Ultrastructural study of induced keratinization in sulcular gingival epithelium in rhesus monkeys

Einar K. Kristoffersen, Raul G. Caffesse, Carlos E. Nasjleti and Tore Ø. Kristoffersen

Department of Periodontology, School of Dentistry, University of Bergen, Bergen, Norway, and Department of Periodontology, School of Dentistry, University of Michigan, Ann Arbor, Michigan, USA

Kristoffersen, E. K., Caffesse, R. G., Nasjleti, C. E. & Kristoffersen, T. Ø. Ultrastructural study of induced keratinization in sulcular gingival epithelium in rhesus monkeys. *Acta Odontol. Scand.* 1983, 41, 227–240. Oslo. ISSN 0001–6357.

Gingival sulcular epithelium—which in primates normally does not keratinize—can be induced to undergo keratinization by prolonged intensive antibacterial therapy. Three months before biopsy the teeth of two adult male rhesus monkeys were scaled and polished, and for 5 days the monkeys were given intravenous injections of 75 mg Achromycin® daily. Their teeth were subsequently subjected to daily cleaning and polishing. The presumably in-situ-keratinized sulcular epithelium was examined by transmission electron microscopy. It was also compared with oral gingival epithelium from the same two animals and with oral and sulcular epithelium from a rhesus monkey that had not been exposed to local or systemic antibacterial therapy. The results confirmed earlier histological studies, which have shown that under the conditions described the sulcular epithelium becomes parakeratinized. In addition, several other ultrastructural changes were observed, some of which suggest that the treatment given may result in the formation of a more efficient permeability barrier in the sulcular area. The possible clinical significance of such a barrier is briefly discussed. □ *Ultrastructure; keratinization; gingival epithelium; rhesus monkey*

Tore Ø. Kristoffersen, Department of Periodontology, School of Dentistry, University of Bergen, Årstadvei 17, N-5000 Bergen, Norway

The epithelium of the gingival sulcus in primates normally does not achieve the same degree of keratinization as does the epithelium covering the oral surface of the gingiva (17, 30). The keratinizing potential of gingival sulcular epithelium in rhesus monkeys has been studied in a series of publications (2–5, 15). When the sulcular epithelium was exposed to the oral environment through surgical procedures and left in this position for 2 to 3 weeks, it underwent keratinization (3). Intense antibacterial therapy consisting of mechanical removal of soft and calcified bacterial deposits (scaling), systemic antibiotic therapy for 1 week, and daily polishing of teeth for several weeks also induced increased keratinization of the sulcular epithelium in situ (5). In these investigations the process of keratinization was studied by histological methods. No systematic ultrastructural investigation of such 'induced' keratinization of the gingival sulcular epithelium has been reported.

In the present study, presumably in-situ-keratinized sulcular epithelium from rhesus monkey has been studied by transmission electron microscopy. It was also compared with homologous oral gingival epithelium and with oral and sulcular epithelium from a rhesus monkey that had not been exposed to treatment aimed to increase gingival keratinization.

Materials and methods

Three adult male rhesus monkeys (*Macaca mulatta*), each weighing approximately 6.8 kg, were used in this study. All of them had complete dentitions. A mild generalized gingivitis and a moderate accumulation of calculus were originally present.

Three months before biopsy the teeth of two of the monkeys (ME1 and ME2) were scaled and polished with rubber cups using Nupro® paste. In addition, a 5-day antibiotic

treatment with tetracycline hydrochloride (Achromycin®), 75 mg intravenously daily, was given. After the termination of the antibiotic treatment the teeth of these monkeys were subjected to daily polishing and cleaning with rubber cups, using Nupro paste for 3 months (the therapy was not performed during week-ends). The third monkey did not receive any local or systemic antibacterial therapy and served as untreated control (MC3).

The initial scaling, daily prophylaxis, and biopsy were carried out using ketamine hydrochloride, 200 mg intramuscularly, as a sedative. Biopsies were taken from the buccal area of the lower incisors. They were immediately cut in pieces approximately 2 mm wide and fixed for 1 h at 4°C in 1% paraformaldehyde and 1.25% glutaraldehyde, buffered with 1 M phosphate buffer to pH 7.2 and containing 1% sucrose to adjust the tonicity to 380 mOsmol. The tissue pieces were postfixed for 1 h at 4°C in 1% OsO_4, adjusted for pH and tonicity as described above. After this they were taken through successively increasing concentrations of ethanol for dehydration. Specimens were then infiltrated with propylene oxide, embedded in Epon (19), and sectioned on a Porter-Blum MT-1 or an LKB 8800 ultramicrotome. The sections were picked up on copper grids, stained with uranyl acetate and lead citrate, and examined in a Zeiss EM 9S or a JEOL JEM-100 B electron microscope.

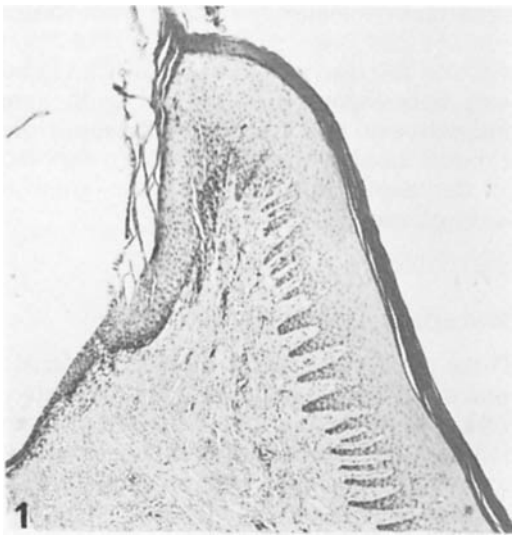


Fig. 1. Rhodamine-B-stained section of gingiva of rhesus monkey ME1. Dark (red) staining parakeratinized layer extending well down into the sulcus. (×40.)

For histological examination portions of representative biopsy specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm, and stained with rhodamine B (6), hematoxylin-eosin, or Mallory's trichrome stain.

Results

At the termination of the antibacterial therapy, examination of histological sections from the experimental animals ME1 and ME2 revealed a clearly defined parakeratinized layer extending well into the gingival sulcus (Fig. 1). In paraffin-embedded sections, but not in those embedded in Epon, the superficial layers that stained red with rhodamine B tended to split up and fray out from the surface (Fig. 1). In the untreated animal, MC3, no histological evidence of formation of a keratinized layer in the sulcular epithelium (SE) was found in our specimens. The oral epithelium (OE) in all monkeys appeared to be orthokeratinized. In ME1 and ME2 few inflammatory cells could be seen subjacent to the sulcular epithelium. A sparse inflammatory cell infiltrate was seen immediately under the junctional epithelium (JE) in these monkeys. In MC3 a widespread inflammatory infiltrate was evident approximating the sulcular and junctional epithelia.

The ultrastructural observations will be described sequentially, starting at the connective tissue interface and moving towards the epithelial surface. For the SE the description will be focused on the midportion of the SE—that is, an area midway between the gingival crest and the bottom of the sulcus. For the OE an area localized at approximately the same distance from the gingival crest was studied.

Basal lamina and adjacent connective tissue

A well-defined, continuous basal lamina was found in both the sulcular and oral

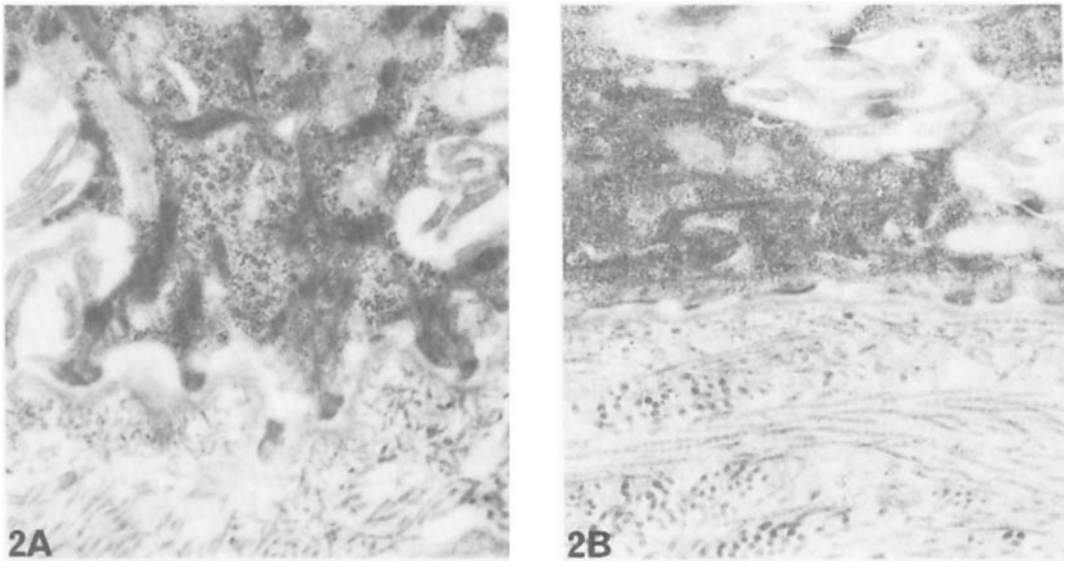


Fig. 2. Stratum basale–connective tissue interface from experimental animal ME1. (A) Oral epithelium. Well-defined, continuous basal lamina with hemidesmosomes. Anchoring fibrils in the intermediate zone between well-organized fibers of the connective tissue and basal lamina. Interdigitating microvilli along cell circumference. High density of free ribosomes, filaments, and dense filament bundles. (×20,000.) (B) Sulcular epithelium. Structure similar to that of OE. (×20,000.)

regions of ME1 and ME2 (Fig. 2). An intermediate layer between the basal lamina and the well-oriented collagen fibrils could also be observed. This layer contained small, randomly oriented, curved anchoring fibrils. A few scattered medium-sized lymphocytes and occasional macrophages were seen in the connective tissue underlying the SE (Fig. 3B). In MC3 the region of the OE exhibited a similar structure. However, the basal

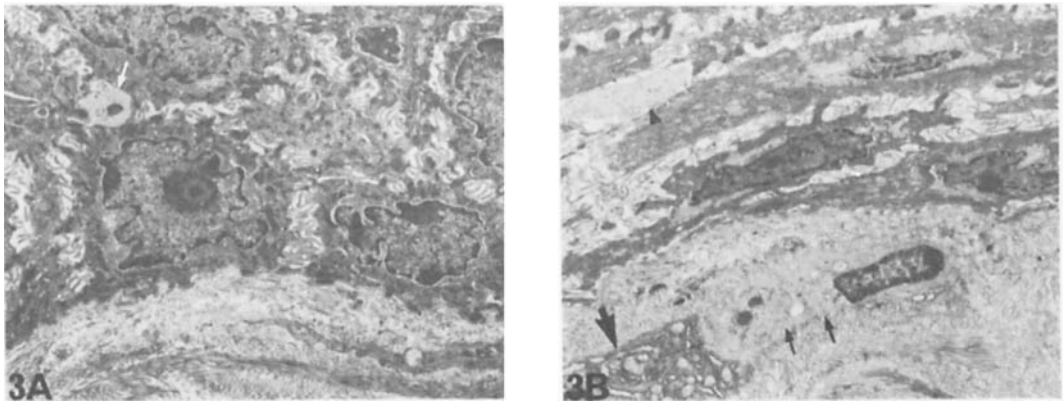
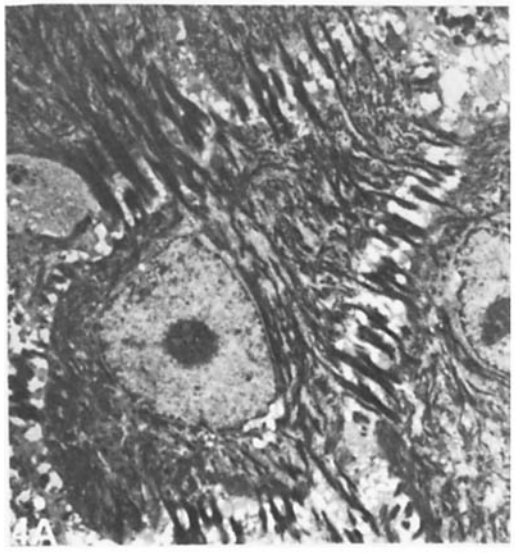


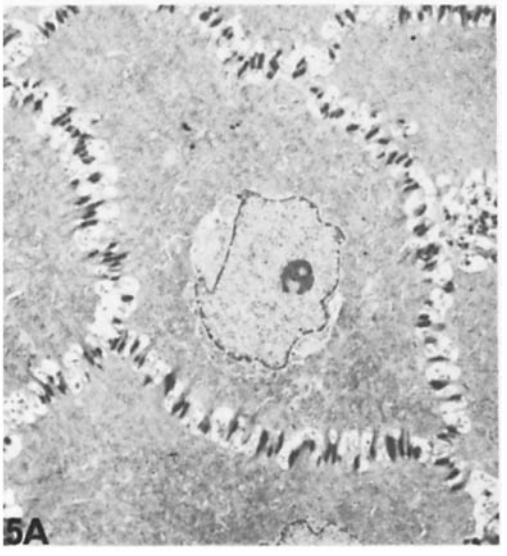
Fig. 3. Basal-suprabasal layers from experimental animal ME1. (A) Oral epithelium. Cuboidal cells. Dense filament bundles. Relatively few desmosomes along cell circumference. A projection of a non-keratinocyte at upper left (arrow). (×3600.) (B) Sulcular epithelium. Slightly flattened cells. Note bundles of fibrils in cytoplasm. Few desmosomes. Projection of a non-keratinocyte (arrow head). A cell with structural characteristics of a medium-sized lymphocyte (small arrows) in close approximation with a fibroblast (only parts of the latter is seen; large arrow) in the connective tissue. (×3600.)

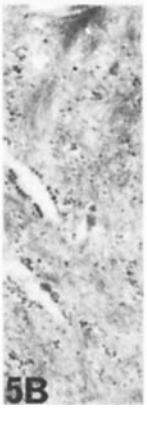
lamina of the SE in MC3 sometimes appeared discontinuous, and underlying connective tissues showed fewer and less well oriented collagen fibrils and focal dense inflammatory infiltrates of mostly medium-sized lymphocytes, macrophages, and small lymphocytes.

Stratum basale/suprabasale

Basal cells in both OE and SE had much







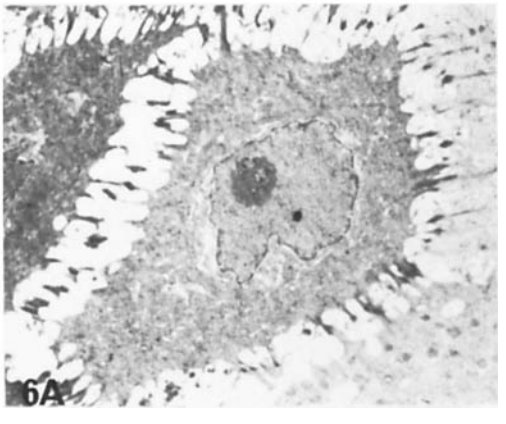


Fig. 4. Lower spinous layer, oral epithelium, experimental animal ME2. (A) Cell of the lower spinous layer. Note pattern of filament bundles. Numerous desmosomes along the periphery. Part of a projection of a melanocyte visible at left. (×3300.) (B) Same cell as in *A*. Longitudinal and cross-sections of filament bundles. Many monosomes and polysomes. (×15,000.)

Fig. 5. Lower intermediate layer, sulcular epithelium, ME2. (A) Characteristic cell of lower intermediate layer. Filament bundles are less electron-dense than in Fig. 4A. Numerous desmosomes. (×3300.) (B) Same cell as in Fig. 5A. Meshwork and relatively electron-lucent bundles of fibrils. RER and ribosomes. (×15,000.)

Fig. 6. Lower intermediate layer, sulcular epithelium, control animal MC3. (A) Cell of lower intermediate layer. Wide intercellular spaces, apparently fewer desmosomes along circumference than in Figs. 4 and 5. (×3300.) (B) Same cell as Fig. 6A. Loose filament meshwork. RER, monosomes, and polysomes. A membrane-coating granule (arrow). (×15,000.)

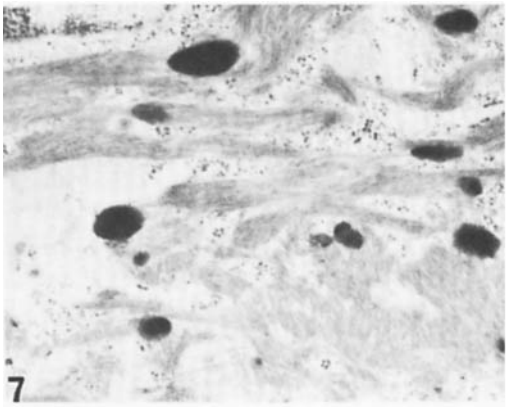
the same appearance in all specimens (Fig. 3). They were usually cuboidal with interdigitating microvilli. In SE from all monkeys basal cells sometimes appeared more flattened than in OE. Along the basal surface several hemidesmosomes could be seen at random intervals. The cytoplasm contained an abundance of rough endoplasmic reticulum (RER) and free ribosomes and moderate amounts of mitochondria and filaments. In basal cells of OE, filaments were characteristically aggregated into filament bundles (Fig. 2A). The filaments of sulcular basal cells were also mostly aggregated in bundles, somewhat less electron-dense than in the OE. Filaments appeared to be present in approximately equal amounts in oral and sulcular basal cells.

Relatively few desmosomes could be seen at the cell circumference in the basal layers of all epithelia. The intercellular spaces appeared somewhat wider in the SE of MC3 than in the experimental animals. A suprabasal layer was variable and was most readily seen in the OE.

Stratum spinosum and corresponding intermediate layers

These cell layers constituted the largest portion of all epithelia, particularly in the OE. The cells were mostly diamond-shaped but became increasingly flattened towards the surface (Figs. 4A, 5A, and 6A). The cytoplasm was dominated by RER, free monosomes and polysomes, and by a great number of tonofilaments, other cell organelles present such as mitochondria and Golgi apparatus being almost obscured by these structures. In this stratum membrane-coating granules (MCG) could be observed in all epithelia (Fig. 6B). The OE filaments, characteristically condensed into dense bundles, formed conspicuous networks around the nuclei and at the periphery in association with desmosomes (Fig. 4). In the SE they were more evenly distributed throughout the cytoplasm, and filament bundles appeared less electron-dense. The number of filaments appeared to be lower in the SE of MC3 (Fig. 6) than in corresponding epithelium in ME1 and ME2 (Fig. 5). Desmosomes, which were greatly increased in number, attained their maximum length in these cell layers. They appeared to be shorter in the SE than in the OE of all monkeys and shorter and less numerous in the SE of MC3 than in the experimental animals (Figs. 13A, 14A, and 15A).

The width of the intercellular spaces also



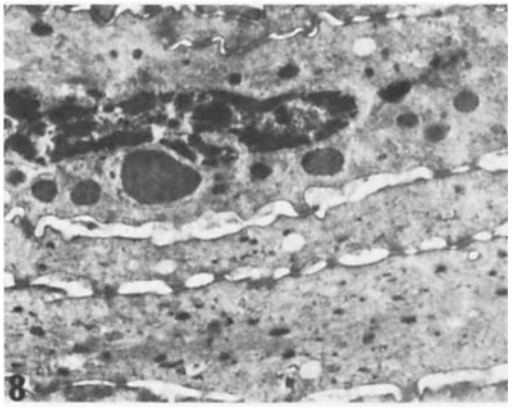


Fig. 7. Stratum granulosum, oral epithelium, experimental monkey ME2. Dense filament bundles and numerous dense, filament-associated keratohyaline granules. RER, monosomes, and polysomes. (×24,000.)

Fig. 8. From transitional zone between intermediate and surface layers, sulcular epithelium, monkey ME1. Cells in lower half of the picture contain many dense filament-associated granules. In the cell in which part of nucleus is seen, many granules are large, globular, and less electron-dense. Several are surrounded by an electron-lucent zone in which at higher magnification ribosomes were often seen. (×8500.)

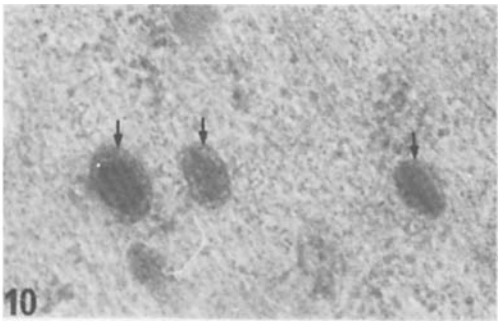


Fig. 9. Junction subsurface/surface layers, sulcular epithelium from monkey ME2. Note lipid droplets, thickened cytoplasmic membrane, and granular amorphous material in intercellular space (arrows). (×20,000.)

Fig. 10. Subsurface layer, sulcular epithelium from control animal. Membrane-coating granules (arrows). Meshwork of filaments not readily discernible in this picture. (×60,000.)

attained its maximum in these cell layers in all epithelia, being similar in SE and OE of ME1 and ME2 (Figs. 4A and 5A) but considerably greater in the SE of MC3 than in the other epithelia (Fig. 6A).

Stratum granulosum and corresponding subsurface layers

A characteristic granular layer was seen in the OE in all specimens (Figs. 7 and 11A). Keratohyaline granules were irregularly shaped, mostly ovoid, and usually associated with filaments (Fig. 7). In the SE a distinct stratum granulosum could generally not be observed. In ME1 and ME2, however, scattered keratohyaline granules, similar to but fewer than those in the OE, were seen (Fig. 8). Membrane-coating granules of the variety characteristic of non-keratinizing epithelium (37) were evident particularly in SE of MC3 (Fig. 10). Cells in the upper intermediate layer of the SE became gradually more flattened and elongated and exhibited numerous closely interdigitating microvilli (Fig. 9). The desmosomes, which in the SE of all animals appeared to decrease in length towards the surface (Figs. 15B and 16B), were oriented at an angle to the surface owing to the projecting microvilli (Fig. 9).

Fig. 11. Upper cell layers of oral epithelium, experimental animal ME1. (A) Upper stratum spinosum, stratum granulosum, and stratum corneum. Abrupt change from stratum granulosum to stratum corneum. Note narrow, dense orthokeratinized layer. (×3600.) (B) Keratin pattern of the same epithelium. Homogeneous electron-dense mass, lacking filament patterns. (×100,000.)

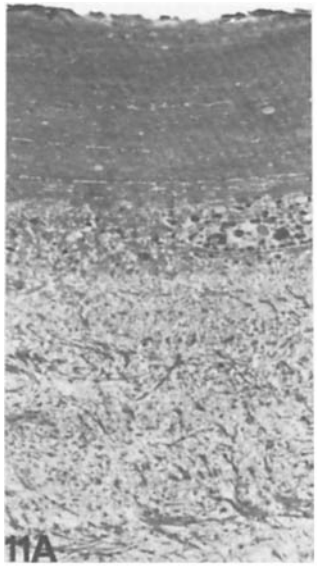
Fig. 12. Surface and subsurface layers of sulcular epithelium from experimental animal ME2. (A) Note gradual transition between layers and greater width of surface layer than in Fig. 11A. Surface layer appears dense and homogeneous with narrow interproximal spaces. Surface cells less electron-dense. Note granules in cells in subsurface layer (cf. Fig. 8). (×3500.) (B) From another specimen, same animal. Gradual transition. Cells in surface layer vary in electron density. Narrow intercellular spaces, interdigitating microvilli even near surface. A pale, apparently swollen cell on the very surface. (×3500.) (C) Filament pattern of typical, relatively electron-dense cell of surface layer. Filaments and free ribosomes can be distinguished. (×100,000.) (D) Filament pattern of less electron-dense cell of surface layer. Filaments are more loosely arranged. (×100,000.)

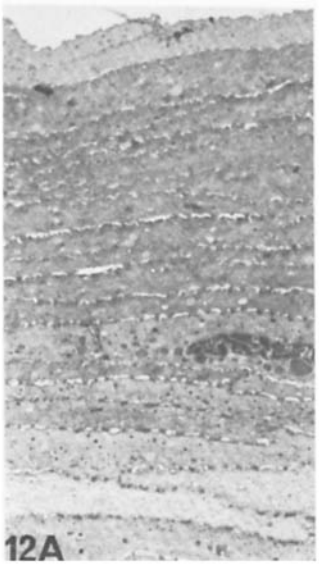
Fig. 13. Surface and subsurface layers of sulcular epithelium from control animal. (A) Both layers consist of flattened cells varying in electron density. Narrow intercellular spaces and apparently well-organized and well-preserved structure were unusual among our control specimens. (×3500.) (B) Commoner pattern of relatively irregular, flattened cells with long microvilli extending into a wide intercellular space. In most sections accumulations of granulocytes, lymphocytes, and monocytes were also observed (not shown here). (×3500.) (C) Filament pattern most commonly observed in cells of the surface layer. Scattered filaments and free ribosomes. (×100,000.)

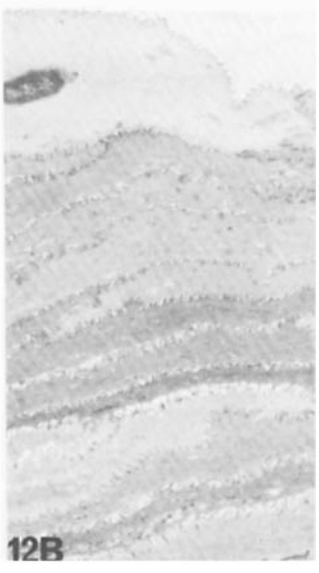
The cell membranes in the oral granular layer, in contrast, showed a more even contour with much less pronounced formation of microvilli. The desmosomes were more parallel to the epithelial surface and were more variable with respect to their length than those of the SE (Fig. 14B).

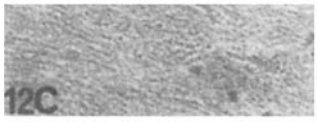
Both in SE and OE cell membranes of this layer showed a thickening (Figs. 14–16), and intercellular spaces appeared narrower than in the deeper layers. In the control animal the SE usually exhibited wider intercellular spaces and less pronounced membrane thickening than in the experimental animals (Figs. 13 and 16).

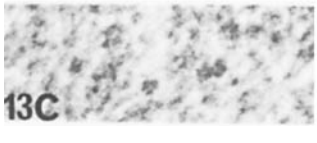
The number of organelles such as mitochondria and RER decreased markedly towards the surface in all epithelia. Several

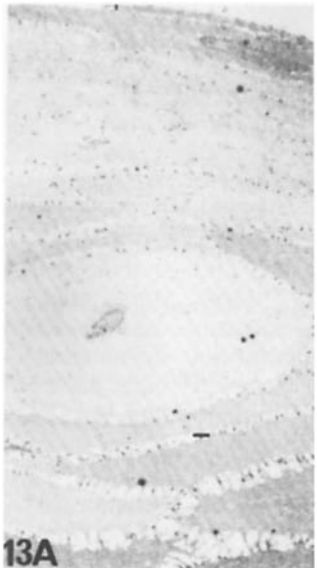


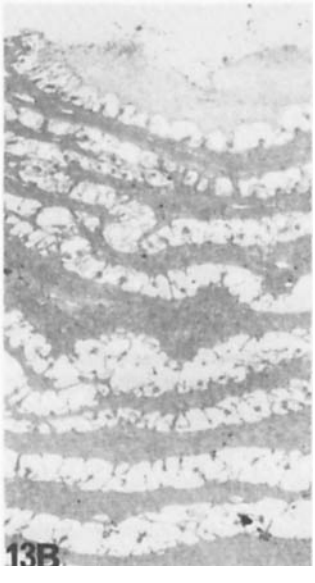












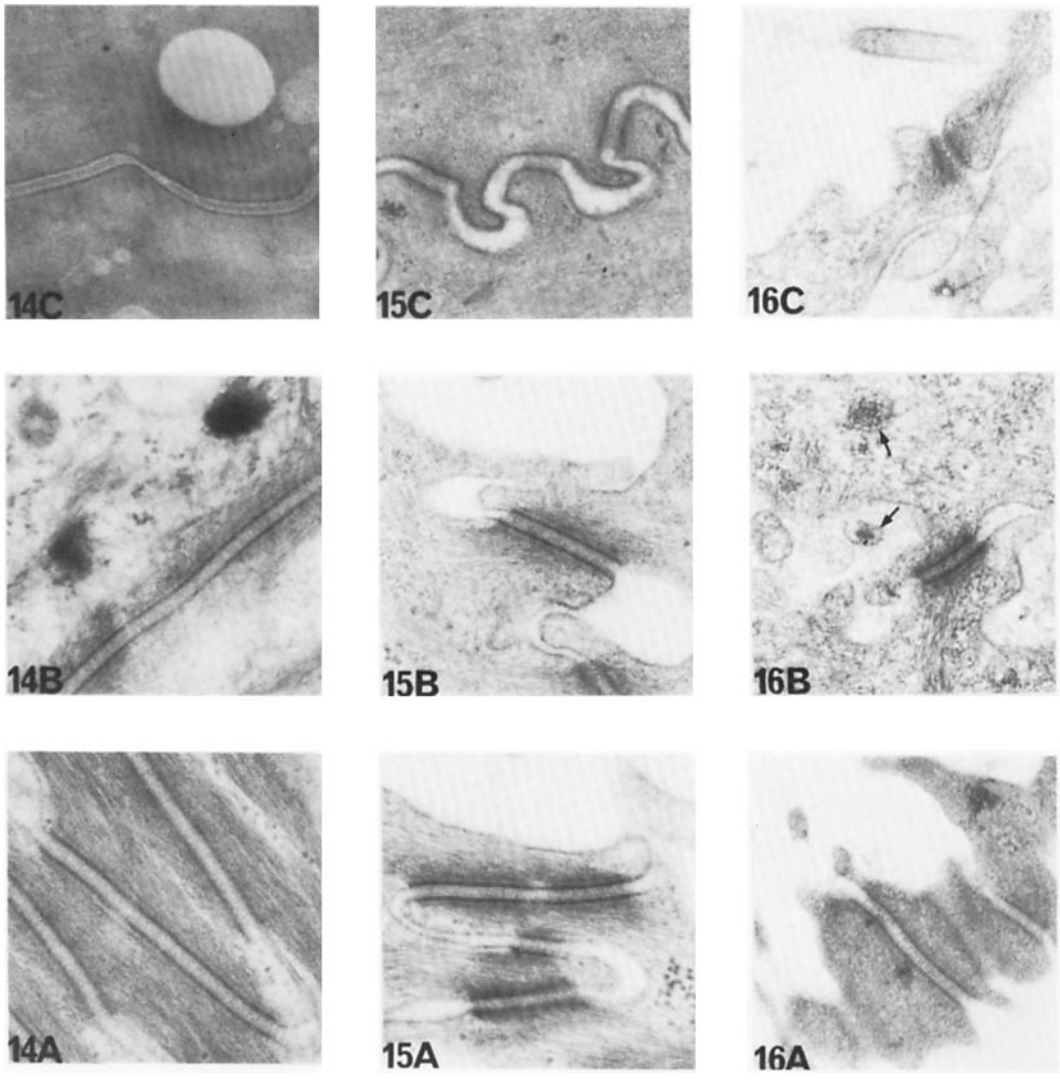
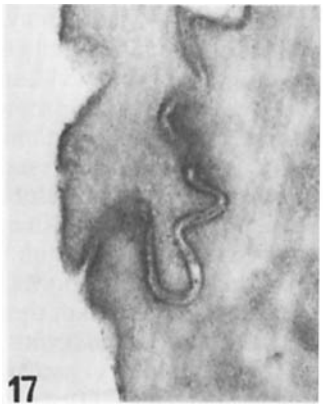


Fig. 14. Desmosomes in various layers of oral epithelium. (All magnifications, ×40,000.) (A) Stratum spinosum. (B) Stratum granulosum. Note filament-associated keratohyaline granules and membrane-coating granule. (C) Stratum corneum. Note granular, dense cytoplasm and 'empty body'.

Fig. 15. Desmosomes from various layers in sulcular epithelium, experimental animal. (All magnifications, ×40,000.) (A) Lower intermediate layer. Slightly widened intercellular space. (B) Subsurface layer. Widened intercellular spaces. (C) Surface layer. Relatively short desmosomes, thickened cytoplasmic membrane. Note 'blurred' appearance of interlamellar plates. Visible filaments in cytoplasm.

Fig. 16. Desmosomes from various layers of sulcular epithelium, control animal. (All magnifications, ×40,000.) (A) Lower intermediate layer. Note wide intercellular space. (B) Subsurface layer. Note the contents in the widened intercellular space, including round or oval bodies surrounded by membranes. Membrane-coating granule in cytoplasm and intercellularly (arrows). (C) Surface layer. Short desmosomes, extremely wide intercellular space.



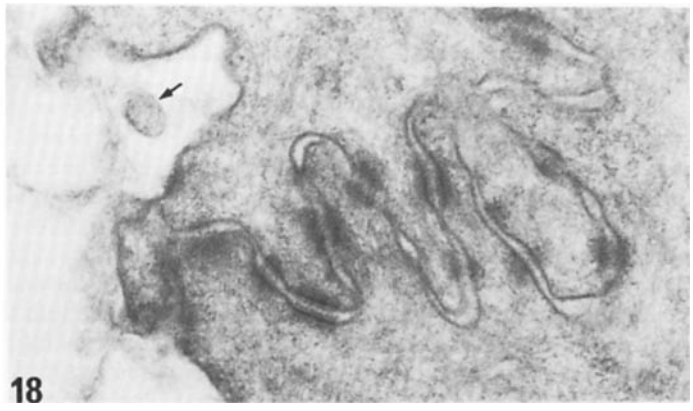


Fig. 17. Junction between surface cells of oral epithelium, experimental animal (surface at left). (×44,000.)

Fig. 18. Junction between surface cells of sulcular epithelium, experimental animal (surface at left). Note thickened cytoplasmic membrane, granular contents of intercellular space, gap junction, many desmosomes. A lamellated or striated round body is seen at the very surface (arrow). (×40,000.)

lipid droplets and 'empty bodies' appeared (Fig. 9), and especially in the SE of the control animal, MC3, glycogen storage granules became apparent. The cytoplasm contained large quantities of tonofilaments, which in the OE were arranged in dense, electron-opaque bundles (Fig. 7). In the SE filaments were much more evenly distributed in the cytoplasm (Figs. 7, 9 and 10).

In the SE of MC3 electron-lucent cells, some of which appeared swollen, were occasionally seen interspersed between other cells of the subsurface layer (Fig. 13A).

Stratum corneum and corresponding surface layers

In the OE the transition between the granular and the keratin layers was characteristically abrupt (Fig. 11A). The cytoplasm of the flat, elongated keratinized oral epithelial cells was markedly electron-dense and homogeneous, containing only scattered 'empty bodies' and few or no remnants of cell organelles (Fig. 11B). Individual filaments were not discernible, and no cell nuclei were seen. Desmosomes were generally long and the intercellular spaces narrow, on rare occasions exhibiting apparent fusion of outer leaflets of membranes (Fig. 14C). Desmosomes and gap junctions were seen connecting adjacent surface cells (Figs. 14C and 17).

In the SE of ME1 and ME2 the transition between the deeper cell layers and the surface layers appeared more gradual than in the OE (Figs. 12A and B). The cells appeared less flattened and showed variable electron density but were always less electron-dense than in the oral keratin layers. The surface layer was considerably wider than the stratum corneum of the OE. Filaments were generally more densely packed than in the subjacent layers but less condensed and more readily discernible than in the oral stratum corneum. However, the cells exhibited highly variable degrees of filament density. The majority were filament-rich and electron-dense, but many cells were lighter, with less densely packed or even loosely arranged filaments (Figs. 12C and D). Cell nuclei were present in many cells throughout this stratum. They were mostly shrunken and indented, and various organelles, especially ribosomes, could be demonstrated even in the surface layers. Interdigitating microvilli remained a dominant feature of the cell circumference (Fig. 15C). Desmosomes became even shorter than in the deeper layers and also often showed morphological changes; the interlamellar layer gradually became indistinct, and the

two electron-dense lamellar plates became continuous with the thickened cell membrane (Fig. 15C). They could, however, be readily recognized, and even adjoining surface cells were connected by desmosomes and gap junctions (Fig. 18). Intercellular spaces were wider than in the corresponding layers of the oral epithelium. Cells on the very surface often appeared swollen and electron-lucent, with markedly thickened cell membranes (Fig. 12B). Occasional globular, often striated, granules were seen in the intercellular spaces, even up to the very surface (Fig. 18).

Considerable interspecimen variation was seen in the surface layers of the SE of MC3 (Figs. 13A and B). The cells frequently appeared electron-lucent and contained granular material, loosely arranged filaments (Fig. 13C), mitochondria, and various amounts of vacuoles. Intercellular spaces were fairly wide almost up to the very surface in most specimens, with apparently relatively few desmosomes, whereas in others the spaces were narrower (Figs. 13A and B and 16C). Finely granular material and sometimes striated or lamellated globular structures appeared intercellularly (Figs. 16B and C). Not all such globular structures could be positively identified. Membrane thickening was generally less pronounced than in the experimental animals.

Non-keratinocytes

Non-keratinocytes constituted a small proportion of the cell populations in all epithelia, except in localized areas in the SE of MC3, where accumulations of neutrophil granulocytes, monocytes, and macrophages and particularly small and medium-sized lymphocytes were observed.

Discussion

Differentiation of oral epithelia is primarily genetically determined but may be modulated, especially through the influence of the underlying connective tissue (for review, see Ref. 29). It is also well established that environmental factors such as smoking and even mechanical factors such as friction may influence some expressions of epithelial differentiation, including the tendency to keratinize (20, 26). Observations from transplantation studies (22) suggest that site-specific connective tissue may be necessary for the expression of the overall tissue architecture but may not have a decisive influence on the degree of keratinization. For keratinization of the sulcular gingival epithelium the importance of the state of the underlying connective tissue bed with respect to the degree of inflammatory change has been emphasized by Ten Cate (40) and by Gelfand et al. (11). Others, while emphasizing the role of inflammatory change in the connective tissue (2, 5, 15), have also pointed out the role played by environmental factors (3, 4). To what extent environmental factors such as bacterial accumulations exert their modulating influence by altering the connective tissue bed or through direct effects on epithelial cells is not known and could not be assessed in the present study.

Several previous studies have described histologically the in vivo changes in gingival sulcular epithelium which may be induced by antibacterial therapy in the monkey (2, 5, 15) and in man (1, 10). In monkeys these changes are characteristic of parakeratinization (2, 5), the surface area of parakeratinized epithelium increasing with time to cover the entire sulcular epithelium to the junctional epithelium before the end of a 40-day experiment (2). Only one report has been found in which ultrastructural features of sulcular epithelium in individuals who had or had not received antibacterial therapy (scaling, root planing, oral physiotherapy) were briefly compared (39). The appearance of keratohyaline granules, the more frequent occurrence of typical MCG, and the disappearance of signs of degeneration were taken as evidence of 'a stronger trend toward keratinization' (39).

The present study has produced ultrastructural confirmation that antibacterial therapy may influence the end product of epithelial differentiation in gingival sulcular epithelium in rhesus monkeys. Our observations are in accordance with the conclusion from histological investigations characteriz-

ing the surface layers as being parakeratinized (2, 5). In addition, several observations were made that may contribute to a better understanding of the patterns of differentiation in sulcular epithelium under various conditions and their possible clinical significance.

In accordance with findings of other investigators (17, 39), cells of the basal and suprabasal layers of the various epithelia studied exhibited certain differences but also strikingly similar features. Thus, the number of desmosomes interconnecting these cells appeared to be lower than in more superficial strata in all epithelia. The size of the nuclei and the cytoplasmic content were also largely similar. The amount of filaments and the tendency for them to be arranged in bundles were a particularly characteristic common feature.

In the strata superficial to the suprabasal layers the similarities were much less prominent. In particular, the organization and appearance of tonofilaments were different in the OE and the SE. In the OE the filament bundles remained compact and electron-dense throughout the spinous layer. According to Schroeder (29) this structural feature in the human oral cavity is characteristically seen in epithelia from the hard palate and oral surface of the gingiva. These epithelia exhibit a differentiation pattern of type 'A' according to Schroeder's classification (29). The end product of this pattern in oral gingival epithelium is a characteristic stratum corneum in which the typical corneocyte exhibits a keratin pattern termed 'A1', characterized by a homogeneous, electron-dense mass lacking filament profiles. The ultrastructural characteristics of the OE of our specimens were in good agreement with those reported for oral gingival epithelium by other investigators (17, 18, 29, 34) and coincide with Schroeder's type 'A' differentiation pattern (29).

In contrast to the findings in OE, the filament bundles in the layers immediately distal to the suprabasal cells in the SE both in the control and in experimental animals became less electron dense and tended to blend into a meshwork of more loosely arranged filaments. This change in the arrangement of filaments from the basal layers to the deeper layers of the intermediate strata resembles that seen in oral epithelia that exhibit differentiation patterns such as Schroeder's type 'B' and 'BC' (29, 31), e.g. those of the alveolar and buccal mucosa. In such epithelia this change appears to represent the 'first and decisive step of cytodifferentiation' (29). With respect to this particular feature, then, the SE of the experimental animals, in spite of the altered structure of the surface layers, did not show appreciable modulation in the deeper layers. The significance of the observation is uncertain. It may be a point in favor of the view that the modulation of the surface characteristics to some extent occurs as the result of a direct influence on the epithelial cells rather than indirectly by altering the connective tissue bed.

The specific reasons for the difference between OE and SE with respect to the pattern of differentiation, including the tendency for filaments to aggregate into bundles in intermediate and upper strata, are not known. Dale et al. (7) have recently presented evidence that a 'stratum corneum basic protein' (SCBP) is an interfilamentous or matrix protein for epidermal keratin. Its phosphorylated precursor must be activated by proteolysis and/or dephosphorylization. The active protein was shown to effect the formation of filament bundles or 'macrofibrils' in a suspension of keratin filaments. The degree of condensation and organization increased with increasing concentrations of SCBP (7). It is not known at present to what extent the organization of keratin filaments into macrofibrils in oral epithelium is influenced by materials such as SCBP in vivo, whether such substances are present and active in all epithelial layers, or to what extent their concentration or activity is strictly dependent on connective tissue specificity and genetic influence or may be influenced by environmental factors, such as bacterial products or other substances of the gingival area.

Moving toward the surface, the SE in the two experimental animals showed structural characteristics that—although no quantitative data were obtained and no identity is implied—in some respects appeared similar

to those described by Schroeder (29) for the type 'B' epithelium of alveolar mucosa. These characteristics included the lack of condensation of filaments into bundles, the appearance of MCG and thickening of cytoplasmic membranes in the upper intermediate layers, and the wide surface layer exhibiting tickened cytoplasmic membranes, relatively narrow intercellular spaces containing an amorphous granular material and cytoplasmic contents of variable electron density, in which filaments, ribosomes, and occasional lipid droplets were seen.

The SE in the control animal showed a greater variability of all cell layers than what was observed in the experimental animals. Local differences in the environment probably account for a great deal of this variability. There was also considerable variability within cell layers of the same specimen. The variable appearance complicates a uniform description or classification of this epithelium.

The observation that desmosomes of the sulcular epithelium varied in length, being shortest in the basal and surface layers and longer in the intermediate layers, is in agreement with the observation of Geisenheimer & Han (12). The numeric density of desmosomes has also been reported to vary among various strata in most oral epithelia, usually being low in the basal layers and higher in the more superficial layers (29), in accordance with our observations. The significance of these phenomena is not entirely understood. It would appear, however, that a high density of cell attachments in the surface layers would favor the structural integrity of the epithelium.

Structural changes in desmosomes in surface layers or oral epithelia have also been reported by others (17, 18, 22), although they have not been consistently found in oral gingival epithelium (25, 29). The significance of such changes remains speculative.

The possible clinical significance of inducing keratinization of the sulcular epithelium has recently been discussed by Squier (36). He suggests that this may be a pointless pursuit, mainly since even non-keratinized epithelia may offer resistance to the penetration of many substances (35, 38) and since keratinization of the highly permeable (28, 30) junctional epithelium may lead to loss of epithelial attachment to the tooth surface and thus be harmful. Although this latter point may be well taken, it should be kept in mind that in spite of the intensive antibacterial therapy over considerable periods of time in this and other (2, 5, 32) studies, keratinization of the junctional epithelium in situ has never been reported as a result of antibacterial therapy and may not be readily achieved (30, 41).

Our investigation has pointed out structural alterations in the experimental animals—other than the degree of keratinization—which may be important in creating a more effective permeability barrier in the sulcular region, as indicated by histological observations of staining reactions and morphology. Although an orthokeratinized stratum corneum probably constitutes a highly impermeable membrane (9), several studies indicate that a significant permeability barrier is present in the stratum granulosum of keratinized epithelia and in corresponding layers of non-keratinized oral epithelia (9, 35, 38). This barrier coincides with the appearance of MCG, which release their contents into the intercellular spaces, (16) and often with thickened cytoplasmic membranes or 'marginal band' (13). The frequent occurrence of MCG and possibly the granular, although sometimes striated or lamellated, globular contents of the intercellular spaces in our control specimens of sulcular epithelium may indicate a tendency to form a permeability barrier (35, 38). The extreme width of the intercellular spaces in most control specimens inspires little faith in the barrier function of this epithelium, however. In the experimental animals the process of forming a permeability barrier appeared to have proceeded further in the surface layers. Membrane-coating granules were more rarely seen than in the deeper strata, possibly indicating that their contents had been discharged into the intercellular spaces (35, 38), which were lined by markedly thickened cytoplasmic membranes. The permeability of such a sulcular epithelium remains to be investigated, but it seems likely that it would be comparable to that of other

epithelia with a similar structure and different from sulcular epithelia such as that seen in our control specimens.

The sulcular epithelium is closely related to and partially formed by the extension of the oral gingival epithelium (17). Our experimental procedure had induced considerable structural changes, particularly in the upper layers of the sulcular epithelium. Nevertheless, the oral and the sulcular epithelial leaflets differed in many respects. The tendency for filaments to aggregate into electron-dense bundles was markedly more pronounced in the oral epithelium in all except the basal layers. The desmosomes were longer even in the spinous layer and tended to increase in length towards the surface, whereas in the sulcular epithelium they often became shorter and less numerous, with wider intercellular spaces. Finally, the cytoplasmic contents of the surface cells also showed characteristic differences. Some of these findings suggest that the parakeratinized sulcular epithelium may be more permeable than the orthokeratinized oral epithelium.

The possible role of a permeability barrier in the pathogenesis or the prevention of gingival inflammation is at present conjectural (8), and the permeability of the sulcular epithelium has not been specifically investigated. It is conceivable that reduced permeability may retard the development of inflammatory changes by preventing or retarding a penetration of noxious plaque products. Mattson & Attström (23) and Mattson et al. (24) have suggested that structural differences of possible significance for the epithelial permeability may contribute to the difference in the rate of development of experimental gingivitis in young and old individuals. Moreover, Schroeder et al. (23) found that one of three dogs in which a slight lymphoid inflammatory infiltrate was present initially showed a similar but more rapid development of experimental gingivitis than dogs without such pre-existing infiltrates. The authors ascribed these observations primarily to the pre-existing infiltrate. There were, however, obvious structural differences in the sulcular and junctional epithelia as well, which could conceivably have contributed to the different rates of development of gingivitis.

Increased epithelial permeability may also permit increased penetration of substances into the sulcular region from within. To the extent that this contributes to gingival exudation, it could conceivably influence the sulcular environment and thereby conditions for bacterial growth. It has been suggested that increased gingival exudation may contribute to the more rapid growth of bacterial plaque observed in individuals with gingivitis than in those with little or no inflammation (14).

Acknowledgements.—We are grateful to Drs. S. S. Han and Knut A. Selvig for valuable suggestions during the preparation of the manuscript.

References

1. Bral, M. M. & Stahl, S. S. Keratinizing potential of human crevicular epithelium. J. Periodontol. 1977, 48, 381–387
2. Bye, F. L., Caffesse, R. G. & Nasjleti, C. E. The effect of different plaque control modalities on the keratinizing potentials of the sulcular epithelium in monkeys. J. Periodontol. 1980, 51, 632–641
3. Caffesse, R. G., Karring, T. & Nasjleti, C. E. Keratinizing potential of sulcular epithelium. J. Periodontol. 1977, 48, 140–146
4. Caffesse, R. G., Nasjleti, C. E. & Castelli, W. A. The role of sulcular environment in controlling epithelial keratinization. J. Periodontol. 1979, 50, 1–6
5. Caffesse, R. G., Kornman, K. S. & Nasjleti, C. E. The effect of intensive antibacterial therapy on the sulcular environment in monkeys. II. Inflammation, mitotic activity and keratinization of the sulcular epithelium. J. Periodontol. 1980, 51, 155–161
6. Clausen, F. & Dabelsteen, E. Increase in sensitivity of the rhodamine B method for keratinization by the use of fluorescent light. Acta Pathol. Microbiol. Scand. 1969, 77, 169–171
7. Dale, B. A., Lonsdale-Eccles, J. D. & Holbrook, K. A. Stratum corneum basic protein: an interfilamentous matrix protein of epidermal keratin. Curr. Probl. Dermatol. 1980, 10, 311–325
8. Dolby, A. E. Host response to dental bacterial plaque. In: Shanley, D. ed. Efficacy of treatment procedures in periodontics. Quintessence Publishing Co. Inc., Chicago, Berlin, Rio de Janeiro 1980, pp. 197–206
9. Elias, P. M. & Friend, D. S. The permeability barrier in mammalian epidermis. J. Cell. Biol. 1975, 65, 180–191
10. Fry, H. R. & App, G. R. Histologic evaluation of

the effects of intrasulcular toothbrushing on human sulcular epithelium. J. Periodontol. 1978, 49, 163–173
11. Gelfand, H. B., Ten Cate, A. R. & Freeman, E. The keratinizing potential of crevicular epithelium: an experimental study. J. Periodontol. 1978, 49, 113–118
12. Geisenheimer, J. & Han, S. S. A quantitative electron microscopic study of desmosomes and hemidesmosomes in human crevicular epithelium. J. Periodontol. 1971, 42, 396–405
13. Hashimoto, K. Fine structure of horny cells of the vermilion border of the lip compared with skin. Arch. Oral Biol. 1971, 16, 397–410
14. Hillam, D. G. & Hull, P. S. The influence of experimental gingivitis on plaque formation. J. Clin. Periodontol. 1977, 4, 56–61
15. Kornman, K. S., Caffesse, R. G. & Nasjleti, C. E. The effect of intensive antibacterial therapy on the sulcular environment in monkeys. I. Changes in the bacteriology of the gingival sulcus. J. Periodontol. 1980, 51, 34–38
16. Larker, R. M. Membrane coating granules: the fate of the discharged lamellae. J. Ultrastruct. Res. 1976, 55, 79–86
17. Listgarten, M. A. Normal development, structure, physiology and repair of gingival epithelium. Oral Sci. Rev. 1972, 1, 3–67
18. Listgarten, M. A. The ultrastructure of human gingival epithelium. Am. J. Anat. 1964, 114, 49–69
19. Luft, J. H. Improvements in epoxy resins embedding methods. J. Biophys. Biochim. Cytol. 1961, 9, 409–414
20. Mackenzie, I. C. The effect of friction on the keratinizing epithelia of the oral mucosa and skin of rodents. Br. Dent. J. 1973, 134, 231–236
21. Mackenzie, I. C. Spatial organization and tissue architecture in normal epithelia. In: Mackenzie, I. C., Dabelsteen, E. & Squier, C. A. eds. Oral premalignancy. University of Iowa Press 1980, pp. 220–241
22. Martinez, I. R. Jr. & Peters, A. Membrane-coating granules and membrane modification in keratinizing epithelia. Am. J. Anat. 1971, 130, 93–120
23. Mattson, L. & Attström, R. Histologic characteristics of experimental gingivitis in the juvenile and adult beagle dog. J. Clin. Periodontol. 1979, 6, 334–350
24. Mattson, L., Theilade, J. & Attström, R. Electron microscopic study of junctional and oral gingival epithelia in the juvenile and adult beagle dog. J. Clin. Periodontol. 1979, 6, 425–436
25. Mery, C. C., Saglie, F. R. & Sabag, N. E. Normal desmosomal morphology in spinous cells from inflamed human gingiva. Arch. Oral Biol. 1980, 25, 693–695
26. Mosadomi, A., Shklar, G., Loftus, E. R. & Channey, H. H. Effects of tobacco smoking and age on the keratinization of palatal mucosa. Oral Surg. 1978, 46, 413–417
27. Müller, W. & Schroeder, H. E. Differentiation of the epithelium of the human hard palate. Cell Tiss. Res. 1980, 209, 295–313
28. Saito, I., Watanabe, O., Kawahara, H., Igarashi, Y., Yamamura, T. & Shimono, M. Intercellular junctions and the permeability barrier in the junctional epithelium. A study with freeze-fracture and thin sectioning. J. Periodont. Res. 1981, 16, 467–480
29. Schroeder, H. E. Differentiation of human oral stratified epithelia. S. Karger, Basel 1981
30. Schroeder, H. W. Histopathology of the gingival sulcus. In: Lehner, T. ed. The borderland between caries and periodontal disease. Academic Press, London 1977, pp. 43–78
31. Schroeder, H. E. & Amstad-Jossi, Mc. Epithelial differentiation at the mucogingiva and alveolar mucosa. Cell Tiss. Res. 1979, 20, 75–97
32. Schroeder, H. E., Graf-de Beer, M. & Attström, R. Initial gingivitis in dogs. J. Periodont. Res. 1975, 10, 128–142
33. Schroeder, H. E. & Münzel-Pedrazzoli, S. Correlated morphometric and biochemical analysis of gingival tissue. J. Microsc. 1973, 99, 301–329
34. Schroeder, H. E. & Theilade, J. Electron microscopy of normal human gingival epithelium. J. Periodont. Res. 1966, 1, 95–119
35. Squier, C. A. The permeability of keratinized and non-keratinized oral epithelium to horseradish peroxidase. J. Ultrastruct. Res. 1973, 43, 160–177
36. Squier, C. A. Keratinization of the sulcular epithelium—a pointless pursuit? J. Periodontol. 1981, 52, 426–429
37. Squier, C. A. Zinc iodide-osmium staining of membrane-coating granules in keratinized and non-keratinized mammalian oral epithelium. Arch. Oral Biol. 1982, 27, 377–382
38. Squier, C. A. & Rooney, L. The permeability of keratinized and non-keratinized oral epithelium to lanthanum in vivo. J. Ultrastruct. Res. 1976, 54, 286–295
39. Takarada, H., Cattoni, M., Sugimoto, A. & Rose, G. G. Ultrastructural studies of human gingiva. I. The upper part of the pocket epithelium in chronic periodontitis. J. Periodontol. 1974, 45, 30–42
40. Ten Cate, A. R. The dento-gingival junction. An interpretation of the literature. J. Periodontol. 1975, 46, 475–477
41. Yamasaki, A., Nikai, H., Niitani, K. & Ijuhin, N. Ultrastructure of the junctional epithelium of germfree rat gingiva. J. Periodontol. 1979, 50, 641–648

Received for publication 1 March 1982