A Quantitative Electron Microscopic Study of Desmosomes and Hemidesmosomes in Human Crevicular Epithelium

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CREVICULAR EPITHELIUM lying between the gingival sulcus and the connective tissue of the free gingiva serves as the main barrier against the ingress of foreign material and the loss of tissue fluid. Located in close proximity to the tooth surface, it may be subject to irritation from plaque, calculus and other local irritants. The integrity of this epithelium depends to a large extent on the various intercellular attachment complexes which are important in cellular adhesion and fluid passage.[1, 2]

Several studies have been devoted to the fine structure of normal and inflamed crevicular epithelium;[3-7] however, there is still a lack of quantitative data relevant to the fine structure of normal crevicular epithelium. Such data could provide a precise picture of normal morphology and facilitate the recognition of early pathological deviations.

The purposes of this investigation were (1) to study the fine structure of clinically normal crevicular epithelium in humans and (2) to provide a statistical analysis of the number, relationship and relative size of desmosomes and hemidesmosomes in the various cell regions.

MATERIALS AND METHODS

The material consisted of gingival biopsies from eight volunteers (average age 24) all of whom exhibited excellent oral hygiene and intact dentitions. None of the subjects showed any evidence of inflammation in the area to be biopsied but in an effort to achieve as clinically normal gingiva as possible each received a soft cup prophylaxis, instruction in oral hygiene and new tooth brushes approximately four weeks prior to tissue sampling.

Under block or infiltration anesthesia, a narrow rectangle of marginal tissue measuring approximately 3 × 5 mm and including the crevicular epithelium up to the level of epithelial attachment was excised with a Bard Parker #12 blade (Fig. 1). Once separated, the tissue was immediately placed in cold 0.1M phosphate buffered paraformaldehyde and cut transversely into two equal parts. After two hours in the buffered paraformaldehyde, the specimens were transferred through 0.1M phosphate buffer into 1% phosphate-buffered osmium tetroxide for two hours at 4°C.[11] All fixatives and buffers were kept at pH 7.3 and contained 4.5% sucrose.[9] Following fixation, the specimens were rapidly dehydrated in increasing concentrations of ethanol, infiltrated with propylene oxide, and embedded in Epon*[8] with the crevicular epithelium facing up for ease of orientation.

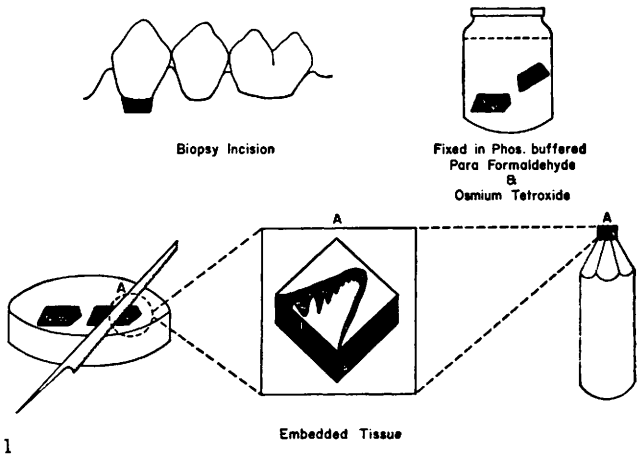

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FIGURE 1. *Schematic diagram illustrating the major steps in tissue preparation.*

After polymerization, the blocks were cut with a jewelers saw into four or five 1 mm cubes each containing a piece of transversely sectioned free gingiva. Individual cubes were remounted on standard Epon cylinders for trimming and sectioning. Each block was then trimmed so that a small area of crevicular epithelium measuring approximately ⅓ mm square was isolated. In order to verify the histologic identity of the region, sections of 1 μ in thickness were cut and stained with toluidine blue for light microscopic observation.

Thin sections (400-700Å) were prepared on a LKB ultramicrotome and placed on copper grids, some of which were Formvar-coated and carbon reinforced. Each grid was stained with uranyl acetate and lead citrate[22] prior to observation in a Hitachi HS-8 electron microscope.

A planimeter† was used to measure the circumference of individual cells from enlarged photomicrographs taken at standard magnifications. Cells were grouped

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*Epon Resin 812, Shell Chemical Co., New York.
†Tacro Inc., Germany.

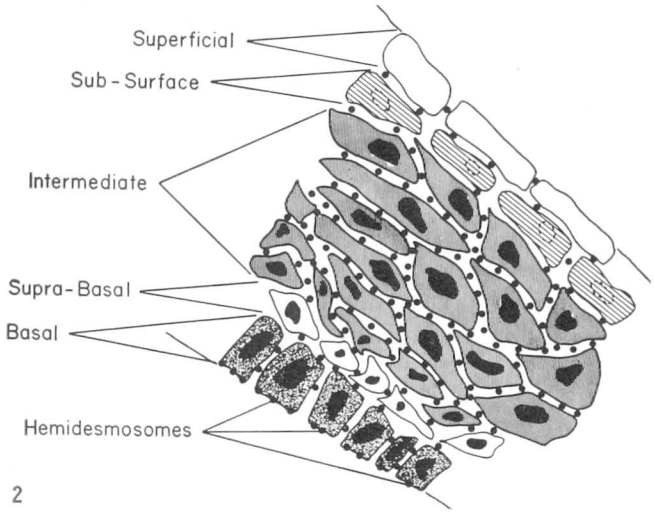

FIGURE 2. *Schematic diagram illustrating the division of crevicular epithelium into six cell regions.*

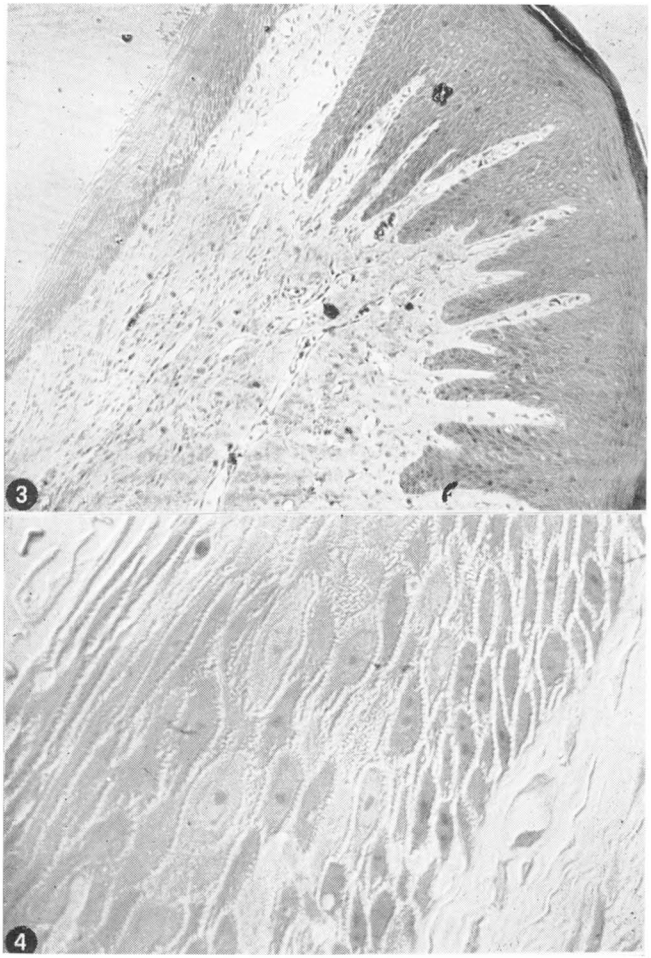

FIGURE 3. *Light micrograph of normal free gingival margin demonstrating an intact crevicular epithelium and almost complete absence of inflammatory cells. Tissue embedded in Epon and stained with toluidine blue. Original magnification X200.*

FIGURE 4. *Light micrograph of a representative area of crevicular epithelium. Tissue embedded in Epon and stained with toluidine blue. Polarizing microscope. Original magnification X650.*

into six regions according to location (Fig. 2) and the number of desmosomes and their individual lengths recorded. In addition, total cell circumference was measured for all cells except along the outer portion of the surface cells and the admesodermal border of the basal cells. The latter was separately measured for use in calculating the relationships of hemidesmosomes to cell membrane length. All dimensions were originally recorded in millimeters and changed to Å of mμ units during statistical analysis.

FINDINGS

Morphological Observations

Under the light microscope, 1 μ-thick sections of crevicular epithelium and the adjacent keratinized portion of the free gingival margin demonstrated a notable lack of inflammatory cells (Fig. 3). High power magnification of the crevicular epithelium showed it to vary from 5 to 15 cells in thickness, uninterrupted by connective tissue papillae (Fig. 4).

At the electron microscopic level, the crevicular epithelium was found to resemble nonkeratinizing oral mucosa. Cells of the basal layer had a large ovoid nucleus, basally placed mitochondria, a small number of desmosomes, and "foot-like" cellular extensions into the underlying connective tissue (Fig. 7). Typical intermediate cells were found to exhibit irregular cell borders, a reduced number of mitochondria, indented nuclei and many desmosomes (Fig. 10). Crevicular cells lacked keratohyalin granules and bundles of dense intracellular fibrils which characterized attached gingiva (Fig. 13). In place of the stratum corneum, the superficial layers were composed of cells in various stages of degeneration; these cells were often lacking a well defined nucleus but usually contained numerous electron dense bodies, variable sized vesicles and intact desmosomes (Figs. 16, 17).

Typical desmosomes with an indistinct intercellular layer were found in all cell layers (Figs. 8, 12, 17). They were occasionally observed in association with tight or intermediate junctions in the intermediate and basal regions (Fig. 17). Hemidesmosomes were irregularly spaced along the basal (admesodermal) surface of the basal cells (Fig. 5). Although the lateral borders of the hemidesmosome were less well defined than those of the desmosome, favorably sectioned material showed an intermediate dense line as well as the peripheral density (Fig. 6). Between hemidesmosomes, small pinocytotic vesicles were occasionally noted along the basal surface.

Most tissue sections were completely devoid of inflammatory cells or contained only an occasional lymphocyte or polymorphonuclear cell (Fig. 11). Plasma cells were never observed. There were no desmosomes

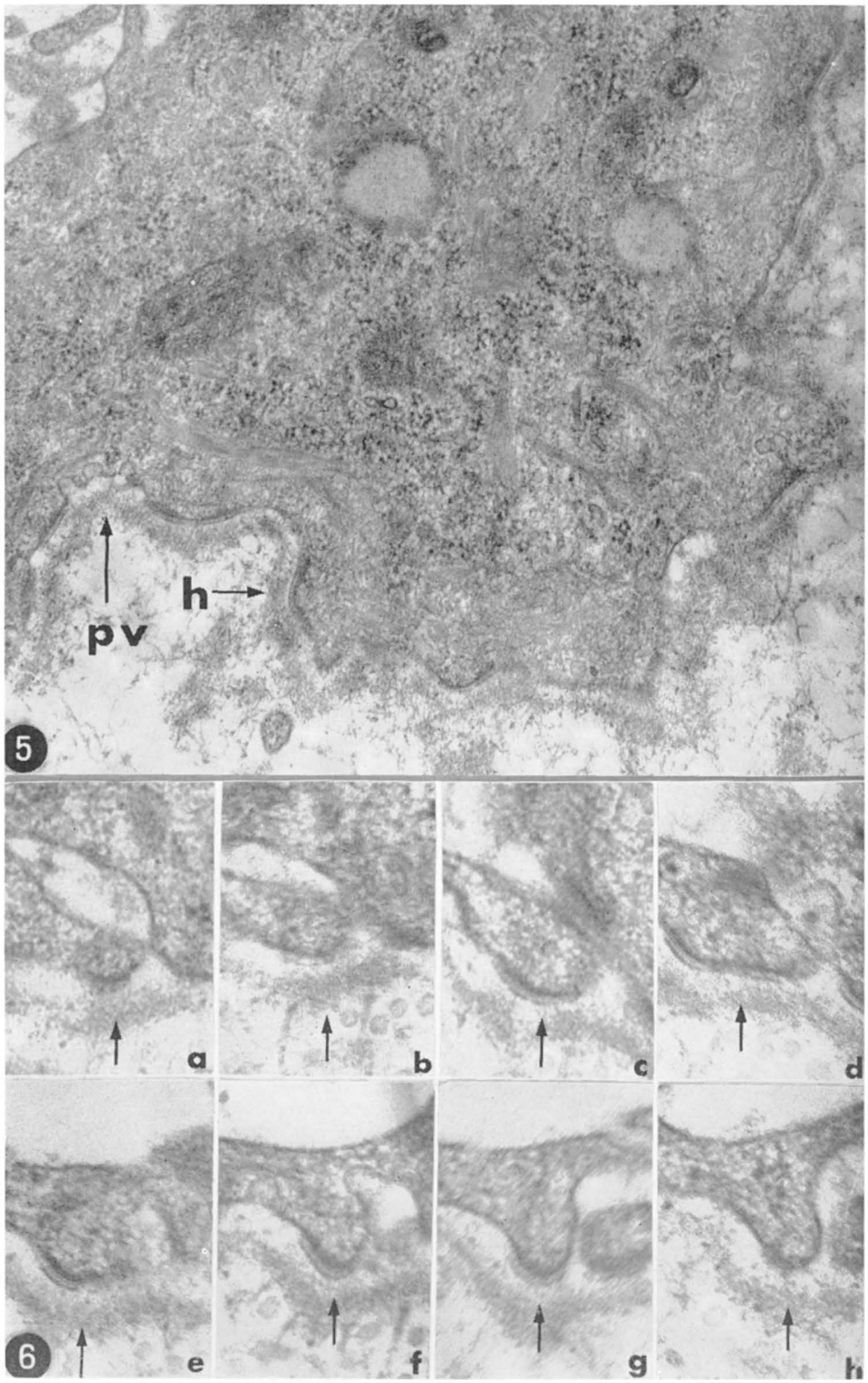

FIGURE 5. *Basal surface of basal cell and electron microscopic basement membrane. Pinocytotic vesicles (PV) and hemidesmosomes (H) are irregularly distributed along the plasma membrane. Original magnification X34,200.*

FIGURE 6. *Serial sections of a single hemidesmosome (arrow) sectioned at 600Å intervals. This particular hemidesmosome demonstrates a symmetrical pattern with a peak at (d). Approximate size 4,200 Å. Original magnification X48,900.*

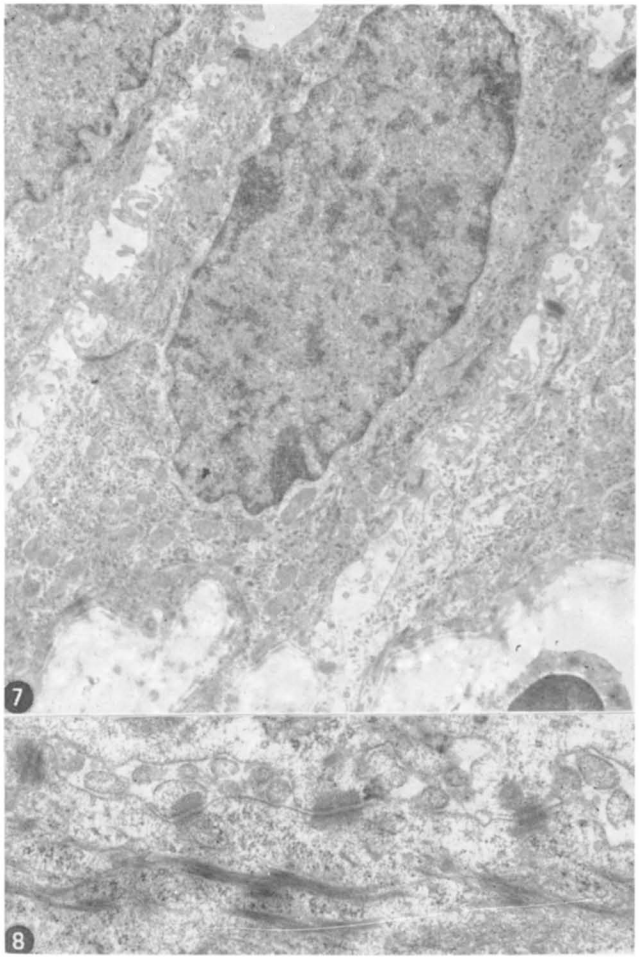

FIGURE 7. *Basal cell of crevicular epithelium containing a large ovoid nucleus, basal concentration of mitochondria and scarcity of desmosomes. Original magnification X8,000.*
FIGURE 8. *Area between the basal and supra-basal layers demonstrating a narrow intercellular compartment and intracellular tonofilaments sectioned longitudinally and in cross-section. Original magnification X24,000.*

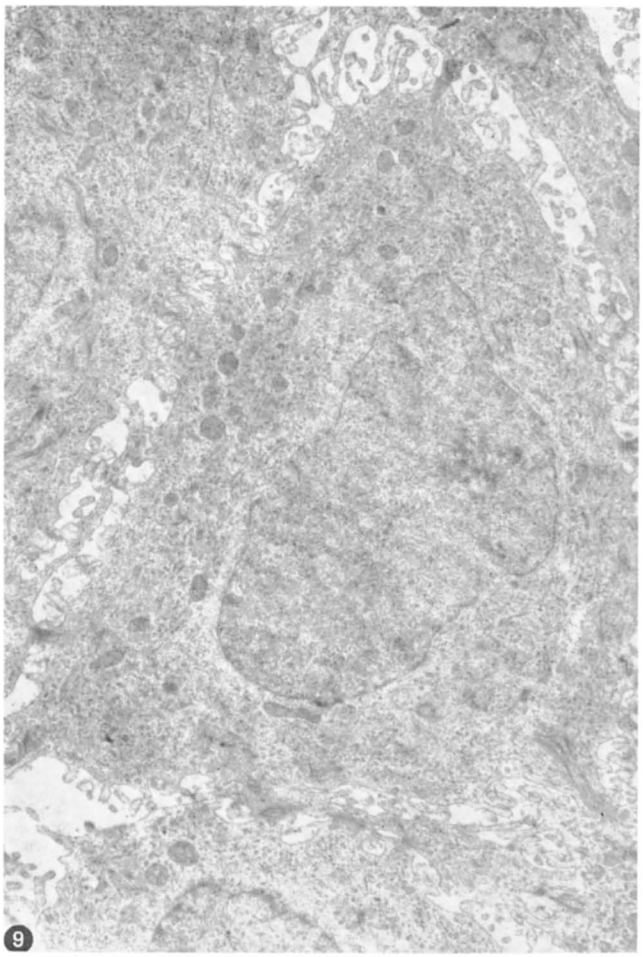

FIGURE 9. *Supra-basal cell demonstrating morphological characteristics similar to both basal and intermediate cells. Original magnification X12,000.*

present between inflammatory clear cells and epithelial cells (Figs. 14, 15). The clear cells appeared to compress the desmosomes of the neighboring epithelial cells into the adjacent intercellular spaces while migrating through the epithelium. In those areas where the epithelial and clear cells were in contact, the individual plasma membranes often continued parallel to each other for varying distances in a manner similar to intermediate junctions. Ruptured or degenerating desmosomes were never observed in this area.

The intercellular space, in general, appeared to be slightly dilated (Figs. 9-11, 16). A narrow space was seen less frequently and usually only for a limited distance (Figs. 8, 12). Cellular debris or precipitate were rarely observed and there was no evidence to indicate a direct bacterial penetration. In general, the intercellular compartment tended to be narrowest in the intermediate layer, slightly wider in the basal layer and widest in the most superficial regions. Junctional complexes were usually absent in the areas immediately adjacent to the basement membrane.

Samples of keratinized gingiva were fixed and processed concurrently with crevicular epithelium in an effort to determine if the widened intercellular compartment was not a fixation artefact. The keratinized tissue showed no evidence of an enlarged intercellular space (Fig. 13) indicating that the dilated intercellular spaces found in the crevicular epithelium were not artefacts of fixation.

In most sections, the basement lamina consisting of the lamina densa and lamina lucida was clearly visible as it closely followed the admesodermal surfaces of the basal cells (Fig. 5). The lamina densa had a granular appearance and was often associated with fine fibrils which either crossed the lamina lucida or extended into the adjacent connective tissue as individual fibrils or "loop-like" tufts. These fibrils appeared to be concentrated in areas adjacent to the hemidesmosomes and could be seen to extend into the attachment plaques.

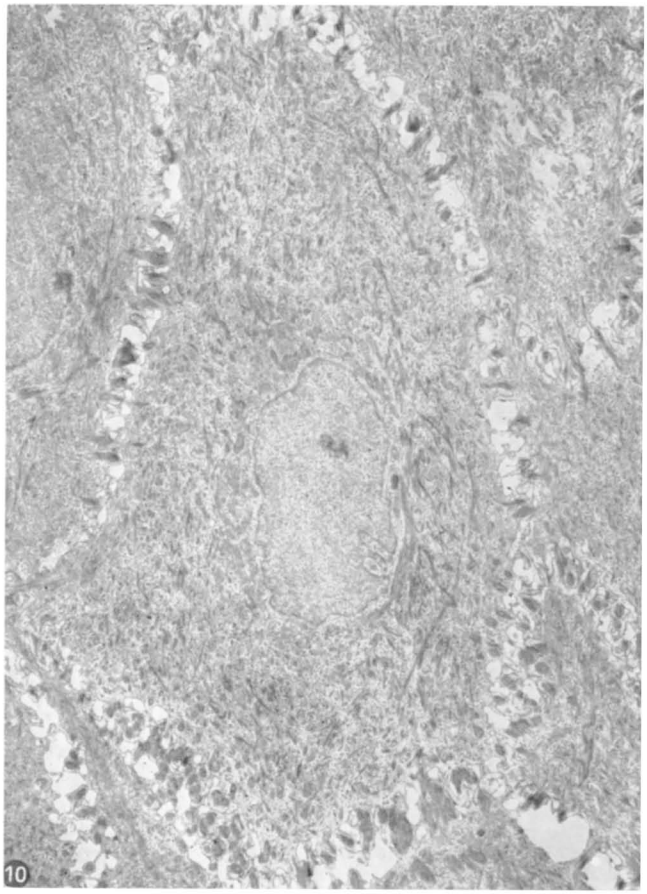

FIGURE 10. *Typical cell from the intermediate layer of crevicular epithelium showing a random distribution of tonofilaments and numerous desmosomes. Original magnification X10,000.*

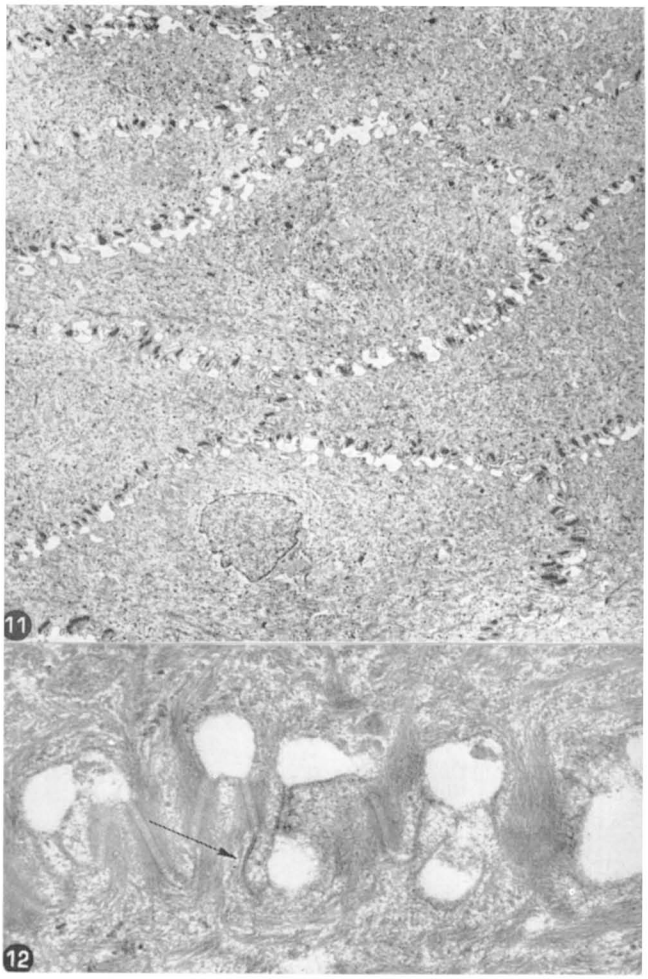

FIGURE 11. *Survey photomicrograph of the intermediate region illustrating a moderately dilated intercellular space and irregular cellular projections. Original magnification X38,-000.*

FIGURE 12. *Tight junctions (arrow) associated with desmosomes in the intermediate layer of crevicular epithelium. Original magnification 49,200.*

Serial sections of selected desmosomes and hemidesmosomes used to estimate the size and shape of these structures indicate that the desmosome is 4,000-4,500 Å "long" and 3,000-3,500 Å "wide," while hemidesmosomes (Fig. 6) tend to be smaller, measuring approximately 3,500 Å by 3,000 Å.

Statistical Analysis

Statistical evaluation was based on measurements of over 500 cells from eight patients. The parameters measured included the number of desmosomes per micron, the percentage of cell circumference composed of desmosomes or hemidesmosomes, here called the desmosome or hemidesmosome attachment index (DAI or HAI), and finally the mean of observed desmosome lengths. Measurements were taken from individual cells of the various layers and grouped according to patient. However, since the means for the pooled data were in agreement with those from individual patients only the means and standard deviations related to the pooled data will be presented.

In order to facilitate the identification of individual cells, the crevicular epithelium was arbitrarily divided into six regions based on the relationship of each region to the surface or basal cells (Fig. 2). The surface region consisted of cells directly bordering the crevice. The sub-surface region denoted the second cell from the surface and, therefore, was made up of those cells that were in direct contact with the surface layer. The intermediate region was usually several cell layers thick being located between the sub-surface and supra-basal layers. The supra-basal region was the single layer of cells directly bordering the basal cell region. The basal region referred to the traditional single layer of basal cells. The basal or admesodermal surface of the basal cells represented the hemidesmosome region. As will be seen, the establishment of sub-surface and supra-basal regions helped to produce highly significance differences between the remaining basal, superficial and intermediate regions.

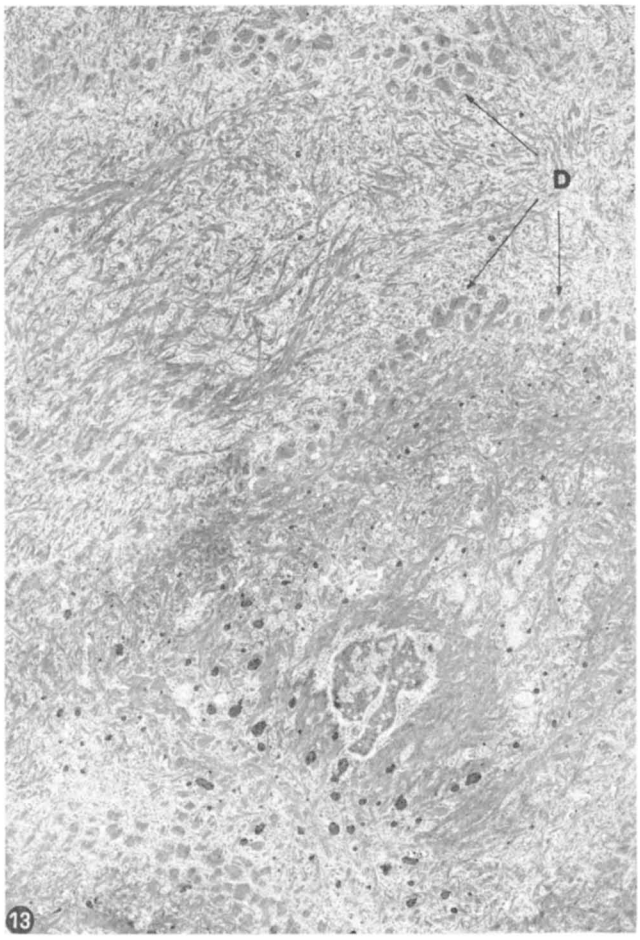

FIGURE 13. *Survey micrograph of stratum spinosum and granulosum from human keratinized gingiva. The absence of a dilated intercellular compartment and the dense accumulations of intracellular tonofilaments characterize this type of tissue. Original magnification X9,600.*

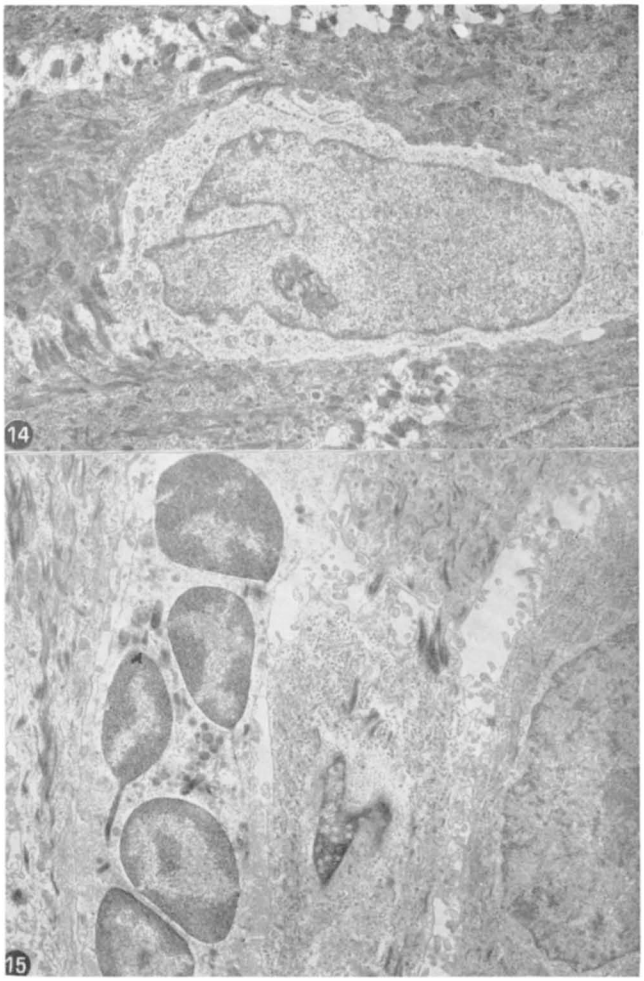

FIGURE 14. *Clear cell, possibly an inactive melanocyte, surrounded by epithelial cells. Desmosomes in the adjacent intercelluar space appear compressed while they are absent between the clear cell and epithelial cells. Original magnification X6,400.*

FIGURE 15. *Portion of a PMN migrating through the lower strata of crevicular epithelium containing a lobulated nucleus and numerous dense bodies. Original magnification X7,600.*

Based on the sample results, the number of desmosomes and hemidesmosomes per micron varied significantly ($p < .001$) between the different cell layers (Table 1). Desmosomes were most numerous in the intermediate layer (0.83), least in the basal (0.22) and supra-basal regions (0.37) and in between in the sub-surface (0.47) and surface (0.44) layers. Hemidesmosomes (1.49) were more numerous than desmosomes and demonstrated the greatest variability. Statistical comparisons between various pairs of regions using the two tailed Student's *t*-test demonstrated highly significant differences between the major regions, namely the hemidesmosome, basal, intermediate and surface layers.[12]

It was also noted that the desmosome attachment index (DAI) was the highest in the intermediate region (34%), least in the basal layer (7%) and intermediate in the supra-basal (14%) sub-surface (14%) and surface (13%) layers. The hemidesmosome attachment index (HAI) was also high (27%) resembling the desmosome attachment index (DAI) of the intermediate layer. The differences analyzed as a group (Table 2) were highly significant ($p < .001$). Comparison between pairs of regions using the two tailed Student's *t*-Test showed significant differences between the major categories.[12]

The mean observed desmosome length as found in the sample was greatest in the intermediate region (433.4 mμ) and less in the supra-basal (394.2 mμ), basal (333.9 mμ), sub-surface (322.9) and surface (318.5) regions in descending order (Table 3). The mean observed hemidesmosome length (218.3 mμ) was considerably smaller than any of the desmosome values. These differences analyzed as a group (Table 3) were highly significant ($p < .001$). Statistical comparison between pairs of regions using the two tailed Student's *t*-test indicated highly significant differences in nearly all the comparisons between major regions.[12]

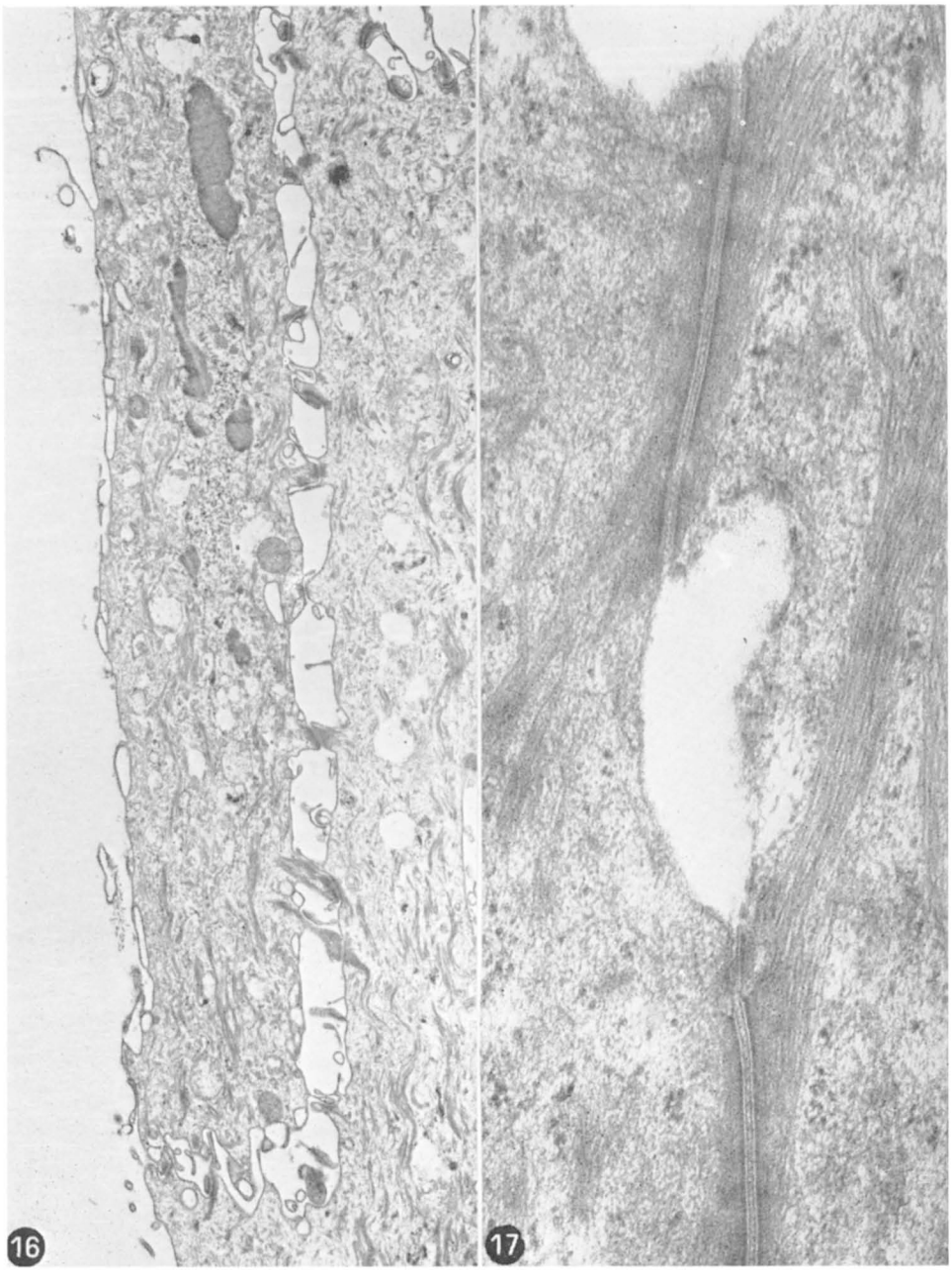

FIGURE 16. *Superficial cells showing marked intracellular degeneration and apparent persistence of desmosomes. Original magnification X8,400.*
FIGURE 17. *Intercellular area from the superficial region. Typical desmosomes with associated tonofilaments inserting into the inner leaflets of the attachment plaques are present. Original magnification X57,000.*

DISCUSSION

One possible explanation for the small number of published ultrastructural studies of crevicular epithelium may be related to the difficulty encountered in accurately orienting the tissue specimens. Our technique is similar to techniques recently published by Gavin[4] and Schroeder,[5] except that we transect the excised specimen only once prior to embedding in Epon. This modification minimizes soft tissue handling, allows excellent orientation and permits adequate tissue fixation.

The recent work of Loe[13] indicates that clinically healthy marginal gingiva is free of microscopic evidence of inflammation. This concept is contrary to the widely held belief that inflammation is a normal component of healthy gingiva.[10, 14, 15] Our findings, based on electron microscopic observation, support the concept of an inflammation-free marginal gingiva in clinically healthy tissue. Although inflammatory cells were on occasion present, the usual evidence of inflammation, namely a dense cellular infiltrate, dilated vessels, ruptured cells

TABLE 1
Analysis of Variance of Mean Number of Desmosomes per Micron

Region	*Mean (± S.D.)*	*N*
Hemidesmosome*	1..49 (0.62)	73
Basal	0.22 (0.11)	136
Supra-basal	0.37 (0.12)	25
Intermediate	0.83 (0.36)	252
Sub-surface	0.47 (0.27)	26
Surface	0.44 (0.18)	52

Source of Variation	*Sum of Squares*	*DF*	*Mean Square*	*F Ratio*	*P*
Between	89.05	5	17.81	153.21	<.001
Within	64.87	558	0.12		
Total	153.92	563			

*This mean represents that of hemidesmosomes counted along the admesodermal surface of the basal cells.

TABLE 2
Desmosome Attachment Index (DAI): Analysis of Variance of Percentage of Cell Circumference Composed of Desmosomes

Region	*Mean % (± S.D.)*	*N*
Hemidesmosome*	27 (7.31)	73
Basal	7 (3.42)	136
Supra-basal	14 (5.92)	25
Intermediate	34 (12.01)	252
Sub-surface	14 (7.04)	26
Surface	13 (5.88)	52

Source of Variation	*Sum of Squares*	*DF*	*Mean Square*	*F Ratio*	*P*
Between	7.86	5	1.57	190.58	<.001
Within	4.60	558	.008		
Total	12.46	563			

*The percentage refers to that of circumference composed of hemidesmosomes (Hemidesmosome Attachment Index; HAI).

TABLE 3
Analysis of Variance of Mean Observed Desmosome Lengths (Millimicron)

Region	*Mean (± S.D.)*	*N*
Hemidesmosome*	218.3 (157.6)	73
Basal	333.9 (178.2)	136
Supra-basal	394.2 (128.0)	25
Intermediate	433.4 (106.0)	252
Sub-surface	322.9 (133.3)	26
Surface	318.5 (92.9)	52

Source of Variation	*Sum of Squares*	*DF*	*Mean Square*	*F Ratio*	*P*
Between	3,060,390	5	612,078	33.56	<.001
Within	10,176,400	558	18,237		
Total	13,236,800	563			

*The length represents that of hemidesmosomes measured along the admesodermal surface of the basal cells.

and large amounts of edematous exudate were never observed.

The slightly dilated intercellular compartment which has also been reported in previous studies of nonkeratinized epithelium[4, 5, 16] may indicate a preinflammatory change related to the bacterial flora[17] and/or tissue lysosomes.[3] The consistent lack of intercellular spaces in the neighboring gingival epithelium in all of our samples indicates that the widened intercellular space was not a fixation artefact since the pH, tonicity and other conditions of fixation were held constant. In a previously cited study,[5] dilated intercellular spaces were observed despite the fact that the teeth and soft tissues were removed as a unit.

The material used in this study consistently demonstrated a well defined basement membrane. This is in agreement with the findings of Gavin[4] but differs from the observations of Schroeder[5] who found the basal lamina to be "structurally indistinct." The differences reported might be due to the presence of inflammatory changes in the unselected samples and/or to the effects of tissue decalcification.

The less distinct appearance of the hemidesmosomes and the greater variability of their size compared to desmosomes, may be taken to indicate a somewhat different structural composition of the two complexes. On the other hand, these differences may be related to location or to the difficulty involved in identifying and precisely measuring individual hemidesmosomes. Stern[18] has also noted similar differences between desmosomes and hemidesmosomes in his study of oral epithelium.

In carrying out this investigation, certain assumptions and techniques were necessary to insure meaningful comparisons of the quantitative data. Measuring the cell circumference, counting and measuring observed desmosomes, and assigning cells to arbitrarily defined layers become important only when executed under precisely standardized conditions. The planimeter used to measure cell circumference was adequate inasmuch as it faithfully traced the overall outline of cells while the most minute undulations of the plasma membrane which could not be traced, stayed constant throughout the various regions. Precise structural definitions were necessary for assigning cells to various regions because the crevicular epithelium varied in thickness and only a portion of the full thickness was observed in any given electron micrograph. The criteria and definitions used were based primarily on relative location and were presented in the preceding section.

In addition to location, cellular morphology proved to be a valuable aid in grouping cells. The superficial cell, as noted, showed advanced stages of degeneration, while the cells immediately below were characterized by

swollen mitochondria, disruptions in the nuclear membrane and rarefaction of cytoplasm, all of which are early degenerative changes. The cells of the supra-basal layer had characteristics of both the intermediate cells and basal cells; namely, a triangular or polyhedral shape, numerous mitochondria and a large centrally placed nucleus. These cells may represent true intermediate cells or portions of basal cells whose relationship to the basement membrane could not be visualized in a single plane.

The small number of desmosomes present between basal cells may be related to frequent cell division and the relatively rapid outward migration of the basal cell. The large number of desmosomes and the flattened cellular appearance found in the intermediate layer may reflect the degree of contribution this particular region makes to the protective aspects of epithelial function. Indeed, in this region intracellular fibrils become more prominent while the polysomes and mitochondria become fewer when compared to the more basal cells, thus indicating that the intermediate cells have differentiated fully to perform such physical functions. The reduced number of desmosomes in the superficial regions suggest that cell desquamation may depend on a reduction in the number of desmosomes as well as the rupture of those remaining. It is tempting to speculate that a critical level of attachment, for example DAI 15%, may indicate that the remaining desmosomes are no longer adequate to anchor the superficial cell to the underlying epithelium.

The hemidesmosome and desmosome attachment indices have not been previously described in the literature. Both are simple, reproducible and objective methods of quantitation which give some indication as to the relationship between attachment complexes (hemidesmosome or desmosome) and the length of cell circumference. Interestingly, hemidesmosomes along the admesodermal surface were more numerous per unit length than desmosomes of the intermediate region while the respective attachment indices were the reverse. This may be related to the fact that although hemidesmosomes were more numerous, they tended to be smaller in length than desmosomes of the intermediate layer and, therefore, made up less of the cell circumference on a percentage basis.

The mean value of observed desmosome lengths represents an average of random sections through individual desmosomes. These structures are reportedly elipsoidal in shape[19] which suggests that the observed means are probably less than the real long diameter. However, the observed mean values should reflect, insofar as such values show statistically meaningful differences, the real differences or similarities present in the various regions. Variation in desmosome size has also been observed by Han et al[20] who reported that desmosomes of the stratum intermedium in rat enamel organs were considerably larger than those of the inner or outer enamel epithelium. A decrease in desmosome size in the superficial layers of oral mucosa has also been reported by Van Bulow.[21] Although there is little direct evidence to attribute any functional significance to these findings, it is possible that the observed reduction in desmosome size reflects a further weakening of the intercellular attachment mechanism thereby facilitating cell desquamation.

Summary

Eight young adult patients with excellent oral hygiene were used as subjects in this investigation. Each provided a narrow band of free gingiva including an intact crevicular epithelium. The excised tissue was fixed and processed for electron microscopic observation in a conventional manner after which small areas of the crevice were studied with a Hitachi HS-8 electron microscope.

Photomicrographs were taken of individual cells and a linear planimeter was used to record the circumference of each photographically enlarged cell. The number and lengths of individual desmosomes and hemidesmosomes were also recorded. The cells from each patient were grouped into six regions based on their relationship to the surface and underlying connective tissue: surface, sub-surface, intermediate, supra-basal and basal layers and the admesodermal surface of the basal cells which comprised the hemidesmosome region. The quantitative data relative to the different regions was then statistically analyzed by computerized program.

The results of this investigation support previous reports on the fine structure of crevicular epithelium and represent the first original quantitative data on the size, number, and relative distribution pattern of desmosomes in this tissue. Desmosomes were found in all cell layers being most numerous in the intermediate, least in the basal and in between in the superficial layers. The mean observed desmosome length and the percentage of cell circumference composed of desmosomes followed the same pattern except that desmosomes tended to be smaller in the more superficial regions rather than the basal layer. Hemidesmosomes, on the other hand, were more numerous per unit length, smaller, and more variable than similar measurements of desmosomes. In general, the material observed in this study demonstrated a notable lack of inflammatory cells or other obvious signs of inflammation. The relationship of these findings to cell desquamation and the functional significance of the various layers are discused.

Conclusions

1. In the intermediate layer of crevicular epithelium

there are more desmosomes and their average length is greater than in the basal or superficial regions.

2. Hemidesmosomes are more numerous per unit length of cell circumference than desmosomes; however, they are smaller and more variable in length than desmosomes.

3. Electron microscopic observations of clinically normal marginal gingiva indicates that this tissue may be free of inflammation when rigid standards of oral hygiene and tissue selection are applied.

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Abstracts

THE EFFECT OF CHLORHEXIDINE MOUTH RINSES ON THE HUMAN ORAL FLORA

Schiøtt, C. R., Löe, H., Jensen, S. B.,
Kilian, M., Davies, R. M., and Glavind, K.
J. Periodont. Res. 5:84, 1970

Rinsing twice daily with 10 ml of a 0.2% solution of chlorhexidine gluconate, four subjects were compared to four controls who practiced no oral hygiene. The number of bacteria in saliva was estimated by a cultural technique and impression preparations were used for the study of bacteria on the gingiva and tooth surface. An increase of 300% in the bacterial count was noted in the controls. An 85%-90% reduction in the number of bacteria per ml saliva was noted in the chlorhexidine group for a period of 22 days. When the rinses were stopped, bacterial colonization of the tooth surfaces returned to previous levels as shown by the impression techniques. *The Royal Dental College, Vennelyst Boulevard, 8000 Aarhus, Denmark.*

THE DEVELOPMENT OF THE VASCULAR BED OF THE MARGINAL PERIODONTIUM

Kindlová, M.
J. Periodont. Res. 5:135, 1970

The vascular bed of the periodontium was studied using corrosive latex casts and development of the periodontium on histologic section. The 49 Wistar rats used were sacrificed from ages 20 to 32 days for study of the developing crown through eruption to the time when the tooth was in occlusion. The vascular bed of the periodontium developed from three different tissues, the alveolar mucosa, the enamel organ, and the connective tissue of the alveolar process. These vascular areas form one system in the developed periodontium, however, they do retain individual characteristic arrangements. The inflammatory infiltration at the junction of the enamel organ with the oral epithelium is also described. *Institute of Dental Research, Biological Department, Vinohradska 48, Prague, Czechoslovakia.*