

HISTOLOGICAL AND HISTOCHEMICAL INVESTIGATIONS OF LUPUS ERYTHEMATOSUS IN THE SKIN AND THE ORAL MUCOSA

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Abstract. Skin and oral lesions of chronic discoid lupus erythematosus from 6 patients have been investigated histologically and histochemically. Intra-individual comparisons between skin and oral lesions and intrasection comparisons between clinically affected and unaffected regions were performed. Enzyme histochemical recordings consisted of oxido-reductase and hydrolase activities. A high degree of correlation was noted in the intra-individual comparisons. The intrasection comparisons provided information concerning metabolic dynamics of the disease. The data obtained support the view that serious vascular changes are involved in the development of the changes in the overlying epithelium. The enzyme histochemical results agreed generally with histological data and proved to be a valuable diagnostic aid.

Key words: Lupus erythematosus; Skin; Oral; Histopathology; Histochemistry; Hydrolases; Oxidoreductases

Chronic discoid lupus erythematosus is usually regarded as a collagen disease which may affect the skin and the oral mucosa, either simultaneously or separately. The histological appearance of the skin lesions has been described in detail by, among others, Lever (14). The histology of the oral manifestation of the disease has been presented in case reports (5) and in comparisons with other types of lupus erythematosus (4). The incidence frequency of the oral manifestations and their carcinomatous potential has been extensively reviewed by Andreassen (3).

Systematic, intra-individual histological and histochemical comparisons between skin and oral lesions of chronic discoid lupus erythematosus seem to be lacking in available literature. Such investigations have been performed on lichen planus lesions (11). The aim of the present study was to use the same standardized tissue analyses as with lichen planus in order to facilitate comparative evaluation of skin and oral manifestations of chronic discoid

lupus erythematosus. Enzyme histochemical comparisons were to be made within each section between clinically affected and unaffected tissue areas in order to gain an impression of the dynamics of the disease.

MATERIAL AND METHODS

For intra-individual comparisons, biopsy specimens were obtained from both skin and oral lesions of 6 patients known to suffer from chronic discoid lupus erythematosus. The skin lesions to be analysed were localized as follows: shoulder—3—, arm—1—, neck—1—, and cheek—1—. All patients had lesions in the buccal oral mucosa biopsies.

Clinically, the skin lesions were erythematous, infiltrated patches with signs of peripheral hyperkeratosis. The oral pathologic changes included slightly elevated erythematous regions without induration. They were all surrounded by white borders or striae radiating from the margins.

In order to facilitate intra-section comparisons between tissues involved in the lesions and their neighboring less affected cells, long and spool-shaped biopsies were performed including adjacent, clinically healthy areas.

Immediately after excision the biopsy specimens were immersed in ice-cold Histocon (Histo-Lab Bethlehem Trading Ltd, Sweden). The tissues were transported to the laboratory in this solution according to techniques described previously (12, 13). All biopsy specimens were submitted to standardized treatment in ice-cold Histocon for 24 hours.

The tissues were mounted on cryostat chucks and frozen in isopentane chilled close to its freezing point by liquid nitrogen. They were stored in a freezer at -80°C prior to sectioning.

Cold microtome sections (8 microns) were obtained in a cryostat (System Dittes-Duspiva, Germany) at -20°C . Efforts were made to come as close as possible to serial sectioning throughout the specimen.

The histological studies comprised staining with haematoxylin and eosin and the van Gieson technique of cold microtome sections prefixed for 10 minutes in 4% neutral buffered formaldehyde. Staining with PAS after McManus was also performed after fixation of the sections.

The enzyme histochemical analyses comprised the following enzyme activities and original methods:

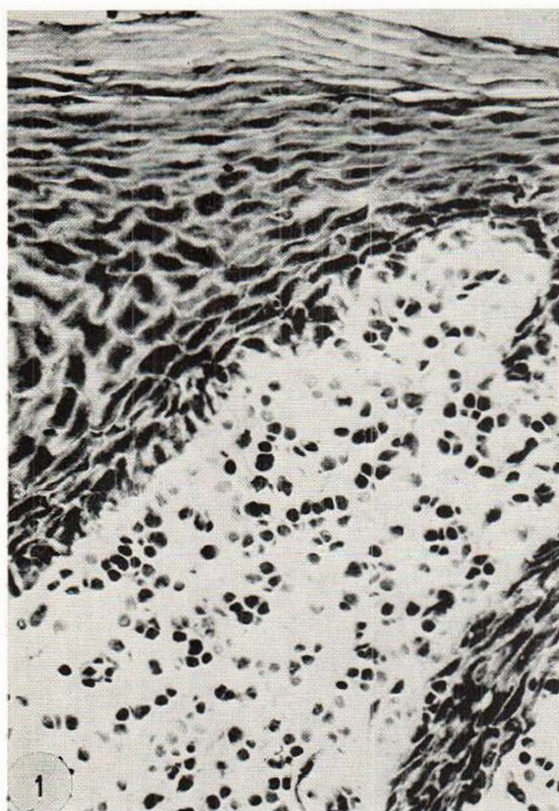


Fig. 1. Diffuse inflammatory condition in an oral lesion of chronic discoid lupus erythematosus. The picture includes single rows of lymphocytes along the borders of blood vessel ectasias, reflecting a diffuse distribution of such ectasias. Note the absence of a stratum granulosum despite hyperkeratosis and degeneration of the basal cells of the epithelium. Haematoxylin and eosin staining, $\times 285$.

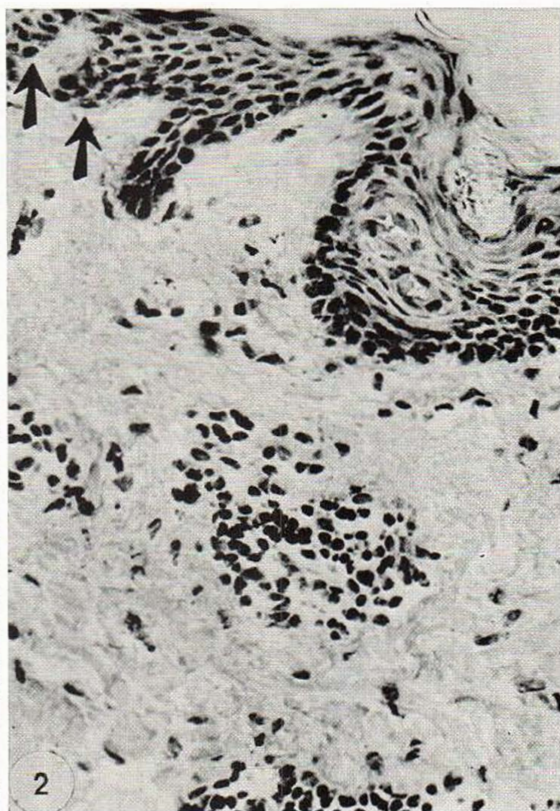


Fig. 2. A skin lesion from the same patient as in Fig. 1. Deep, perivascular accumulations of inflammatory cells are seen in the connective tissue. Note the tendency to keratotic plugging and the focal degenerative changes in the basal cells of the epithelium (arrows). Haematoxylin and eosin staining, $\times 285$.

Oxido-reductases

- NADH₂-diaphorase (EC 1.6.1.1) (8)
- glutamate dehydrogenase, GDH (EC 1.4.1.2) (15)
- NADPH₂-diaphorase (EC 1.6.2.3) (8)
- glucose-6-phosphate dehydrogenase, G-6-P.DH (EC 1.1.1.49) (1)

Hydrolases

- acid phosphatase, APase (EC 3.1.3.2) azo dye and Gomori lead techniques (6)
 - leucine aminopeptidase, LAP (EC 3.4.1.1) (15)
 - adenosinetriphosphatase, ATPase (EC 3.6.1.3) (16)
- Controls of possibly interfering, non-enzymic staining reactions were performed after enzyme inhibitory fixation (10% formaldehyde) of the tissue sections and incubation of the sections in media lacking enzyme substrates.

RESULTS

Histological findings. The following histological changes were found to be present in both the skin

and the oral specimens of the same individual:

1. Hyperkeratosis.
2. Normal or decreased thickness of the stratum granulosum.
3. Irregular acanthosis alternating with atrophy of the stratum spinosum.
4. Focal liquefaction degeneration of the stratum basale.
5. Hyperchromasia and polymorphism of single epithelial cells.
6. Single cell keratinization.
7. Migration of inflammatory cells into the epithelium.
8. Thickening of the basement membrane forming focally a homogeneous, eosinophilic and PAS-positive band.
9. Perivascular accumulation of chiefly lymphocytes even deep into the connective tissue.

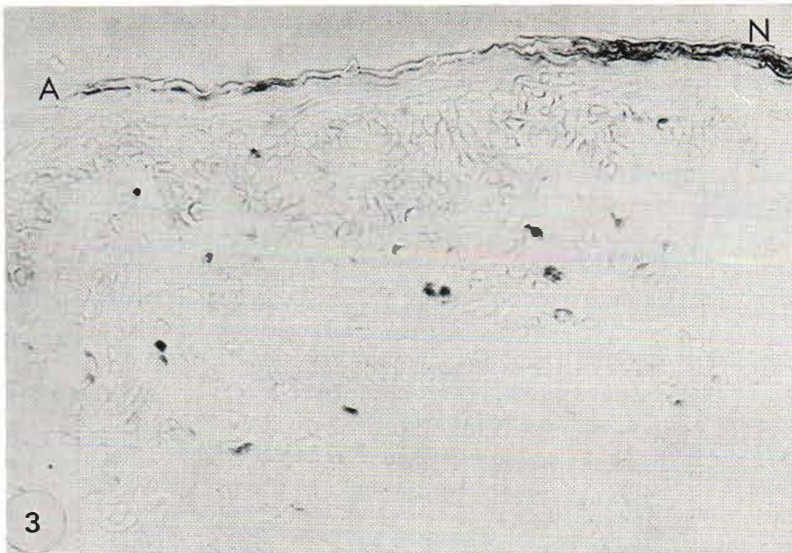


Fig. 3. Gradual loss of acid phosphatase activity in the superficial epithelial cell layers from clinically normal (*N*) to affected (*A*) skin areas. Enzymatically active macrophages and melanophages are visible in the underlying connective tissue. Azo dye technique. $\times 285$.



Fig. 4. Decrease of anaerobic oxidative metabolism from clinically healthy areas (*N*) towards affected (*A*), hyperkeratotic areas in the periphery of a skin lesion. Note the diminished staining reactions in the affected epithelium and the marked metabolic reactions in inflammatory cells and macrophages in the connective tissue. NADPH₂-diaphorase method, nitrogen atmosphere. $\times 285$.

10. Vasodilatation and edema.

11. Fibrinoid degeneration of the blood vessel walls with PAS-positive reactions.

In the skin the degeneration of the basal cells seemed to start in the hair follicles. The skin appendages showed atrophic changes. In the upper dermis, melanophages were frequently found, corresponding to areas with diminished amounts of melanin in the degenerated basal cells of the epithelium.

In the oral mucosa from 2 patients a pseudo-carcinomatous hyperplasia was observed. Generally

speaking, the inflammatory picture in the lamina propria and submucosa was more diffuse (Fig. 1) than in the skin lesions (Fig. 2). However, upon closer examination this diffuse picture included a lining up of lymphocytes in single rows along the multiple blood vessel ectasias. The oral lesions had a more pronounced telangiectasia than did the skin lesions. Sometimes, secondary infections seemed to be involved in accentuating the oral inflammation.

Enzyme histochemical findings. The changes described below are based on intra-section comparisons between the established lesions and the sur-

rounding, visibly unaffected tissues. The figures refer to the histological results of neighbouring sections (see, Histological Findings).

1. Decreased APase activity in the superficial epithelial cell layers despite increased keratinization (Fig. 3). The APase-positive band was broadened in areas with keratotic plugging but was less intensively stained than outside the lesions.

2. Unchanged or even decreased NADPH₂-diaphorase and G-6-P.DH activity in the stratum granulosum area (Fig. 4).

3. Marked decrease in all oxidative enzyme activity recorded in the presence of atrophy of the stratum spinosum (Fig. 5). Presence of positive histochemical reactions was usually confined to a narrow, perinuclear localization.

4. Focal absence of the normally intense NADH₂-diaphorase and GDH activities in the basal cells of the epithelium (Fig. 6).

5 and 6. Strongly increased cytoplasmic reactions of, especially, NADPH₂-diaphorase and G-6-P.DH activity in individual cells within hyperplastic epithelium (Fig. 7).

7. Increased activity of G.6.P.DH in migrating leucocytes.

8. Increased activity of LAP in the connective tissue closely adjacent to the homogeneous, sub-epithelial region (Fig. 8).

9. Presence of both hydrolytic and oxidative enzyme activity within the inflammatory infiltrates. Marked APase and LAP activity in the rather few macrophages and in the melanophages (Fig. 9).

10. Increased LAP activity in pericytes of the blood vessel walls in the presence of early degenerative changes including dilatation (Fig. 10). Loss of all oxidative enzyme activity in fibroblasts and fibrocytes in areas with edema and connective tissue necrosis.

11. Loss of all enzyme activities in the degenerated and necrotic blood vessel walls. The most pronounced changes were noted with the ATPase technique (Fig. 11).

The APase activity in the superficial cell layers of the skin epithelium was generally more prominent than in the oral epithelium.

Increased histochemical reactions of oxidative enzyme activity were recorded in the oral epithelium in areas with focal hyperplasia. Frequently, however, the individual cells did not show any marked increase in their cytoplasmic reactions, compared with neighboring regions but the increased number

of cells gave a false impression of enhanced enzyme level. The rich vascular plexus in the oral specimens provided a more varied pattern of enzyme activity in the vessel walls than in the skin specimens. Loss of ordinary metabolic reactions in the walls was occasionally observed even when they were covered by only a single row of inflammatory cells.

Generally speaking, there was a high level of concordance in the intra-individual comparisons between skin and oral specimens. The few differences observed could be explained by variations in the anatomy and environment of the two localizations. The interindividual comparisons showed a somewhat more differentiated picture. However, most prominent histological characteristics of the disease were present in all biopsy specimens.

DISCUSSION

Taking the special histological structure of the oral mucosa and the effects of moistening from saliva into consideration, the clinical pictures of the oral and cutaneous lesions were frequently very much alike. Similar observations have been reported by Andreassen (3). The oral histological findings in the present investigations were on the whole in agreement with those described by Andreassen & Poulsen (4).

The hyperkeratosis observed in the light microscope was not accompanied by any marked increase in the thickness of the stratum granulosum. This indicated a rapid rate of keratinization compared with the slow rate of keratinization and hence thickened granular layer in, for example, lichen planus (14). The weak APase activity and the unchanged or even decreased NADPH₂-diaphorase and G-6-P.DH activities in the superficial cell layers of the lupus erythematosus lesions further support the concept of a keratinization differing from that in lichen planus (11), and also from that in other hyperkeratotic lesions, such as leukoplakia (10).

A high level of concordance was generally noticed when comparing morphological and metabolic signs of atrophy in the stratum spinosum. A marked loss of cytoplasmic oxidative enzyme activity was observed in atrophic cells. The perinuclear predilection of the remaining positive histochemical reactions may conform with the distribution of organelles in the cells. Mitochondria are known to be arranged

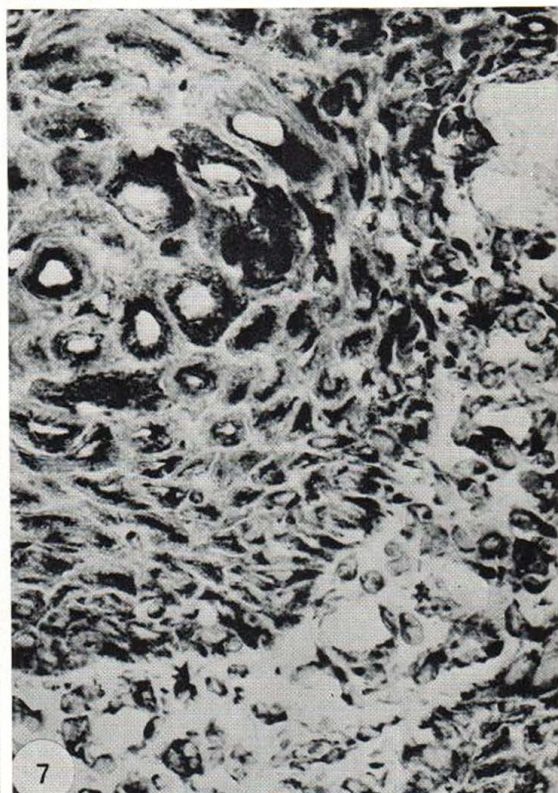
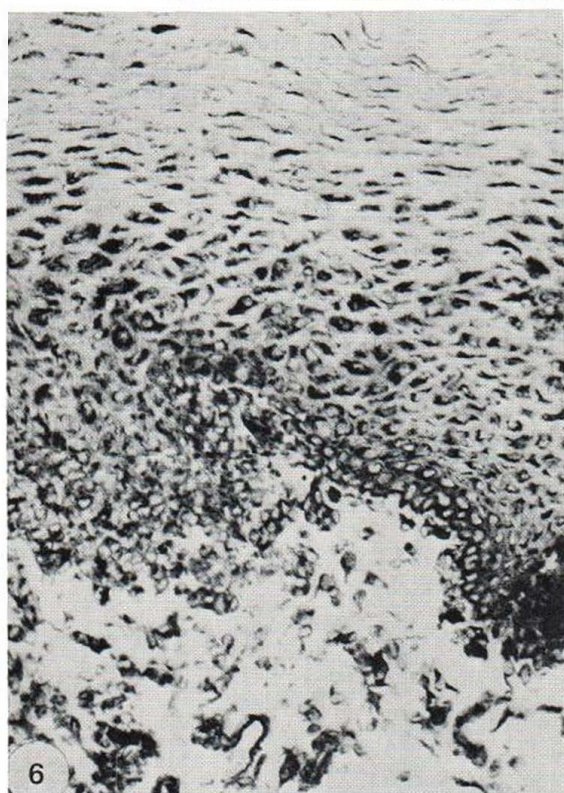


Fig. 5. Decrease of aerobic oxidative metabolism in the stratum spinosum and most of the basal epithelial cells at the border between clinically normal (N) and affected (A) skin tissue. Positive histochemical reactions in the epithelial cells are confined to a narrow perinuclear localization in areas with atrophy. NADH₂-diaphorase method, oxygen atmosphere. $\times 285$.

Fig. 6. Focal loss of glutamate dehydrogenase activity in the ordinarily strongly stained basal cells of the epithelium. The figure represents a hyperplastic oral epithelium. $\times 285$.

Fig. 7. Increased cytoplasmic reactions of glucose-6-phosphate dehydrogenase activity in certain cells of a pseudo-carcinomatous oral epithelium. $\times 445$.

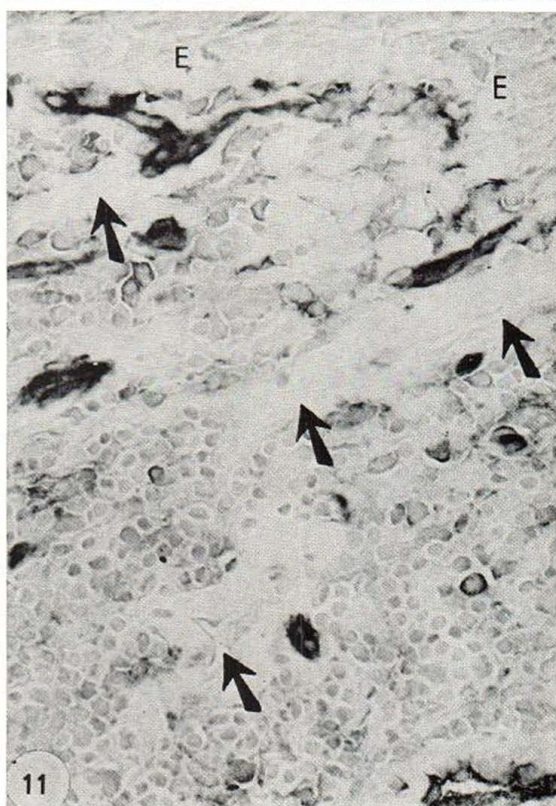
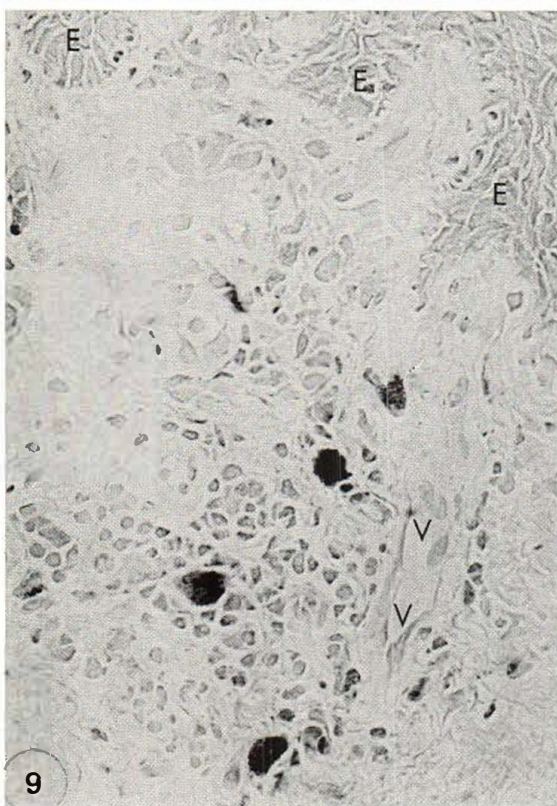
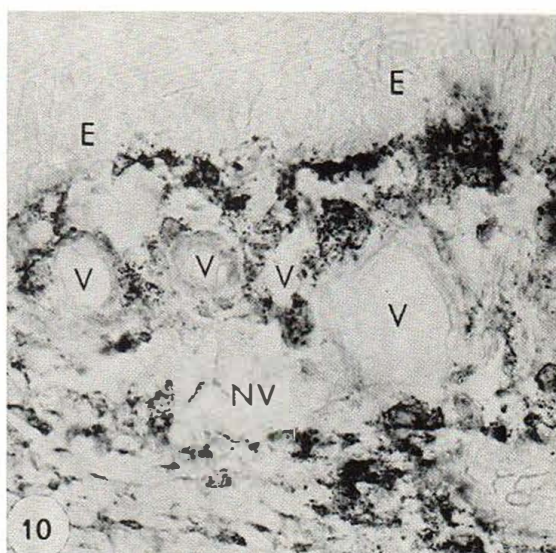
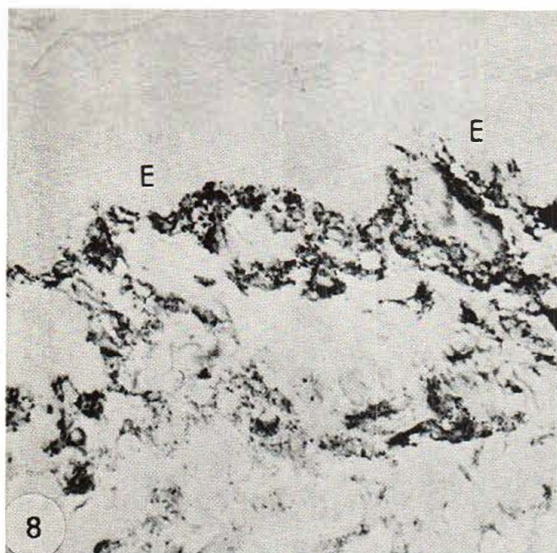


Fig. 8. Intense activity of leucine aminopeptidase in the sub-epithelial zone of a skin lesion. Positive reactions are also visible in scattered macrophages in the connective tissue. Skin epithelium, E. $\times 285$.

Fig. 9. Intense acid phosphatase activity in macrophages in an inflammatory infiltrate surrounding a dilated blood vessel (V). Oral epithelium, E. Azo dye technique, $\times 445$.

Fig. 10. Increased leucine aminopeptidase activity in pericyte areas of dilated blood vessels (V). Note the loss of activity in the necrotic blood vessel (NV). Oral epithelium, E. $\times 445$.

Fig. 11. Marked ATPase activity in unaffected blood vessel ectasias (dark colour) beneath an oral epithelium (E). A heavy infiltrate of inflammatory cells can be discerned surrounding necrotic blood vessels (arrows). $\times 445$.

perinuclearly in the stratum spinosum of the human mucosa (7). In the present study it seemed easier to demonstrate the distribution of epithelial atrophy and cell degeneration by means of enzyme histochemistry than by histology alone.

The focal liquefaction degeneration of the basal cell layer in the epithelium was most prominent in areas where the patchy, perivascular inflammatory infiltrates reached this layer. However, basal cell degeneration was also present in certain areas with dilated blood vessels and necrosis of the vessel walls without any concomitant accumulation of inflammatory cells. The loss of the normally high aerobic activity of NADH₂-diaphorase and GDH in the basal cells may reflect damage, including a diminished access of metabolites to the cells. The loss of metabolic reactions in the basal cells of lupus erythematosus lesions does not seem to be as extensive as in lichen planus (1). Hyperkeratosis in the periphery of the erythematosus areas may represent irritation hyperplasia promoted by sublethal damage.

The thickening of the PAS-positive subepithelial basement zone evidently included damage to the basement membrane itself, as shown by loss of melanin pigment into the connective tissue and visibly unrestricted migration of inflammatory cells into the epithelium. The high level of G-6-P.DH activity in such migrating leukocytes may correspond to their phagocytic properties (9).

The increased hydrolytic enzyme activity in the pericyte areas of the dilated blood vessels and the intense activity in macrophages are analogous to the findings of cell differentiation in granulomas (2). All enzyme activity and differentiation of perivascular cells seemed to have ceased when morphological signs of necrosis of the vessel walls were present. The relatively few APase-positive macrophages visible in the lesions may be due to progressive blood vessel necrosis and hence obstructed differentiation of such cells.

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