges and the width of epithelial ridges in specimens included in group I

BIOPSY No.	CONNECTIVE TISSUE			EPITHELIUM
	Mean height (μ) of 10 ridges	Mean width (μ) of 10 ridges	Number of ridges per 1 mm at the surface	Mean width of 10 ridges
K 15	170	40	10	60
K 19	300	30	10	60
K 21	160	40	12	50
K 36	240	40	12	40
K 37	190	30	14	30
K 52	140	40	12	60
K 53	120	50	10	50
K 54	190	30	12	60
K 55	260	40	14	40
K 56	230	50	10	60
K 59	210	30	14	50
K 61	290	80	10	60
K 64	190	50	10	70

In specimens which showed discontinued connective tissue ridges the intervening epithelial ridges were connected by cross ridges (Fig. 3B, arrows).

Group II. Conically shaped connective tissue papillae were observed in twenty-four out of 26 specimens. The remaining two, in addition, displayed areas with disrupted ridges.

The papillae projected from the connective tissue at right angles to the surface of the gingiva. Most specimens showed an even and regular papillary distribution similar to the design of an egg tray (Fig. 2A) whereas others showed a more irregular distribution (Fig. 4A). Two or more papillae with a common base were frequently seen (Fig. 2A, arrows). Small secondary papillae occurred on top of thick papillae in some cases (Fig. 4A, arrows).

From one specimen to another the mean height of ten connective tissue papillae varied between 110 and 380 μ , the mean width between 40 and 90 μ and the density between 48 and 200 pr. 1 mm² surface area (Table II, Fig. 2A, 4-5).

Towards the gingival margin the connective-tissue papillae became shorter and thinner and were often fused, forming connective tissue ridges (Fig. 5B, arrows).

The subsurface of the corresponding epithelium was pitted giving an

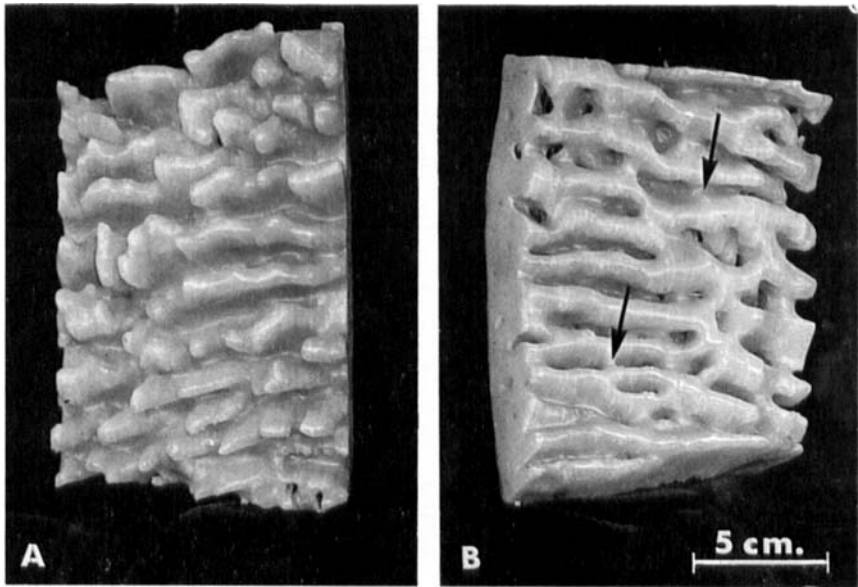
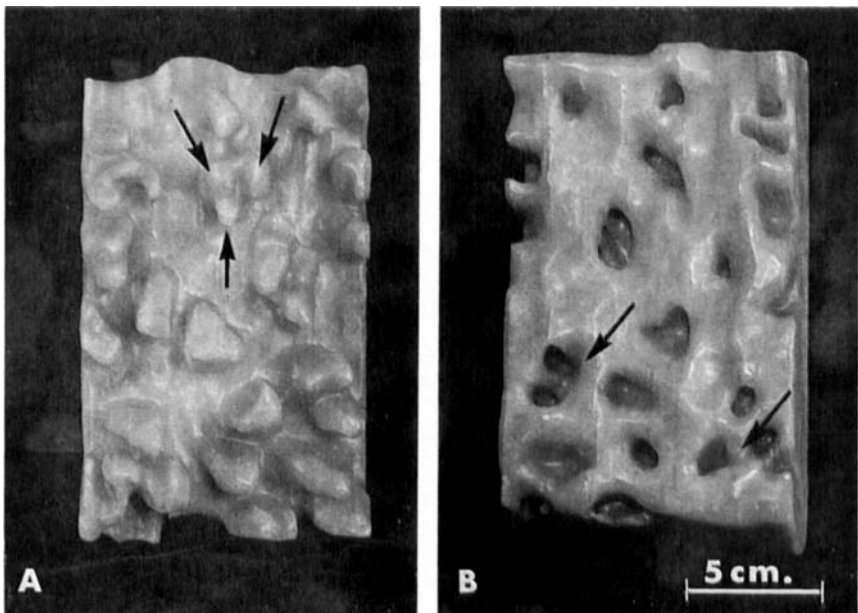


Fig. 3. Models (200 \times) showing the subsurface of the epithelium (B) and the connective tissue (A) of the oral aspect of the free and attached human gingiva adjacent to the buccal surface of 5 (72 year old male). The specimen shows short connective tissue ridges running parallel to the gingival margin (A). The intervening epithelial ridges are connected by cross ridges (B, arrows).



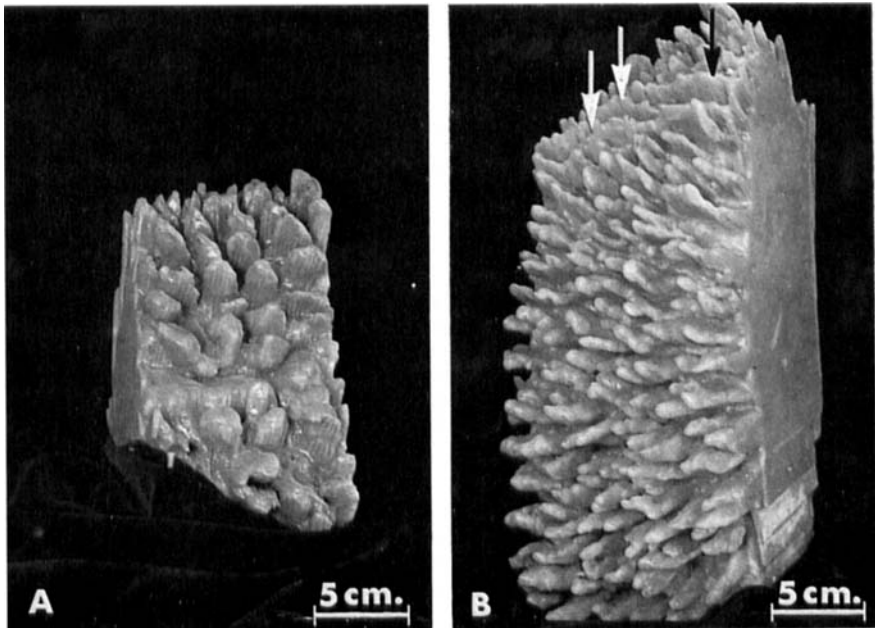


Fig. 5. Models ($200\times$) of the connective tissue of the oral aspect of the free and attached human gingiva adjacent to the labial surface of $\overline{12}$ (20 year old male) (A) and buccal surface of $\overline{15}$ (23 year old female) (B). The one (A) is characterized by short and thick papillae, the other by (B) long and slender papillae. At the gingival margin papillae are fused to connective tissue ridges (B, arrows).

appearance of a honey-comb (Fig. 2B, 4B). In specimens where the connective-tissue papillae were connected by a common base the epithelium showed shallow grooves between the pits (Fig. 2B, arrows, Fig. 4B, arrows). The mean width of ten ridges varied between 40 and 120 μ from one specimen to another (Table II). Comparisons between gingiva of incisors and premolars and between maxillary and mandibular specimens disclosed no regional or individual characteristics.

Some specimens of both groups showed a few thin epithelial pegs extending from the top of the ridges. Most of them were oriented at right-angles to the epithelial surface, but some had a course parallel to the surface. Epithelial cords connecting adjacent or distantly spaced epithelial ridges were found

Fig. 4. Models ($200\times$) showing the subsurface of the epithelium (B) and the corresponding connective tissue (A) of the oral aspect of the free and attached human gingiva adjacent to the buccal surface of $\overline{51}$ (65 year old male). The specimen shows a few thick dispersed papillae (A) on top of which small secondary papillae may occur (arrows). The epithelial subsurface is characterized by dispersed pits (B). Some pits are connected by shallow grooves (arrows).

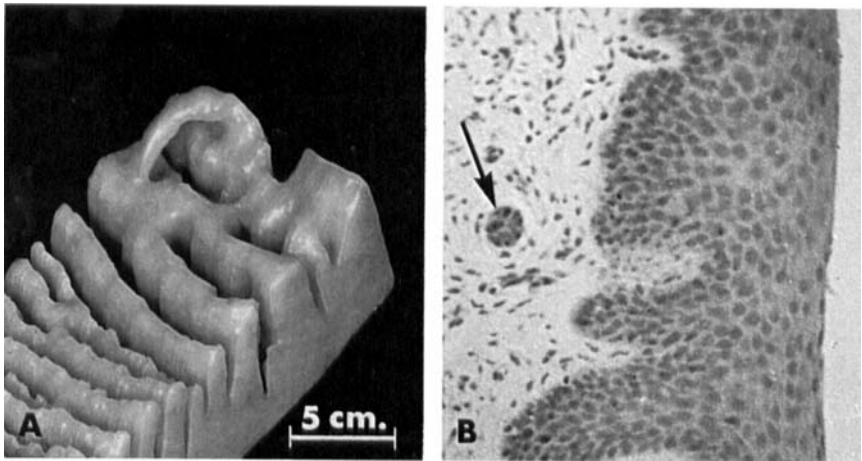


Fig. 6. (A) Model ($200\times$) showing an epithelial cord connecting two adjacent epithelial ridges at the subsurface of the epithelium. (B) Histological section of the gingiva showing a cross-section of an epithelial cord in the subpapillary layer (arrow).

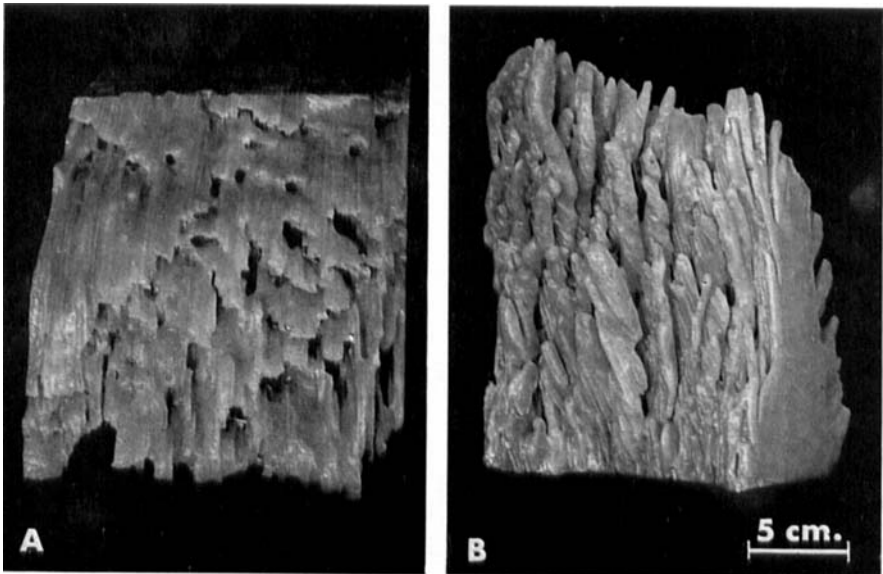


Fig. 7. Models ($200\times$) showing the subsurface of the human crevicular epithelium (A) and the corresponding connective tissue (B). Long and thin connective tissue papillae (B) project into the crevicular epithelium forming deep pits at its subsurface (A).

Table II

The height, width and density of connective tissue papillae and the width of epithelial ridges in specimens included in group II

BIOPSY No.	CONNECTIVE TISSUE			EPITHELIUM
	Mean height (μ) of 10 papillae	Mean width (μ) of 10 papillae	Number of papil- lae per 1 mm ² at the surface	Mean width (μ) of 10 ridges
K 16	240	40	200	50
K 17	270	70	112	70
K 18	190	60	64	80
K 20	190	50	84	70
K 22	180	50	72	90
K 23	270	50	104	50
K 24	160	80	64	50
K 25*	380	60	96	60
K 26*	380	70	76	60
K 28*	360	50	120	40
K 30	220	60	64	50
K 31	170	50	136	60
K 32	260	40	136	50
K 33	200	70	56	90
K 38	240	50	80	70
K 39	200	50	96	80
K 43	170	80	80	60
K 44	290	90	48	60
K 47	320	70	104	40
K 48	190	60	152	50
K 49	110	40	144	70
K 50	140	90	48	120
K 51	170	70	76	70
K 57	240	70	92	40
K 63	310	70	76	70
K 65	250	50	120	60

*Specimens showing a marked surface stippling.

in seven of the specimens (Fig. 6A). In histological sections, these bridges may appear as epithelial islands in the subpapillary layer of the connective tissue (Fig. 6B, arrow).

The epithelium-connective tissue interface corresponding to the crevicular epithelium showed varying numbers of connective tissue papillae of differing thickness and length. Except for two specimens, which showed only a sparse number of papillae in the marginal region, long and thin papillae,

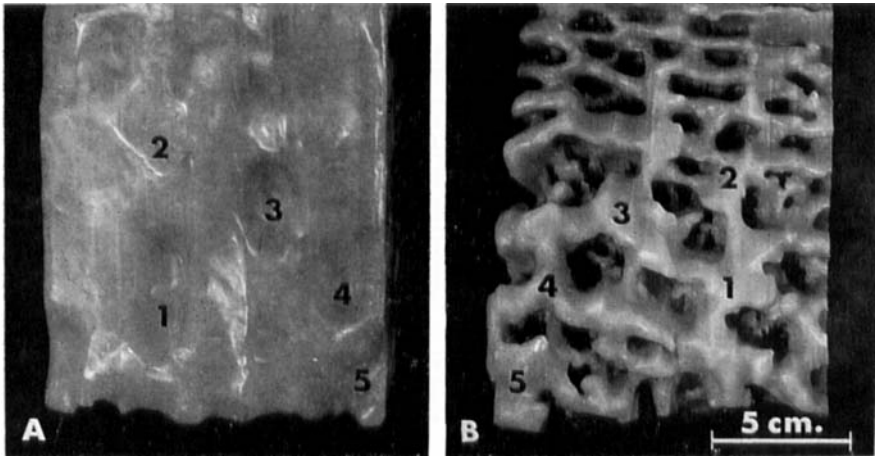


Fig. 8. The surface (A) and subsurface (B) of a model ($200\times$) representing the epithelium of attached human gingiva with a marked stippling on the surface. The depressions (A; 1, 2, 3, 4, 5) coincide with intersections of underlying epithelial ridges (B; 1, 2, 3, 4, 5).

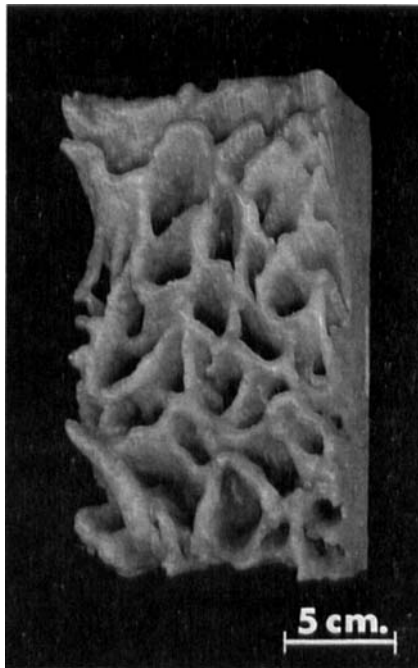


Fig. 9. Model ($200\times$) of the connective tissue of the oral aspect of the free and attached gingiva of Rhesus monkey. The specimen shows an arrangement of connective tissue ridges, giving an appearance closely resembling that of human epithelium overlying a connective tissue with papillae.

oriented almost parallel to the epithelial surface, were found throughout the entire crevicular area (Fig. 7B). At the gingival margin shorter papillae were arranged in parallel rows or were fused into ridges. The subsurface of the crevicular epithelium was characterized by an irregular distribution of deep pits (Fig. 7A).

In specimens with a marked surface stippling the depressions in the surface (Fig. 8A) correlated with areas at the subsurface where several epithelial ridges intersected and formed epithelial masses (Fig. 8B). The connective tissue of these specimens was characterized by closely packed tall and slender papillae (Table II). In no case could any structural characteristic of the epithelium-connective tissue interface be related to the free gingival groove.

In most specimens from rhesus monkeys the epithelium connective tissue interface of the oral aspect of the gingiva showed connective tissue ridges connected by cross-ridges. This gave the connective tissue an appearance closely resembling that of the subsurface of human epithelium overlying a connective tissue with papillae (Fig. 9). The epithelium displayed an arrangement of pegs which resembled a papillary connective tissue surface. In a few specimens, however, only continuous ridges parallel to the gingival margin were present in both connective tissue and epithelium.

DISCUSSION

The present study has demonstrated that the morphology of the epithelium-connective tissue interface of the adult human gingiva varies widely. In general, the preferential pattern of arrangement of the connective tissue seems to be that of papillae projecting into the epithelium, although ridges also occur. No systematic regional or individual characteristics have been found.

The rare finding of epithelial pegs agrees with that of previous investigators (*Emslie & Weinmann, 1949; Emslie, 1951*). The presence of epithelial pegs parallel to the surface of the epithelium and the occurrence of epithelial cords connecting adjacent or distantly spaced ridges, has not been reported previously.

Whether the configuration of the epithelium-connective tissue interface was characterized by a ridge form or by the presence of papillae could not be predicted by viewing the histological sections. This supports the statement of *Emslie & Weinmann (1949)* that histological sections may be relatively poor representations of the true relationships between epithelium and connective tissue.

It is well established that inflammation causes dissolution of the connective tissue, and may stimulate epithelial proliferation (King, 1944; Waerhaug, 1952; Marwah *et al.*, 1960; Egelberg, 1966; Levy *et al.*, 1969). This may lead to the formation of epithelial projections into the connective tissue and alter the original morphology of the border area. It is thus theoretically possible that the morphological variations observed in the present study could be due to different degrees of gingival inflammation in the tissues. Since the tissue specimens were taken from both clinically healthy and slightly inflamed gingivae, the microscopic preparations displayed either an absence of inflammation or various degrees of inflammatory infiltrate in the areas of the gingival margin and the dento-gingival junction. However, in no specimen did the inflammatory process extend into the oral aspects of the tissues and it seems unlikely that the observed variations in the morphology of this area should have been caused by some distant effect of the inflammatory process at the dento-gingival junction. On the other hand, the absence of inflammation at the time of biopsy cannot rule out previous experience of pathology. Emslie & Weinmann (1949) and Emslie (1951) observed that the subsurface of gingival epithelium overlying areas with severe chronic inflammation exhibited a coarse honeycomb pattern. From this it could be inferred that the inflammatory changes cause an alteration of the ridge-form to papillae. The present finding of a higher frequency of connective tissue ridges at the gingival margin, where inflammation was regularly present, seems to refute this interpretation.

It is generally accepted that in normal gingiva the epithelium-connective tissue interface at the dento-gingival junction is smooth and that the presence of epithelial proliferations into the connective tissue represents signs of irritation (Sicher, 1966). The present finding of epithelial projections in an otherwise normal crevicular epithelium may possibly be accounted for by previous experience of gingival inflammation indicating that, once formed, the irregular configuration of the epithelium-connective tissue border of the epithelial attachment may persist.

In skin areas not subjected to great mechanical stress (the face, the sides of extremities, the back, etc.), the epithelium-connective tissue interface shows a scanty number of short and thick connective tissue papillae. In skin covering more stressed areas (the sole and back of the foot, the palm and back of the hand, the patella, the olecranon, etc.) the interface is characterized by numerous densely packed, long and slender papillae which at the sole of the foot and the palm of the hand are arranged in rows and connected by low connective tissue ridges (Greb, 1940; Horstmann, 1952).

In some respects the morphology of the epithelium-connective tissue

border of the gingiva resembles that of regions of the skin which are exposed, to great mechanical stress. It has been shown, however, that in the lip, tongue-palate and skin, the configuration of the epithelium-connective tissue interface is in the main established before birth. This indicates that regional variations in the morphology of the epithelium-connective tissue interface may not represent functional adaptations, but are genetically determined (*Fleischauer & Horstmann, 1951; Horstmann, 1954*). Results from heterotopic transplantations of various types of epithelia seem to confirm this stability of the subsurface characteristics in spite of changes in the physical environment and mechanical stress to the tissues (*Billingham & Medawar, 1948a, 1948b, 1950*). However, to what extent the configuration of the epithelium-connective tissue interface is genetically determined or influenced by function is still a matter of conjecture. The finding of morphologic variations in the epithelium-connective tissue interface implies that the area of contact between epithelium and connective tissue varies. It has been suggested that this may reflect different nutritional demands of the epithelium (*Emslie & Weinmann, 1949; Emslie, 1951; Horstmann, 1954; Sicher, 1966*). Evidence to substantiate this theory is sparse (*Horstmann, 1954*) and the present material does not contribute to the elucidation of the problem.

The observation that the gingival groove had no characteristic counterpart at the subsurface of the epithelium is in keeping with *Emslie & Weinmann's* (1949) finding in the monkey. This contradicts the opinion that the epithelium is likely to be involved in the formation of this anatomical feature (*Orban, 1948*) and supports the view that the presence of a gingival groove is dependent on a special arrangement of the collagen fibres arraying from the cementum into the free and attached gingiva (*Ainamo & Löe, 1966*).

The depressions in the surface of keratinized gingival epithelium (the stippling) corresponded to areas of the inner surface where epithelial ridges intersected. This finding is in agreement with those of previous investigators (*Emslie, 1951; Owings, 1964*). It was evident, however, that numerous long and slender connective tissue papillae were associated with specimens showing a prominent stippling. No explanation for this finding is available.

Previous studies have indicated that in the epithelium-connective tissue interface of gingiva no fundamental differences exist between man and monkey (*Emsley, 1951*). In the present study, however, the arrangement in some monkey specimens differed quite definitely from that of the human. The epithelium had the appearance of human connective tissue and was characterized by epithelial pegs. To what extent this is representative cannot be decided from the sparse material investigated. Also the configuration of the epithelium-connective tissue interface of monkey-gingiva seems to vary.

It is generally accepted that what in histological sections of skin and mucous membranes appear to be epithelial pegs, actually are cross-sections of epithelial ridges (*Sicher, 1966; Ham & Leeson, 1961*). The present finding in the monkey of an epithelium-connective tissue interface characterized by epithelial pegs indicates, that this is not always true.

SUMMARY

The morphology of the epithelium-connective tissue interface was studied in three-dimensional wax models ($200\times$) of gingival epithelium and connective tissue of 24 male and 13 female adult Caucasians and three Rhesus monkeys. The models were made from buccal and labial gingival biopsies, comprising the tissue from the gingival margin to the alveolar crest. Measurements were made of the density, height and width of connective tissue papillae and the width of epithelial ridges in each model. The study has demonstrated that the morphology of the epithelium-connective tissue interface varies widely in the adult human gingiva. Most specimens showed conical connective tissue papillae projecting into the epithelium although more or less continuous ridges also occurred. Epithelial pegs were rarely seen. Epithelial pegs connecting two adjacent or distantly spaced epithelial ridges were found in a few specimens. The finding of connective tissue papillae in the area of the crevicular epithelium of non-inflamed gingivae suggests that once formed such papillae may persist. Depressions in the surface of the epithelium (stippling) coincided with intersections of epithelial ridges, whereas the gingival groove could not be related to any structural characteristic at the subsurface of the epithelium. In some monkey specimens the epithelium-connective tissue interface displayed an arrangement characterized by epithelial pegs.

RÉSUMÉ

CONCEPTION TRIDIMENSIONNELLE DE LA LIMITE ÉPITHÉLIUM-TISSU CONJONCTIF DANS LA GENCIVE

La morphologie de l'interface épithélium-tissu conjonctif a été étudiée sur des modèles de cire tridimensionnels ($200\times$) de l'épithélium gingival et du tissu conjonctif de sujets adultes Caucasiens, 24 du sexe masculin et 13 du sexe féminin, et de 3 singes Rhésus. Les modèles ont été faits d'après des biopsies de la gencive vestibulaire, comprenant le tissu depuis le rebord gingival jusqu'à la crête alvéolaire. La densité, la largeur et la hauteur des

papilles du tissu conjonctif et la largeur des rebords épithéliaux de chaque modèle ont été mesurées. Cette étude a montré que la morphologie de l'interface épithélium-tissu conjonctif variait fortement dans la gencive humaine adulte. La plupart des échantillons présentaient des papilles de tissu conjonctif coniques faisant saillie dans l'épithélium; cependant il existait aussi parfois des rebords plus ou moins continus. On voyait rarement des digitations épithéliales. Des digitations épithéliales reliant deux rebords épithéliaux adjacents ou espacés ont été trouvées dans quelques échantillons. Le fait qu'on ait trouvé des papilles de tissu conjonctif dans la région de l'épithélium du sillon gingivo-dentaire dans la gencive non enflammée indiquerait que ces papilles, une fois formées, pourraient persister. Les dépressions à la surface de l'épithélium (aspect granité) coïncidaient avec les intersections des rebords épithéliaux, tandis que la rainure gingivale ne pouvait être mise en relation avec aucune structure caractéristique sous la surface de l'épithélium. Dans quelques échantillons provenant de singes, l'interface épithélium-tissu conjonctif présentait une structure caractérisée par des digitations épithéliales.

ZUSAMMENFASSUNG

DIE DREIDIMENSIONALE MORPHOLOGIE DER GRENZFLÄCHE ZWISCHEN EPITHEL UND BINDEGEWEBE IM ZAHNFLEISCH

Die Morphologie der Grenzfläche zwischen Epithel und Bindegewebe ist in dreidimensionalen Wachsmodellen ($200\times$) vom Epithel und Bindegewebe des Zahnfleisches von 24 männlichen und 13 weiblichen erwachsenen Kaukasien und drei Rhesus-Affen untersucht worden. Die Modelle wurden hergestellt von buccalen und labialen Zahnfleischpräparaten, die das Gewebe zwischen dem Zahnfleischrand und dem Rand des Alveolarknochens enthalten.

Messungen der Dichte, Höhe und Dicke der Bindegewebspapillen und Dicke der Epithelleisten wurden in jedem Modell vorgenommen. Die Untersuchung hat ergeben, dass die Morphologie der Grenzfläche zwischen Epithel und Bindegewebe im Zahnfleisch des erwachsenen Menschen sehr verschiedenartig ist. Die meisten Präparate wiesen zahlreiche im Epithel hervorragende kegelförmige Papillen auf, obwohl auch mehr oder weniger ununterbrochene Leisten ersichtlich waren.

Epithelzapfen kamen nur selten vor. In wenigen Präparaten erschienen Epithelzapfen, die zwei nahe oder ferner liegende Epithelleisten verbanden.

Das Vorkommen der Bindegewebspapillen im Gebiet des inneren Saum-

epithels in gesundem Zahnfleisch lässt vermuten, dass, einmal gebildet, solche Papillen bestehend bleiben. Kleine Vertiefungen in der Oberfläche des Epithels (Stippelung) entsprachen Schnittpunkten zwischen Epithelleisten, während die Furche am äusseren Saumepithel keiner strukturellen Anordnung auf der Epithelunterseite entsprach. In einigen der Präparate von Affen war das Grenzflächenrelief von zahlreichen Epitelzapfen charakterisiert.

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Address:

*The Dept. of Periodontology,
The Royal Dental College,
DK-8000 Aarhus C, Denmark*