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ULTRASTRUCTURAL FEATURES OF ORAL DISCOID LUPUS ERYTHEMATOSUS

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Abstract. Tissue from buccal and labial mucosal lesions of discoid lupus erythematosus (DLE) was studied in the electron microscope. The material comprised 10 patients, 7 of whom presented skin lesions as well. The epithelium showed a change in differentiation pattern from non-keratinizing to keratinizing epithelium. Civatte bodies (filamentous bodies) were present intercellularly in the basal and suprabasal cell layers, and in the juxta-epithelial lamina propria. Dyskeratotic cells, suggesting immature stages of Civatte bodies, were recorded in the lower stratae of the epithelium. The epithelium–connective tissue interface was abnormal, presenting detachments, gaps and multiplications of the lamina densa. Cytoplasmic tubular structures were observed in epithelial and endothelial cells, as well as in fibroblasts, lymphocytes and macrophages.

The oral tissue changes recorded were common in both groups of patients studied, i.e. with and without skin lesions, and similar to earlier reported changes of DLE skin lesions.

Key words: Lupus erythematosus; Discoid; Mouth mucosa; Oral manifestations; Electron microscopy

Oral manifestations of discoid lupus erythematosus (DLE), referred to as "oral discoid lesions", may occur with or without involvement of the skin (24). Oral discoid lesions may occasionally resemble, clinically and histologically, oral lichen planus or oral leukoplakia (24, 25). However, typical cases show characteristic clinical, histologic and immunologic features (1, 23, 24, 27). The histologic and immunologic changes are essentially similar to changes seen in cutaneous DLE lesions (1, 23, 27).

Ultrastructurally, cutaneous lesions of DLE and systemic lupus erythematosus (SLE) exhibit two major features: (1), changes confined to the basal lamina and the uppermost corium (17, 18, 29), corresponding to the PAS-positive basement membrane, and deposits of immunoglobulins (29); and (2), intracytoplasmic tubular structures in the cells of epidermis and dermis (12, 16, 19, 20).

No ultrastructural data are available on oral mucosa affected by DLE. The purpose of the present paper is to describe ultrastructural features of oral discoid lesions in patients with and without skin manifestations.

MATERIAL AND METHODS

The material consisted of 10 patients with typical oral discoid lesions. The diagnosis was based on clinical and histologic criteria previously described (24). Seven patients presented cutaneous lesions, while 3 patients had no signs of skin lesions, according to a thorough examination by a dermatologist. For further clinical data se Table 1. None of the patients had systemic lupus erythematosus (SLE) according to the criteria set up by the American Rheumatism Association (2), by Dubois (3) or by Hahn, Bagby & Osterland (9). No patients LE cells in serum. Two patients (nos. 5 & 10) had antinuclear antibodies (ANA) in serum. One of these patients (no. 10) may possibly develop SLE later on because of ANA in titre 1:4000, elevated sedimentation rate and elevated IgG in serum.

During the 6 months prior to biopsy the following treatments had been given: topical steroids in 6 patients (nos. 1–3, 5, 8 & 9), topical nystatin in 2 (nos. 2 & 8) and topical amphotericin B in one (no. 10). Treatment with general antimalarials (chloroquine 0.20–0.25 g/day) had been given to 3 patients (nos. 1, 3 & 7) for 13 years, 14 months and 5 months, respectively.

At the time of biopsy the oral discoid lesions were examined by a direct immunofluorescence staining technique. The results will be included in a later study.

The specimens were obtained as 5 mm punch biopsies, including the central erythematous area with intermingled white spots as well as the border zone of radiating white striae. The biopsies were immediately fixed in 5% glutaraldehyde and 4% paraformaldehyde adjusted to pH 7.4 with Sørensen's phosphate buffer (13). While still immersed in the fixative solution the specimens were cut perpendicular to the surface into slices approximately 1 mm thick. Following a minimum fixation period of 24 hours the blocks were then postfixed for 2 hours in 2% osmium tetroxide, adjusted to pH 7.4 with Veronal acetate

Table I. *Data of 10 patients with oral discoid lesions*

Patient no.	Sex	Age (y.)	DLE skin lesions	Site of biopsy	Duration of lesions in years
1	F	41	+	Buccal mucosa	4.8
2	F	29	+	Labial mucosa	5.1
3	M	32	+	Labial mucosa	0.9
4	M	35	+	Buccal mucosa	0.3
5	F	34	+	Buccal mocusa	6.3
6	F	40	+	Buccal mucosa	0.3
7	F	31	+	Buccal mucosa	2.8
8	F	56	−	Buccal mucosa	1.8
9	F	56	−	Buccal mucosa	4.2
10	F	32	−	Buccal mucosa	0.6

buffer, and subsequently exposed to en bloc staining in 2% uranyl acetate in NaH-maleate–NaOH buffer for another 2 hours, dehydrated in ethanol and embedded in Epon®. Areas were selected for ultrastructural study from semithin sections stained with either toluidine blue or PAS–toluidine blue (26). Ultra-thin sections were mounted on R–150 copper grids coated with carbon film and stained with uranyl acetate followed by lead citrate (8, 22). Electron micrographs were recorded on 35 mm films in a Philips EM 301 electron microscope.

RESULTS

Light microscopy

For all 10 patients examined, the histologic features were consistent with oral DLE (1) and appeared to be basically identical. The epithelium showed hyperortho- and/or hyperparakeratosis and acanthosis/hyperplasia alternating with atrophy (Fig. 1). Keratin plugging was present. In addition, the epithelium was characterized by prominent nucleoli in basal and many spinous cells, and by the occasional presence of multinuclear epithelial giant cells (Fig. 2). Evidence of liquefaction degeneration in terms of intercellular edema was found in a few areas within each biopsy. At the basement membrane a homogeneous, PAS-positive band of varying width was found. An inflammatory infiltrate, dominated by lymphocytes, was present in the connective tissue. The infiltrate showed a diffuse as well as a perivascular distribution.

Electron microscopy

A similarity in basic pattern of changes in the ten oral discoid lesions as seen in the light microscope was confirmed at the ultrastructural level. Hence the ten specimens will be considered together in the following presentation.

The epithelium, normally being a non-keratinizing epithelium, presented the cytomorphologic features peculiar to keratinizing epithelia. From the stratum basale towards the stratum corneum an increase in amounts of tonofilaments, mainly arranged into bundles, was recorded, coupled with the appearance of membrane coating granules and keratohyalin granules. Concomitantly, involution of energy producing and synthesizing organelles (mitochondria, Golgi apparatus, endoplasmic reticulum, ribosomes, and nuclei) occurred. The epithelium showed a decrease or disappearance of glycogen.

On occasion, intracytoplasmic desmosomes were observed in the upper nucleus-containing cell layers (Fig. 3). Some basal and parabasal cells contained aggregates of branched structures usually appearing tubular in cross section and with a diameter of approximately 200 Å (Fig. 4). The tubules were either lying free in the cytoplasm or within cisternae of the endoplasmic reticulum. Dyskeratotic cells were occasionally seen in the lower epithelial cell layers. These cells were characterized by a spherical outline and lack of desmosomes. Degenerative changes in the nucleus were variable. The cytoplasm contained massive accumulations of electron-dense tonofilament bundles intermingled with vacuoles and lysosomal structures (Fig. 5). Single cells showed filament bundles of relatively low electron density (Fig. 6). The nuclei of basal and many spinous cells often presented very large nucleoli, dominated by the nucleolonema compartment. Occasionally, strands of fine fibrillar material were recorded, lying free in the nuclear euchromatin of spinous cells (Fig. 7). Electron-dense structures as well as accumulations of dense granules were recorded in the euchromatin of granular and upper spinous cells (Fig. 8).

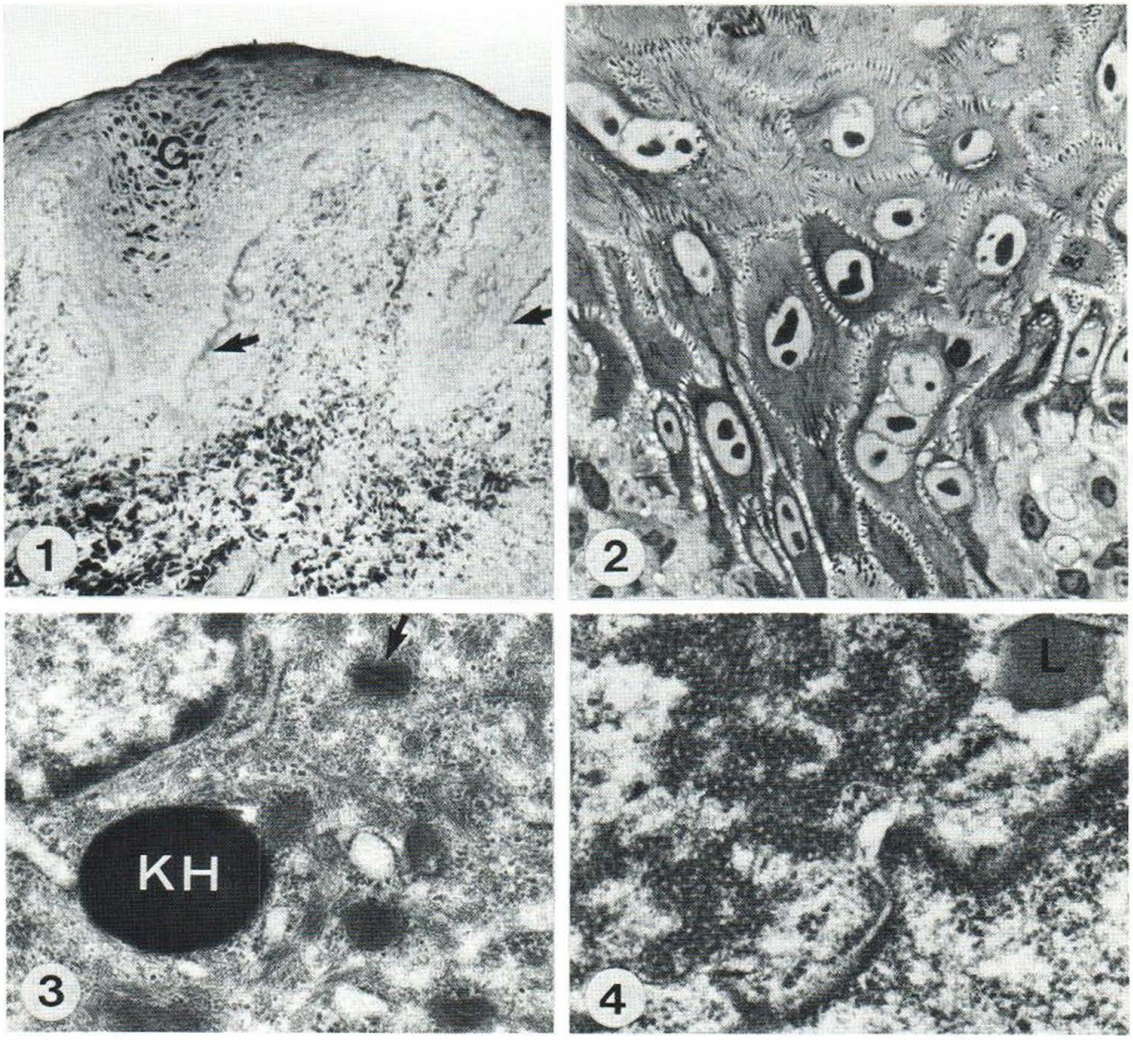


Fig. 1. Light microscopic appearance of oral discoid lesion showing epithelial hyperplasia and atrophy. Arrows indicate thickened basement membrane. *G* marks glycogen. PAS–toluidine blue, ×65.

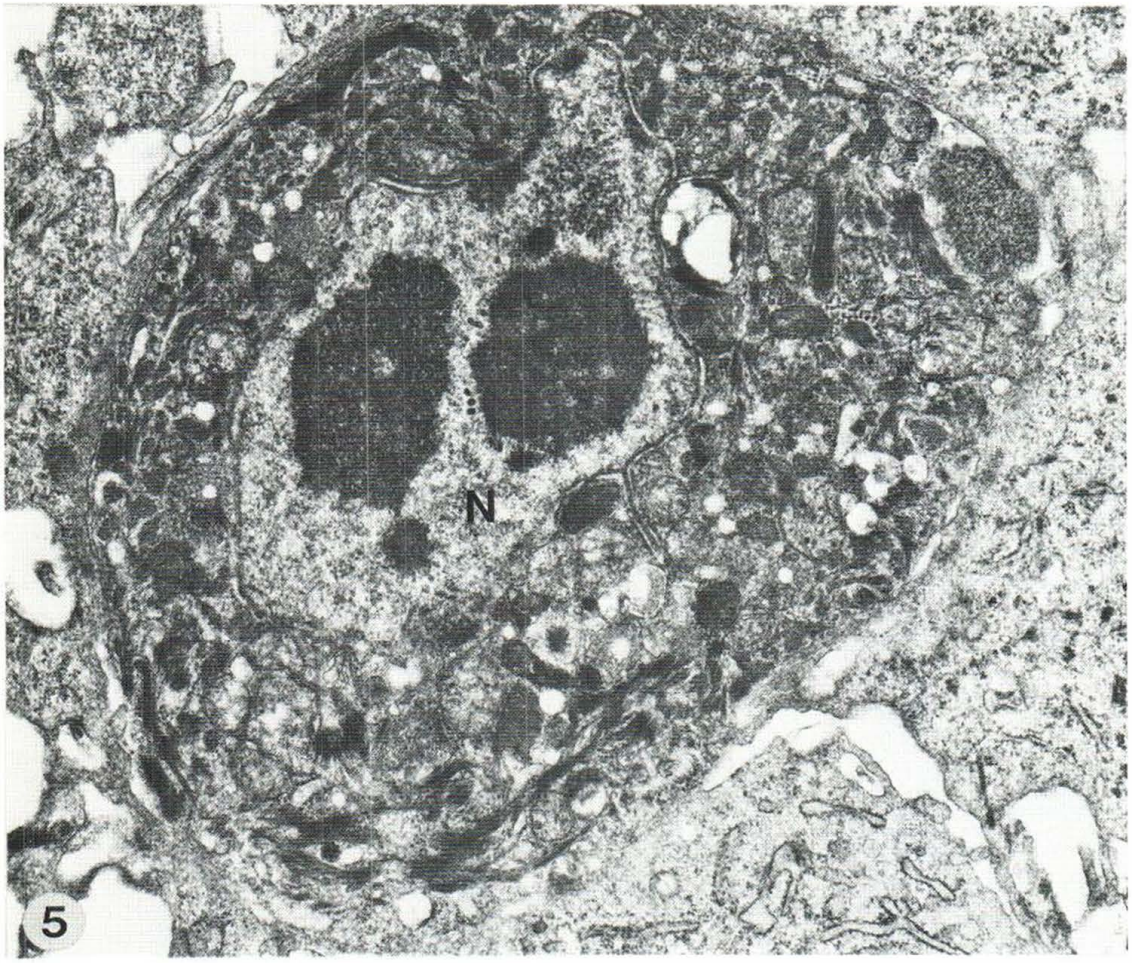
Fig. 2. High-power view of the lower part of the epithelium. Note multinucleated giant cells and prominent nucleoli. Toluidine blue, ×400.

Fig. 3. Part of granular cell with cytoplasmic desmosome (arrow). Keratohyalin (*KH*), ×25 200.

Fig. 4. Part of basal cell. Aggregates of cytoplasmic tubular structures are seen centrally. Lipid (*L*), ×40 000.

Generally, the epithelial cells were closely related and connected by desmosomes, except for localized areas in basal cell regions. The dilated intercellular spaces either appeared empty or contained irregular pools of presumably proteinaceous substance. Intercellularly located inflammatory cells were observed, preferentially in the lower part of the epithelium. On occasion, membrane-limited bodies with tightly packed filaments, 80 Å in diameter, were found intercellularly in basal and parabasal cell layers (Figs. 9 and 10).

The epithelium–connective tissue interface showed morphologic changes in all biopsy specimens, less pronounced in areas with close approximation between inflammatory cells and the epithelium. They consisted of gaps in the lamina densa, alternating with detachments of the lamina densa from the plasma membrane of basal epithelial



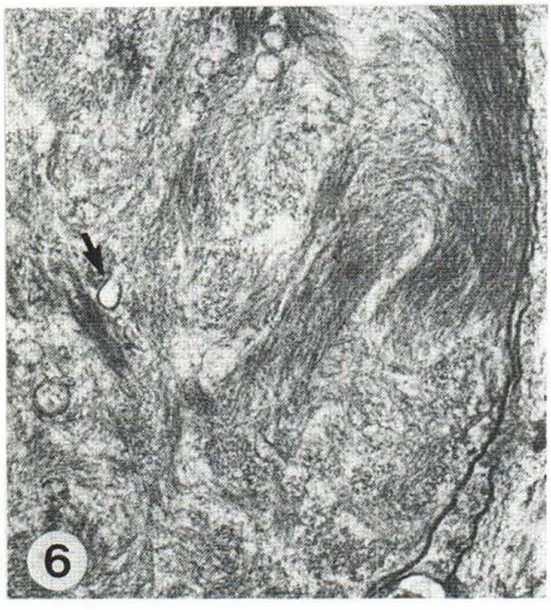
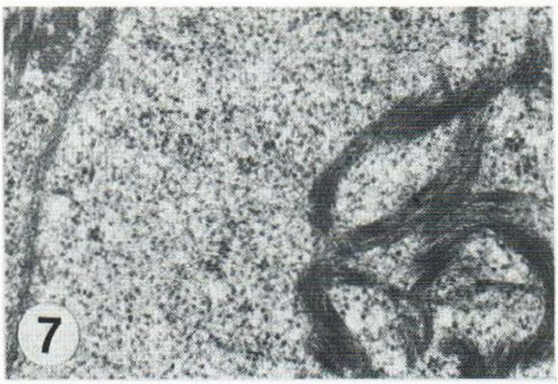
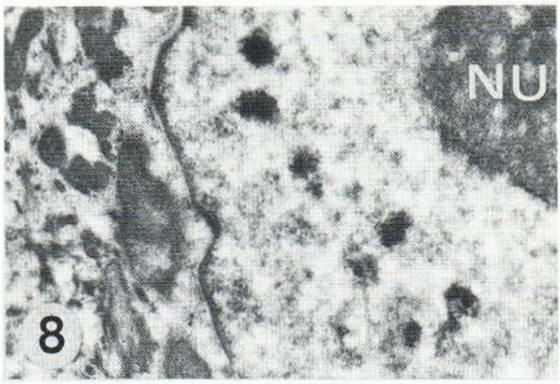
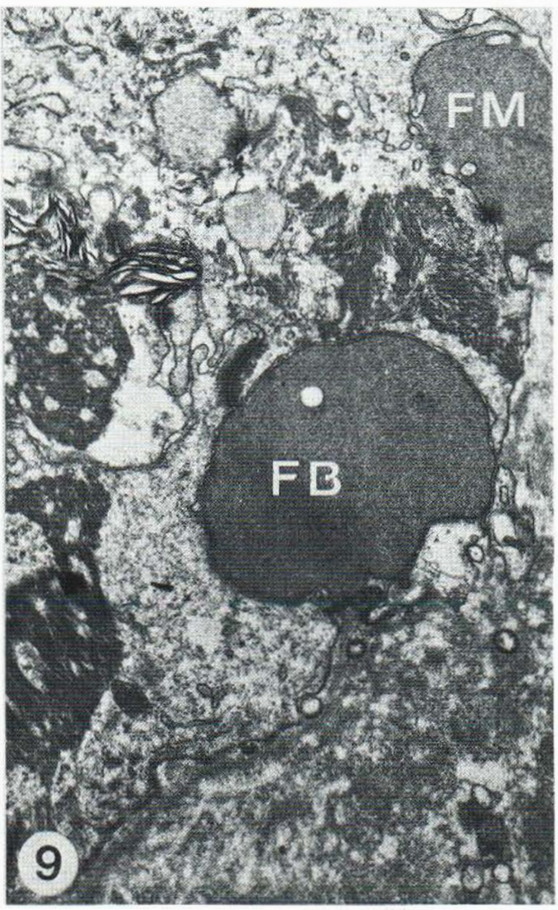


Fig. 5. Dyskeratotic cell in parabasal cell layer. Electron-dense tonofilament bundles intermingled with vacuoles and lysosomal structures are found in the cytoplasm. The nucleus (*N*) contains large chromatin aggregations, ×18 000.

Fig. 6. Detail view of a dyskeratotic cell showing evenly dispersed filaments and vesicular structures (arrow). ×43 000.







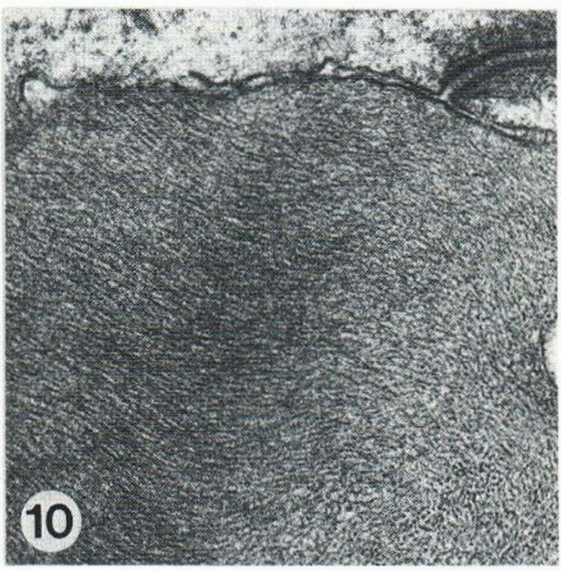


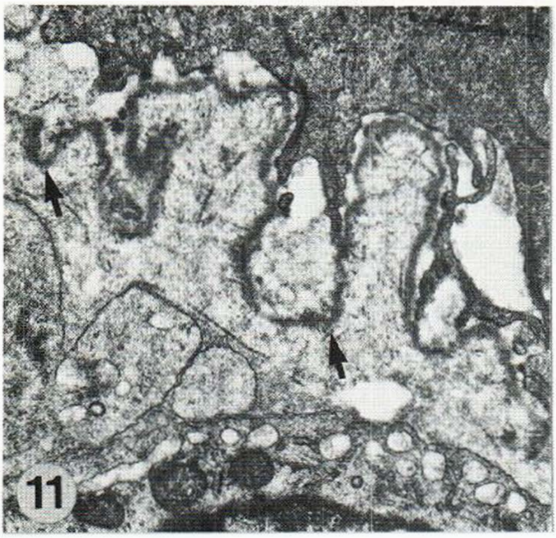
Fig. 7. Nucleus containing strands of fine fibrillar material in the nucleoplasm. ×31 000.

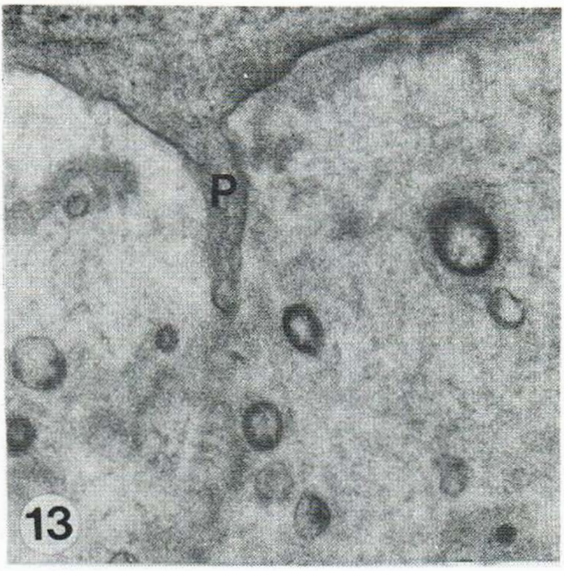
Fig. 8. Nucleus with electron-dense structures within the nucleoplasm. Nucleolus (*NU*). ×42 000.

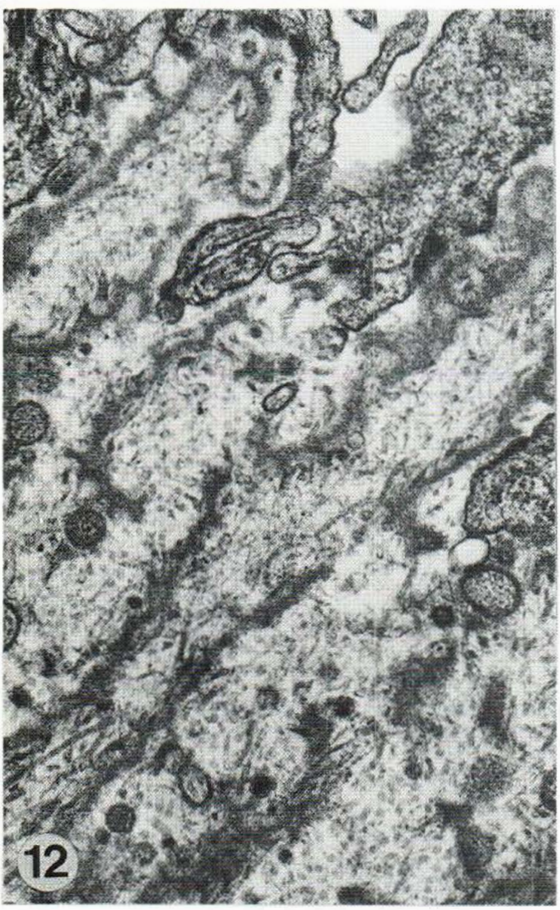
Fig. 9. Basal cell layers with membrane-limited filamentous body (*FB*). A filamentous mass without any limiting membrane is marked *FM*. ×14 500.

Fig. 10. High magnification of the filamentous body seen in Fig. 9. ×54 500.

cells (Fig. 11). Other regions presented lamina densa multiplications, which extended at varying depths into the connective tissue (Fig. 12). The fragments of lamina densa were regularly associated with anchoring fibrils. Membrane-limited structures were observed in the space between the basal cell plasma membrane and the lamina densa, and in relation to lamina densa multiplications. Their morphology ranged from lucent or fine granular bodies, some of which presented a zone of electron-dense material partly or totally lining the inner aspect of the membrane, to homogeneous bodies of varying electron density (Figs. 12 and 13). Slender and bulbous cytoplasmic processes were seen projecting from the suprajacent basal cells into the connective tissue (Fig. 13).







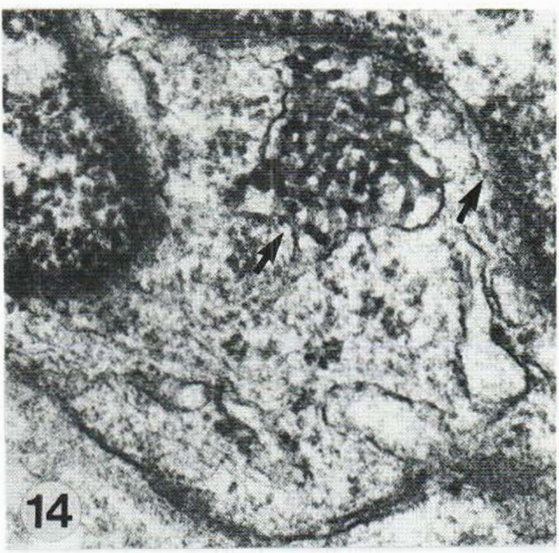


Fig. 11. Epithelium–connective tissue interface, showing detachment of the lamina densa (*arrows*) from basal cell. ×15 000.

Fig. 12. Epithelium–connective tissue interface, demonstrating multiplication of the lamina densa and numbers of membrane limited structures. ×31 500.

Fig. 13. Cytoplasmic projection (*P*) from basal epithelial cell neighboured by lamina densa and membrane-limited structures. ×41 000.

Fig. 14. Part of an endothelial cell. Branched tubular structures are seen enclosed by the outer leaflet of the nuclear envelope (*arrows*). ×57 000.

The juxta-epithelial connective tissue exhibited filaments similar to those constituing the filamentous bodies lying intercellularly in the epithelium. The filaments occurred in patches and without any limiting membranous structures (Fig. 9).

The inflammatory infiltrate, being located mainly perivascularly, consisted of lymphocytes, plasma cells, monocytes/macrophages and single mast cells. Tubular aggregates, similar to those observed in the epithelial cells, were present in all biopsies and confined to endothelial cells and occasionally to fibroblasts, lymphocytes and macrophages. They were either located free in the cytoplasm or partially or wholly enclosed by a single membrane, which was usually studded with ribosomes, and which appeared to be part of, or directly related to, endoplasmic reticulum including the outer leaflet of the nuclear envelope (Fig. 14). The capillary basal lamina was thickened, with interruptions and fragmentations.

DISCUSSION

The establishment of a diagnosis of DLE solely on oral manifestations has been questioned in the literature (24). In the two different groups of patients with DLE described in this study, i.e. those with and without skin lesions, the oral tissue changes were common to both groups and essentially similar to those reported on the cutaneous counterpart (12, 20, 21, 29).

DLE is one of several conditions affecting the oral mucosa in which the epithelial changes reflect a change in differentiation pattern in direction towards keratinization. The cytoplasmic changes in oral discoid lesions from basal towards surface layers are not unlike those previously described in oral lichen planus and leukoplakia (11, 14, 15, 28). The significance of the occasional finding of intracytoplasmic desmosomes in the DLE specimens is as yet not known. Similar structures have previously been observed in a variety of non-neoplastic and neoplastic lesions (for reference: Fejerskov, Roed-Petersen & Pindborg (6)).

Presence of prominent nucleoli in basal cells seems characteristic of both oral DLE (present study) and oral lichen planus (11). However, a difference in epithelial proliferative response may in fact exist, as prominent nucleoli were also demonstrated high up in the spinous cell layers of oral DLE. The intranuclear granular aggregates demonstrated in the upper nucleus-containing cell layers of the DLE lesions are thought to represent stages in the formation of nuclear keratohyalin granules. Similar structures have been described in oral lichen planus by Hashimoto et al. (11), who futher reported the presence of multinuclear squamous cells as found in the present study on oral discoid lesions. The significance of this observation is not known as yet.

The filamentous bodies in this study correspond morphologically to those described in skin lesions of SLE and DLE (18, 19) and lichen planus as well as certain other dermatoses (10). These bodies are called Civatte bodies. The dyskeratotic cells, demonstrated in the oral discoid lesions, are rather similar to cells described in oral lichen planus and also designated Civatte bodies (5). The dyskeratotic cells may represent the mucous membrane counterpart to the immature stages of the filamentous cells described in skin lesions of lichen planus (10).

Multiplications of lamina densa has been described as occurring in skin lesions of patients with SLE and DLE (17, 21). The changes in the lamina densa of oral DLE are basically identical with those reported in oral lichen planus (5, 11), oral leukoplakia (28) and oral mucosa showing epithelial hyperplasia (7). A subepithelial inflammatory cell infiltrate is a feature common to all these lesions. The possibility of lamina densa changes being a non-specific epithelial reaction associated with inflammation is therefore considered likely.

The membrane-limited bodies described as neighbouring lamina densa material may partly be explained as a result of transverse sectioning of cellular projections extending from the basal epithelial cells. Pinched-off cytoplasmic extrusions connected with a detachment process in response to rearrangement of the epithelial–connective tissue interface may further contribute to the appearance of the structures.

The cytoplasmic aggregates of tubular structures are ultrastructurally identical with those reported in skin lesions of DLE and SLE (12, 16, 19, 20) as well as other dermatoses (12). They have been suggested to be viral in nature, based on their morphologic resemblance to the nucleocapsid of paramyxovirus (12, 16), or else to represent a cellular reaction product following injury (4). The demonstration of tubular structures in human experimental skin wounds from steroid-treated as well as normal subjects (4) does not favour a viral nature of the tubular structures.

Hashimoto & Thompson (12) found a decreasing number of tubular structures in DLE skin lesions with increasing age of the lesions. However, this observation was not confirmed by others (20). A decrease in frequency and size of these structures has been found following prolonged chloroquine treatment (20). The present demonstration of tubular structures in all oral discoid lesions occurred irrespective of age of lesions and included cases with and without local corticosteroid therapy as well as general antimalaria therapy prior to biopsy.

The presence of tubular structures has not to our knowledge been reported previously in any disease of the oral mucosa. It remains for further work to show whether the tubular structures in oral discoid lesions are of diagnostic value, especially with regard to oral lichen planus and leukoplakia, which are the most important differential diagnoses for oral DLE.

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