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Clinical and Laboratory Investigations

Ultrastructural changes in four cases of lichen sclerosus et atrophicus

PATRICIA R.MANN AND M.A.COWAN

MRC Unit on the Experimental Pathology of Skin and United Birmingham Hospitals

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SUMMARY

The ultrastructural changes in extragenital lichen sclerosus et atrophicus have been studied in four cases. The most pronounced changes are present in the dermis, where there are collagen degeneration and regeneration and increased amounts of elastin. The basal lamina shows holes, long gaps, and replication. Degenerate dermal material passes into the epidermis, which has increased numbers of Langerhans' cells and decreased numbers of melanocytes. These phenomena are discussed.

Lichen sclerosus et atrophicus (LSA) is an uncommon disorder of the skin and mucous membranes, which has characteristic clinical and histological appearances. Its aetiology is unknown. Electron-microscopic (EM) studies of LSA are few (Forssmann, Holzmann & Cabre, 1964; Nicolau & Balus, 1966). The present EM study was performed on specimens of extragenital LSA obtained from four patients, in order to try to increase the understanding of the aetiology and pathogenesis of the condition. Preliminary findings in two of these patients have been previously reported (Wallace, 1971).

CLINICAL MATERIAL

Case 1

Mrs E.W., aged 62 years, showed multiple, slightly raised, white, infiltrated plaques on the trunk, together with anogenital LSA. Biopsy material was obtained from a typical lesion of the left breast area.

Case 2

Mrs E.D., aged 60 years, had anogenital LSA together with an atrophic, slightly depressed lesion on the inner aspect of the right buttock. The buttock lesion was biopsied.

Case 3

Mrs A.C., aged 69 years. Biopsy was carried out on a shiny, white, infiltrated plaque on the dorsal aspect of the right forearm.

Case 4

Miss B.C., aged 38 years. Biopsy was carried out on a white, linear, slightly depressed lesion on the inner aspect of the right arm, near the elbow.

No treatment whatsoever had been applied to the lesions of Cases 1 and 2. No topical steroid had been applied to the lesions of Cases 3 and 4 for a period of at least 2 months before biopsy for EM was carried out. In Cases 1 and 3 the lesions were active, i.e. the surface was slightly raised, very white and with palpable infiltration. In Cases 2 and 4, the lesions were inactive or resolving, i.e. the surface was level or slightly depressed, less white than in the active lesions and with little palpable infiltration. All the specimens showed the typical light microscopic features of LSA, namely hyperkeratosis, epidermal atrophy with liquefaction degeneration of basal cells, and a zone of pale homogeneous-looking collagen in the upper dermis with a variable lymphohistiocytic infiltrate below it.

METHODS

Pieces of skin were removed by biopsy under local anaesthesia with 1% plain lignocaine. They were cut into cubes of approximately 1 mm and fixed for 1 h in 2% glutaraldehyde in phosphate buffer pH 7·4 with 0·2 M sucrose added. This was followed by washing in several changes of sucrose–phosphate buffer pH 7·4 over a period of at least 24 h before post-fixation in 2% osmium tetroxide dissolved in the same sucrose–phosphate buffer. Dehydration in graded concentrations of ethanol was followed by embedding in Epon. Sections were cut with a glass knife on a Reichert OMU2 ultramicrotome, and 1 μm survey sections were stained with aqueous toluidine blue. Ultrathin sections were collected on uncoated copper grids and stained with methanolic uranyl acetate.

RESULTS

The EM changes were similar in all the specimens, varying only in degree. The dermal changes were present throughout the depth of the dermis, from immediately below the basal lamina to the lower limit of the section, which was still within the hyaline region seen on light microscopy. Cells and blood vessels were sparse in the regions examined. The most striking abnormalities were in the connective tissue fibres and ground substance. These changes were qualitatively consistent, but varied in degree with the clinical activity of the lesions as defined.

Collagen fibres varied greatly in their diameters (10–90 nm) within the same region and showed gross alterations in their alignment to one another (Figs. 1, 2 and 6). They were frequently associated with deposits of granular material (Fig. 1). Normal cross-striations were present in the thicker fibres, and occasional fibres in cross-section looked like empty tubes (Fig. 2). In active lesions (Cases 1 and 3) larger fibres were more common. In some areas the fibres were quite sparse, and few fine fibrils were present. In resolving lesions (Cases 2 and 4) there was much more evidence of new collagen formation, which was laid down in a random fashion as a network consisting of fine fibrils which rarely showed cross-striations (Fig. 3). Larger, striated collagen fibres were still present, but less common.

Masses of varying size with the EM features of elastin were scattered throughout the dermis, often in close proximity to collagen (Fig. 4). Elastin was seen in larger amounts than in normal skin.

Some dermal fibroblasts contained small clumps of mature collagen still showing cross-striations (Fig. 5). In some cases a lysosomal membrane surrounded the collagen, which had presumably been phagocytosed. There were also macrophages which contained collagen fragments and melanin granules.

Basal lamina changes were consistently present and were similar in all the specimens. A general diffuse thickening of the lamina (up to 500 nm) was seen most frequently. In some areas there were small holes in the lamina, and in others there were zones of complete absence of lamina leaving basal keratinocytes in direct contact with the dermis. In other areas there was marked replication of the

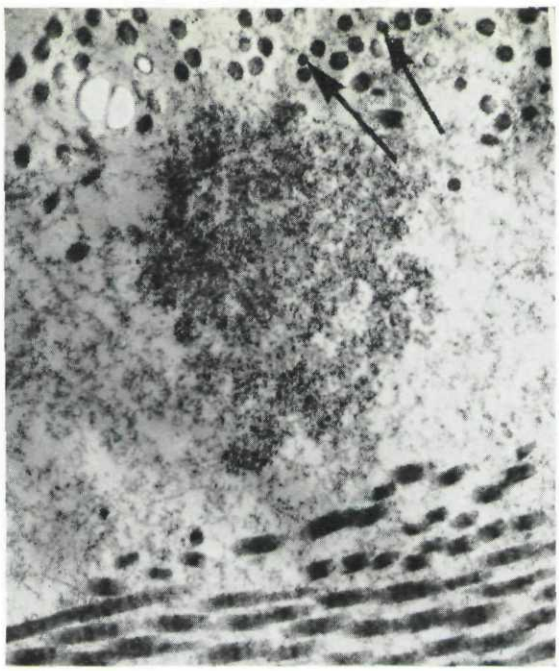

FIGURE 1. Collagen fibres of varying diameters, 40–90 nm (small fibres arrowed), and associated degenerate, granular, dermal material. Clinically active lesion (× 30,000).

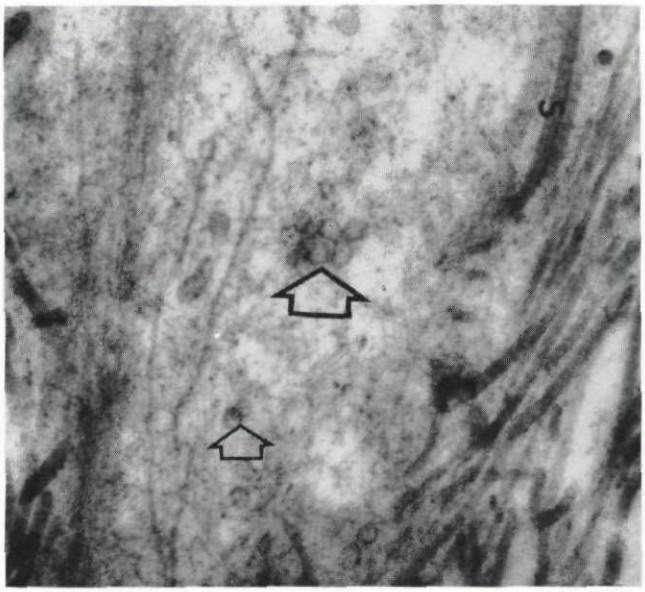

FIGURE 2. Normal collagen fibres with cross-striations (S), and fibres in cross-section appearing as empty tubes (arrow). Diameters vary from 20 to 90 nm (× 31,000).

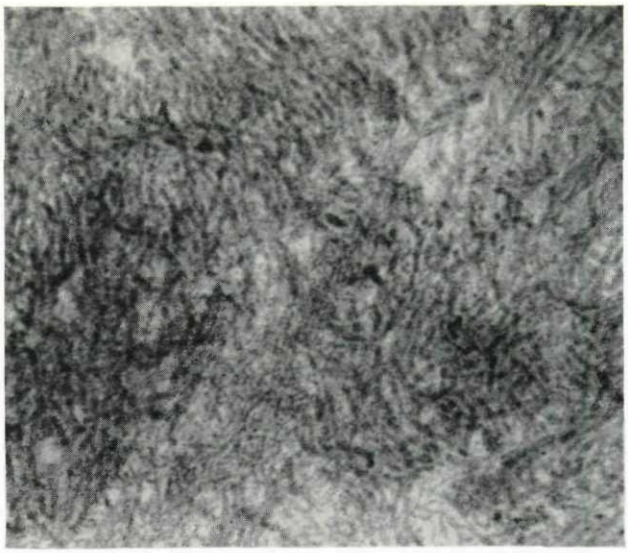

FIGURE 3. Random network of fine collagen fibrils. Diameters vary from 10 to 20 nm. Resolving lesion (× 41,000).

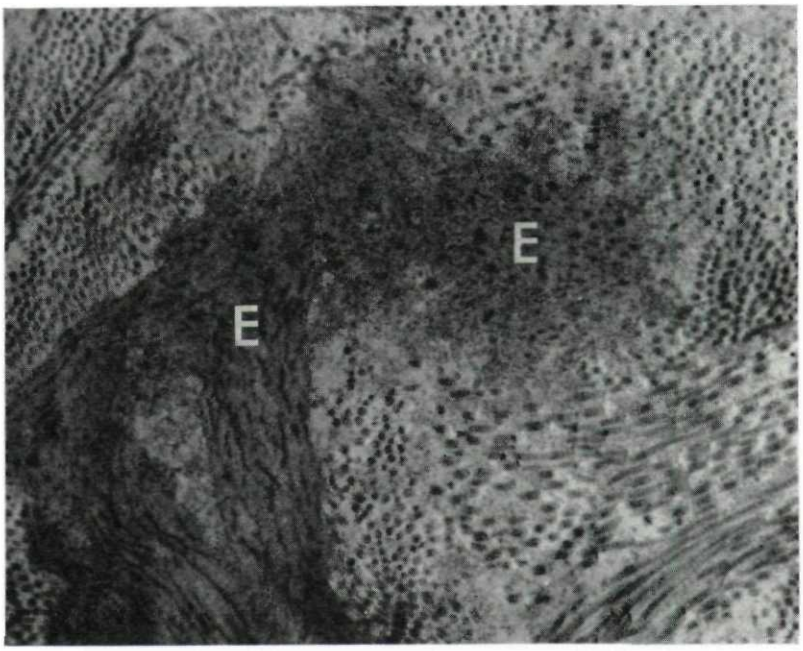

FIGURE 4. Masses of elastin (E) in the dermis (× 13,400).

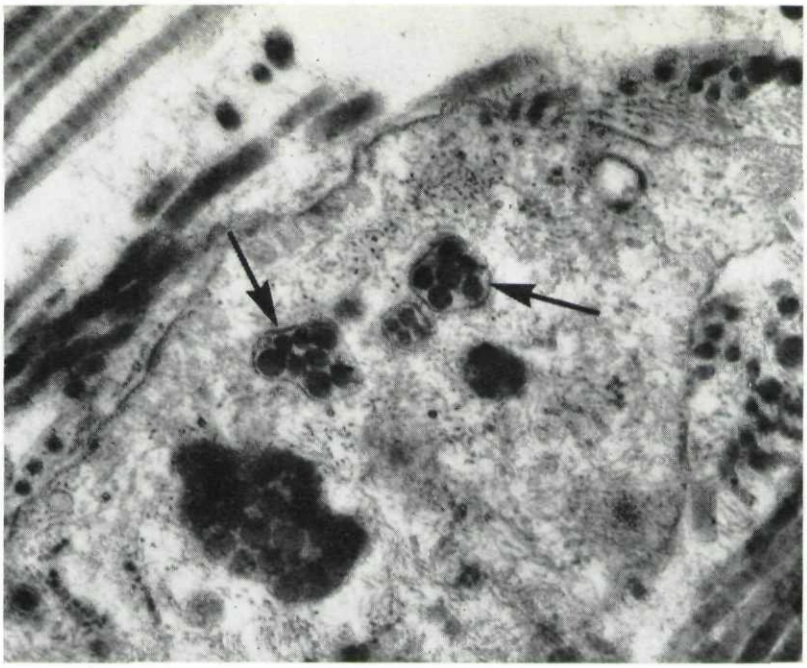

FIGURE 5. Clumps of collagen in cytoplasm of dermal fibroblast. Phagosome membrane arrowed (× 32,000).

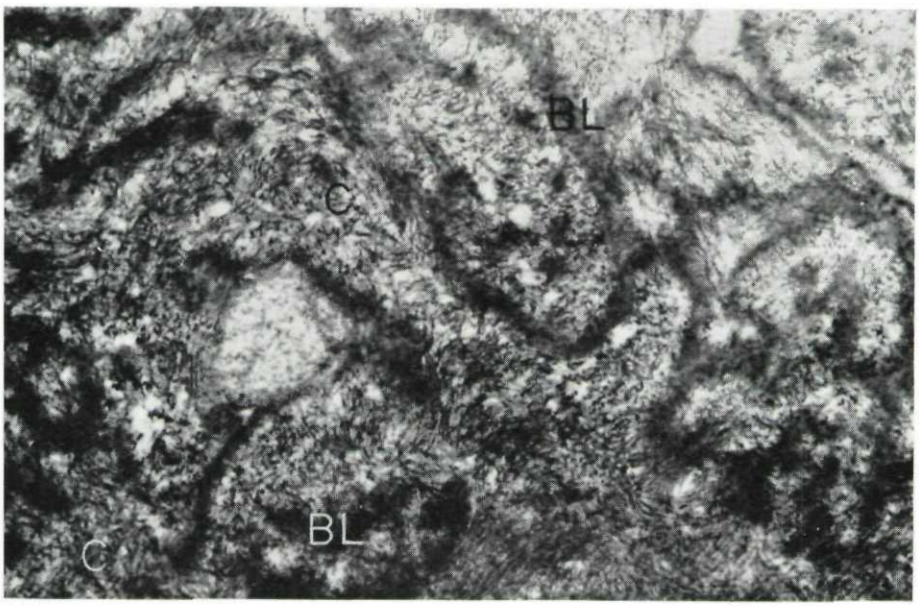

FIGURE 6. Replication of basal lamina (BL). Immature collagen, C (× 6000).

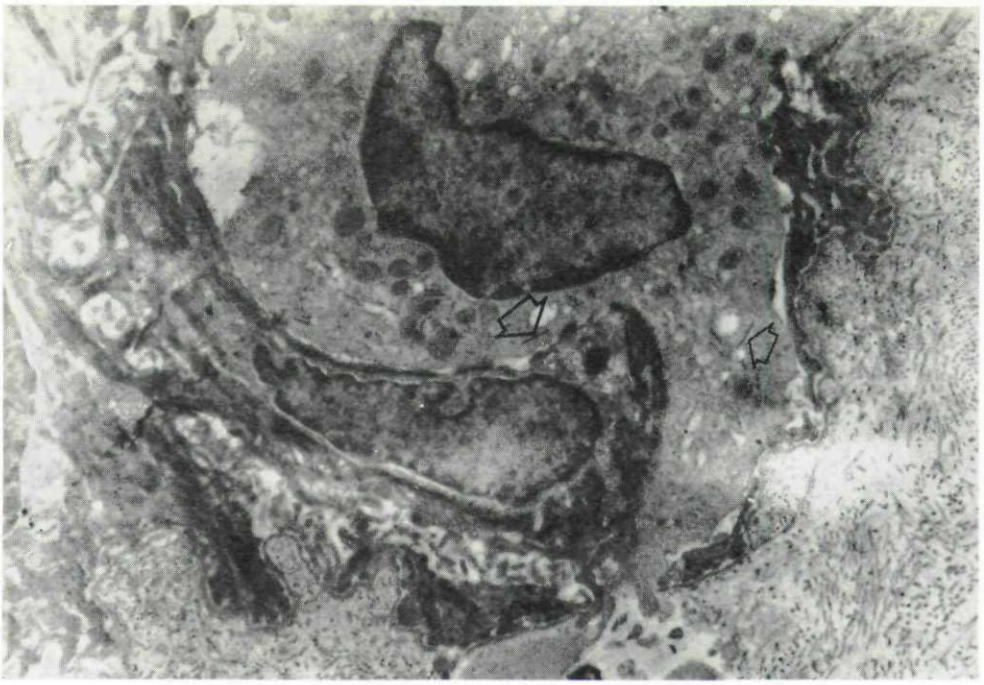

FIGURE 7. Langerhans' cell, part in the epidermis and part in the dermis. Langerhans' granules arrowed (× 7500).

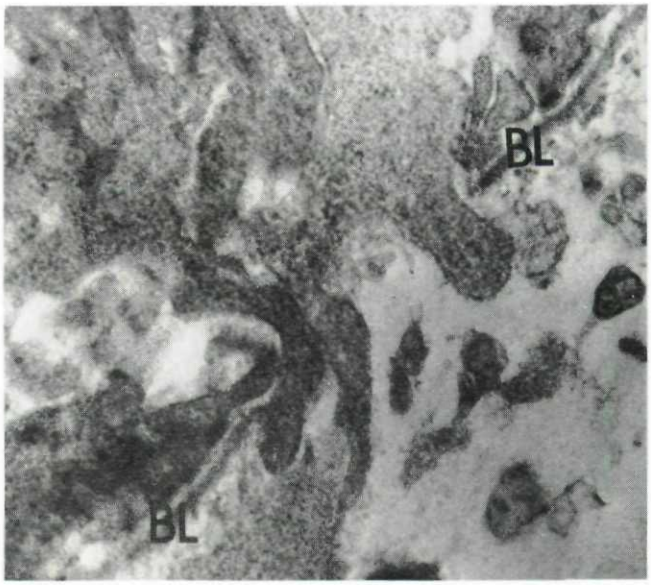

FIGURE 8. Enlargement from Fig. 7 showing hole in basal lamina (BL) (× 35,500).

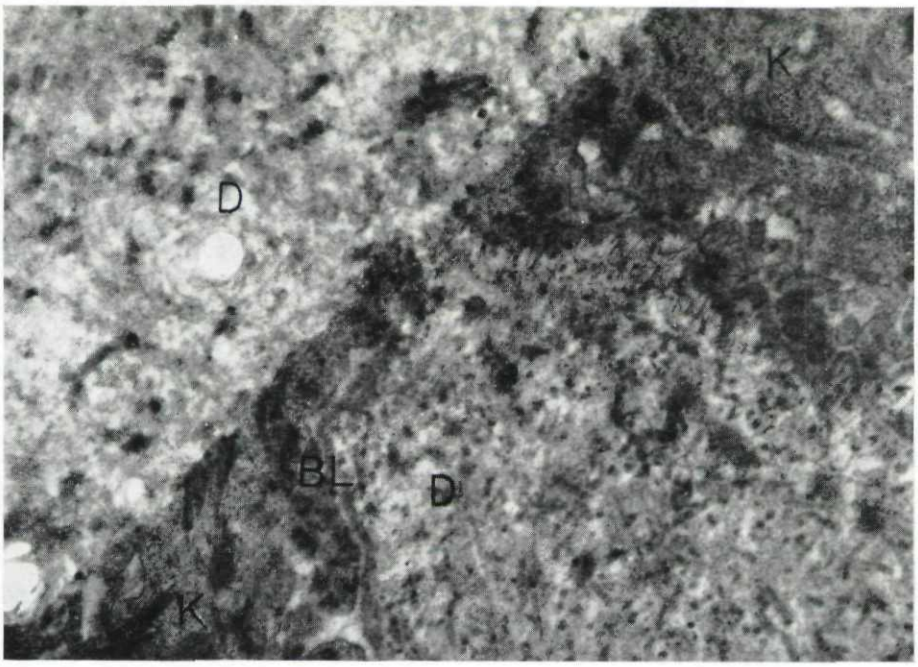

FIGURE 9. Disrupted basal lamina (BL) with degenerate dermal material (D) on either side. Degenerate keratinocytes (K) and hemidesmosomes (H) (× 18,000).

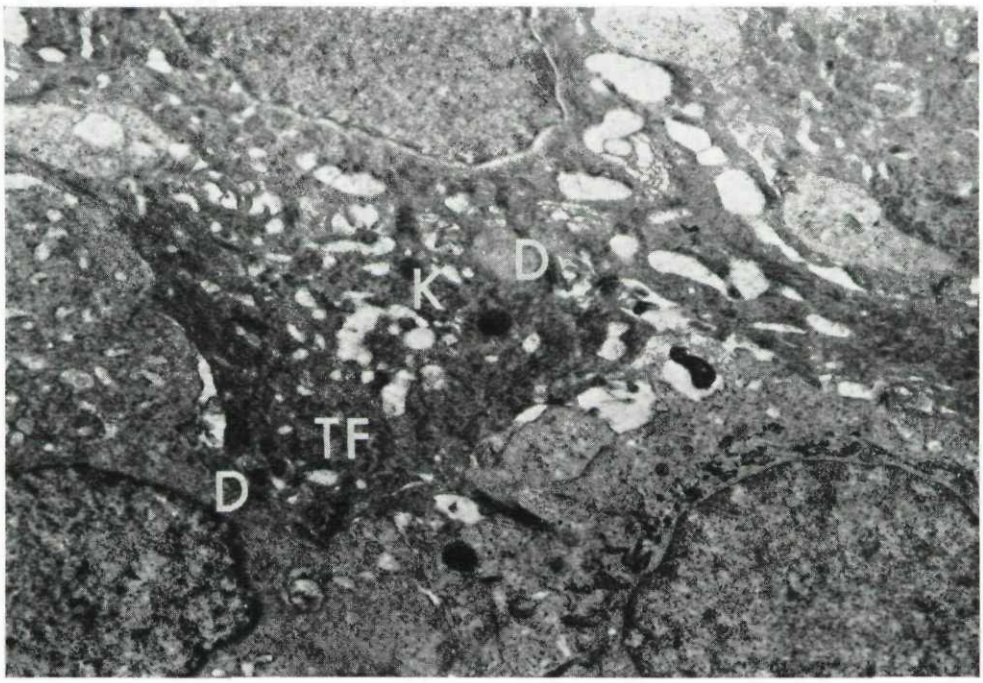

FIGURE 10. Vacuolation and increased electron density of degenerating keratinocyte (K). Retraction of tonofilaments (TF) from desmosomes (D) (× 5000).

basal lamina (Fig. 6). Normal hemidesmosomes were present adjacent to the thickened lamina, but not where the lamina was missing.

Some holes in the basal lamina had cells passing through them, partly in the epidermis and partly in the dermis. These were Langerhans' cells (Fig. 7), or unidentified 'clear cells' (i.e. without Langerhans' granules or melanosomes). Degenerate dermal material containing clumps of collagen and elastin was also seen apparently passing through some of these holes in the basal lamina into the epidermis, where it was sometimes present in large amounts, but less dense than in the dermis (Fig. 9). It had sometimes sheared through basal and suprabasal keratinocytes, leaving a part of these cells above and a part below the material. Where the epidermis was intact the most pronounced changes in keratinocytes were in the basal and suprabasal layers. These keratinocytes showed varying degrees of degenerative change, with shrinkage, vacuolation, and increased electron density (Fig. 10). Keratinocytes in the Malpighian and granular layers appeared normal. Occasional sections showed intra-epidermal macrophages, and other cells which could not be positively identified and had presumably infiltrated the epidermis.

In sections from active lesions, melanocytes could not be seen, but Langerhans' cells of normal morphology were present in apparently increased numbers from the basal zone upwards. In sections from resolving lesions there were fewer Langerhans' cells; melanocytes were present in small numbers and they usually showed degenerative changes. The more active the lesion clinically, the fewer melanocytes and the more Langerhans' cells were present, the latter seeming to replace the former in the stratum basale.

DISCUSSION

The most striking clinical and light microscopic changes in LSA are dermal, and these correlate well with the EM changes seen in the present study, and with findings by other authors (Forssmann *et al.*, 1964). Epidermal changes are also present.

Our study suggests that the major abnormality is in the degeneration and reformation of collagen. Active lesions, as defined, show considerable degeneration of collagen fibres, with marked variability of fibre diameter, and apparently empty collagen tubes. Resolving lesions show much new collagen fibril formation, which is laid down irregularly. Forssmann *et al.* (1964) noted the variability in fibre diameter and 'neofibrillogenesis', and considered LSA to be primarily a disease of new collagen formation, where compaction of new collagen strangulates other dermal structures, leading to a paucity of blood vessels and fibroblasts. The descriptions would suggest that they studied resolving lesions in their two cases. Light microscopic studies also support the occurrence of new collagen formation (Steigleder & Raab, 1961).

The large clumps of elastin present throughout the diseased dermis in our cases were not noted by Forssmann *et al.* (1964) in their EM study. Most descriptions of the light microscopic features of LSA state that elastic tissue is usually absent, or present in only small amounts (Steigleder & Raab, 1961; Montgomery, 1967; Milne, 1972), and it seems possible that the disease process may alter the staining properties of elastin.

Forssmann *et al.* (1964) mentioned thickening of the basal lamina; this finding was consistently present in all our specimens. But they did not report holes in the basal lamina, its absence in places, and its replication in other areas. Basal lamina replication has been reported in various skin conditions, such as psoriasis (Cox, 1969), pityriasis rubra pilaris (Cruickshank & Mann, 1971), and porokeratosis of Mibelli (Abdel-Aziz *et al.*, personal communication, 1972) and when hyperplasia of the epidermis is produced by hexadecane (Cowan & Mann, 1971). This is probably non-specific.

Melanocytes were not seen in active lesions, but were present in small numbers, and showed

degenerative changes, in resolving lesions. This would account for the pearly white appearance of active lesions and the darker colour of resolving ones. These cells seemed to be replaced by Langerhans' cells in the active lesions, and one such cell was seen part in the epidermis and part in the dermis. It has been suggested that there is a reciprocal relationship between the numbers of melanocytes and basal Langerhans' cells in the epidermis. This has been discussed in relation to vitiligo (Wolff, 1972), and the hypopigmented lesions of leprosy (Job, Nayar & Narayanan, 1972). This view is supported by our findings in LSA, but its significance is unknown.

In this study degenerate collagen appeared to be phagocytosed by dermal fibroblasts and pass together with other dermal debris, including elastin, into the body of the epidermis. Thus the skin appeared to eliminate a diseased component. The existence of transepidermal elimination of dermal material is well described, the following constituents being subjected to this process on occasions—elastic fibres (both intact and degenerate), collagen, calcium, bone, amyloid, lepra bacilli, lymphoma cells, and erythrocytes (Mehregan, 1968). In our own cases, the dermal debris was seen only within the epidermis, but this does not exclude the possibility that it might be eliminated through the skin surface. This could account in part for the small punctate lesions which clinically have been called follicular hyperkeratosis, although it is known that histologically true follicular hyperkeratosis may occur.

In contrast to Forssmann's observations, collagen degeneration appeared as prominent as 'neofibrillogenesis', casting doubt on the primary role of the latter. The reduction in the number of capillaries is in agreement with Forssmann's findings, but we would hesitate to attribute this to either collagen degeneration or 'neofibrillogenesis'.

All the evidence suggests that the epidermal changes are secondary to those in the dermis.

ACKNOWLEDGMENTS

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