

ULTRASTRUCTURAL CHANGES IN THE INFLAMMATORY ZONE OF LOCALIZED SCLERODERMA

Takasi Kobayasi and Gustav Asboe-Hansen

*From the Department of Dermatology (with Connective Tissue Research Laboratories),
University of Copenhagen, Rigshospital, Copenhagen, Denmark*

Abstract. Biopsies from the progression zone ("lilac ring") of localized scleroderma of the morphea type were studied with the electron microscope. The most common change of the collagen fibrils was the variety of thickness and their disarray. Uneven thickness and bifurcation of the individual collagen fibrils were also seen. Elastic fibres had a reticular matrix and degenerate fibrils and were covered by a granular coat. Numerous acid glycosaminoglycan (mucopolysaccharide) microfibrils, degranulating mast cells and changes in the dermo-epidermal junction were noticed. These findings indicate fibrogenic and mucinous changes in the "lilac ring". Filamentous bodies, probably identical with "hyalin bodies" were found in the outer dermis of two specimens.

Collagen fibrils (30-40 nm thick), thickening of the basal lamina of the dermo-epidermal junction, vessel walls and perineurium, and considerable amounts of ground substance in the interfibrillar space are the changes in the fibrous area of localized scleroderma that have been described earlier (4, 10, 12). Identical changes have been found in generalized scleroderma of the acrosclerotic type (6, 10). In morphea, an inflammatory progression zone surrounds the fibrotic lesion as a "lilac ring", while in generalized scleroderma the progression zone lies in the deep corium. The progression zone of localized scleroderma was studied in detail, and the results are now reported.

MATERIAL AND METHOD

Skin specimens from 4 patients with localized scleroderma of the morphea type were studied by electron microscopy. The biopsies were taken from the inflammatory zone around the sclerotic lesions. The specimens were fixed in 6% glutaraldehyde in Veronal acetate buffer (pH 7.2) with 7.5% sucrose and then osmicated. After dehydration in alcohol solutions of increasing concentration, the tissue-blocks were embedded in Epon 812 and sectioned

with an ultramicrotome. The ultrathin sections were stained with uranyl acetate and lead citrate and studied with a Siemens electron microscope (Elmiskop 1A) at 80 kV with double condensers.

OBSERVATIONS

Thick collagen bundles were seen in the deep corium, while in the outer dermis and the perivascular areas, the bundles were thinner and separated by wide spaces. Elastic fibres and cells, i.e. fibroblasts, mast cells and histiocytes, were located between the collagen bundles.

The bundles contained *abnormal collagen fibrils* intermingled with normal fibrils. The variability of thickness is the most common abnormality. Fibrils with thicker (150 nm) and thinner (30 nm) diameters than normal fibrils were seen to be grouped within one and the same bundle (Fig. 1). The individual fibrils often evidenced an uneven thickness and bifurcation (Figs. 1, 2). Neither curling nor irregular cut-surfaces were seen. The axial periodicity was a constant 55 nm. Some bundles contained disarranged, non-parallel abnormal fibrils (Fig. 2). Loose bundles of normal collagen fibrils appeared in mucinous areas of one patient (Fig. 7). One specimen showed dense felt-like material covering normal collagen bundles (Fig. 3). Close to some vessels, proto-collagen-like filaments were found together with thin collagen fibrils (Fig. 4) in a parallel arrangement and with regular segmentation. These segments occurring with about 60 nm wide intervals were about 60 nm wide. Some filaments were seen to form 30 nm thick collagen fibrils. Each segment of a filament corresponded with the band of the collagen fibril (Fig. 4).

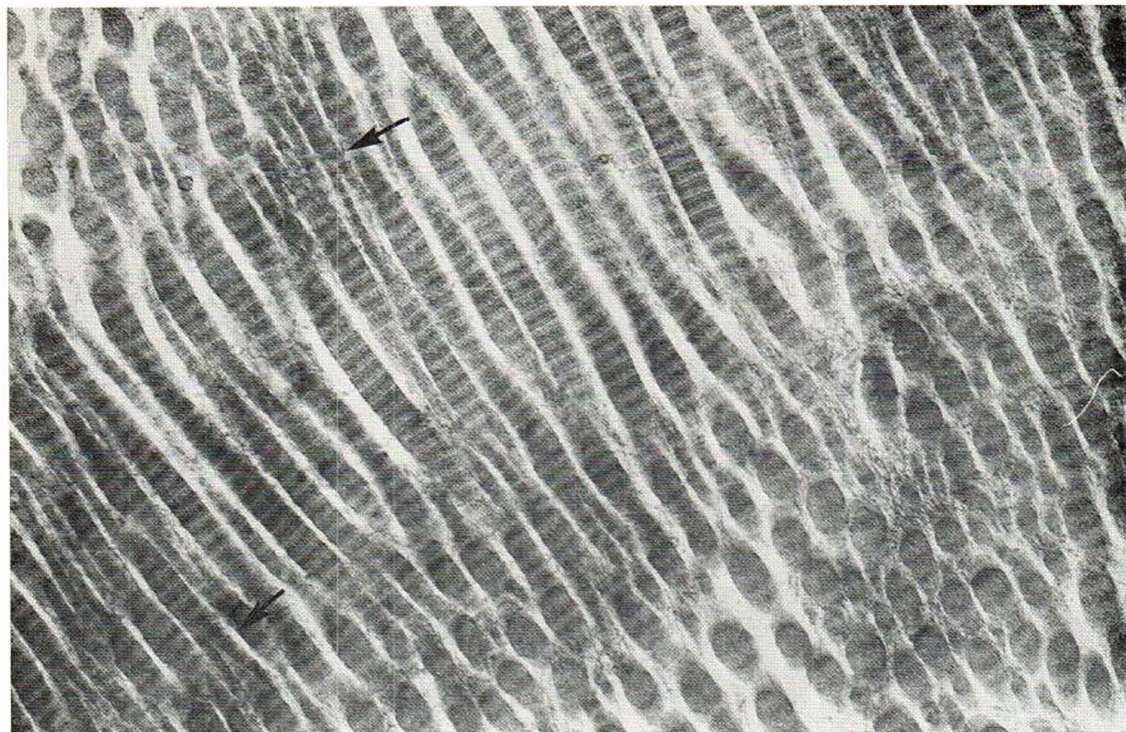


Fig. 1. Abnormal collagen fibrils showing varying thickness (30–100 nm) and bifurcation (arrows). The axial periodicity is normal. $\times 45\,000$.

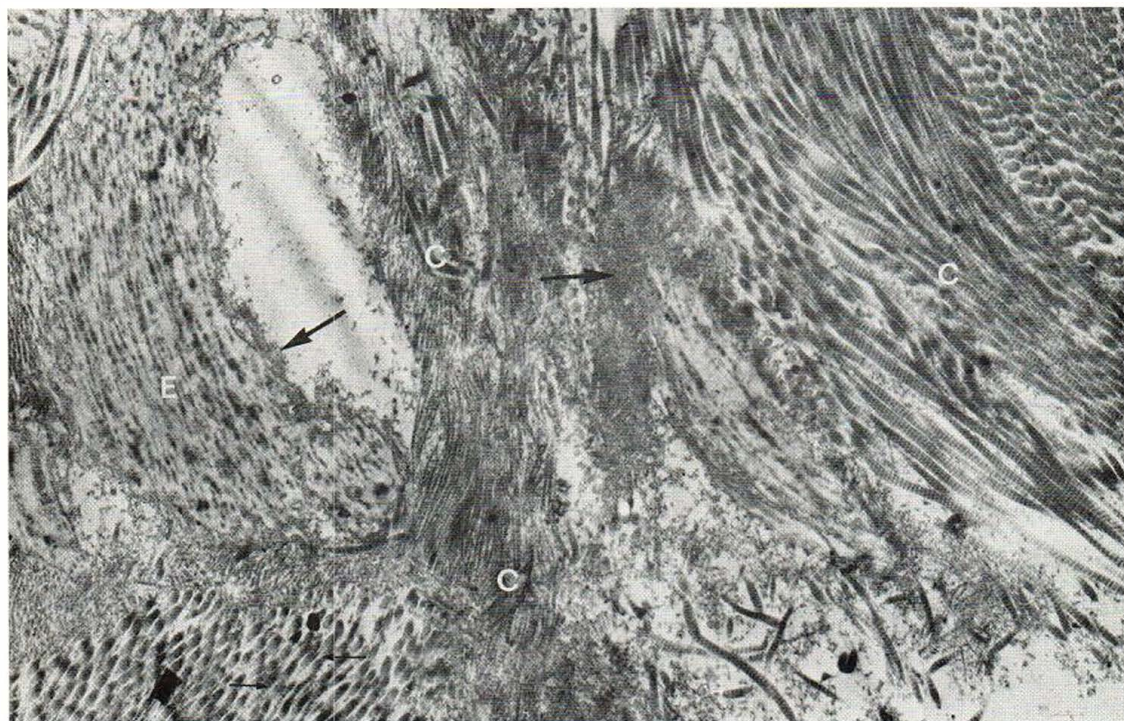


Fig. 2. Deep corium of patient No. 4. The collagen fibrils show bifurcations (thin arrows) and pronounced variations in thickness. (C) Disarranged collagen fibril bundle. (E) Elastic fibre with granular coat (arrows). $\times 22\,500$.

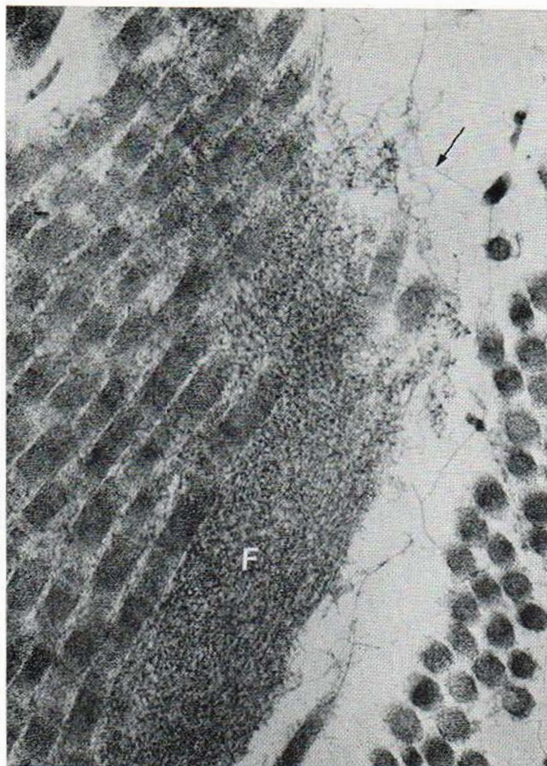


Fig. 3. Felt-like material (F) covering a normal collagen bundle. Arrow indicates acid glycosaminoglycan microfibrils. $\times 46\,800$.

Elastic fibres and fibrils also presented diverse figures: 1) A granular coat surrounding a homogeneous matrix, here and there lacking elastic fibrils (Fig. 2). 2) A reticular matrix with remnants of homogeneous matrix material and a few indistinct elastic fibrils (Fig. 5). 3) Masses of dense straight elastic fibrils (Fig. 6). In mucinous areas, the elastic fibres and fibrils were normal (Fig. 8). In one specimen, distinct elastic fibrils were found within normal collagen fibril bundles.

Mucinous changes were more or less pronounced in all specimens. In one showing strong mucinosis there were no pronounced changes of the fibres. Numerous microfibrils with knobs representing acid glycosaminoglycans (mucopolysaccharides) were seen (Figs. 7, 8, 11).

Solitary filamentous bodies were demonstrated in the outer corium of 2 patients. The bodies faced the dermal fibres direct or were partially surrounded by dermo-epidermal basal lamina material (Fig. 9), while no enveloping double mem-

brane was seen. The filaments, which were arranged in whorls, had a round cut-surface with a diameter of 8 nm.

The dermo-epidermal junction was found to be altered in three specimens (Fig. 10). The basal lamina was relatively thick and showed complicated foldings as well as numerous normal anchoring fibrils. The subepidermal space was filled with fine felt-like fabric of dense granules, while no anchoring filaments were found. The basal-cell membrane and the half-desmosomes were indistinct.

No definite changes were noticed in the vascular and perineural basal laminas.

Mast cells were numerous in mucinous areas (Fig. 11). The cells contained mature and disintegrating granules, and the cytoplasm often appeared honey-comb like.

Fibroblasts and histiocytes were normal.

The dermal alterations are set out in Table I. Within the same areas the pathological changes

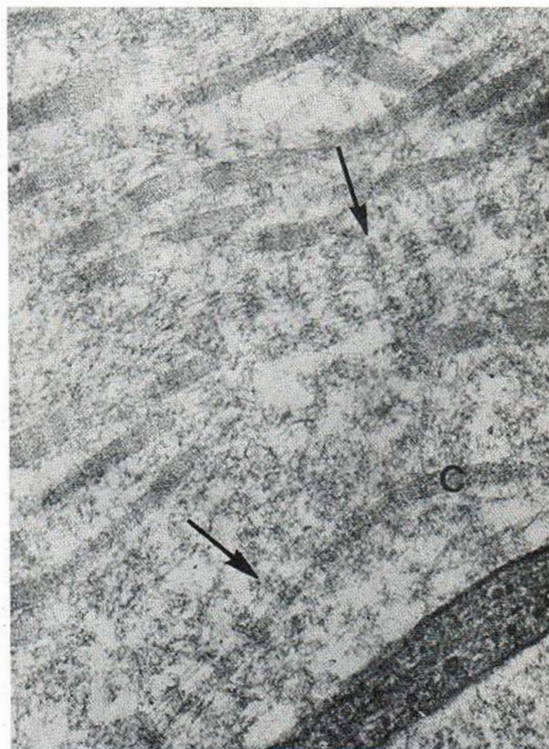


Fig. 4. Proto-collagen-like filaments in parallel arrangement and with regular segmentation (arrows). The proto-collagen filaments seem to be forming a collagen fibril (C). The intervals between segments of both fibrils and filaments are 120 nm. $\times 61\,000$.

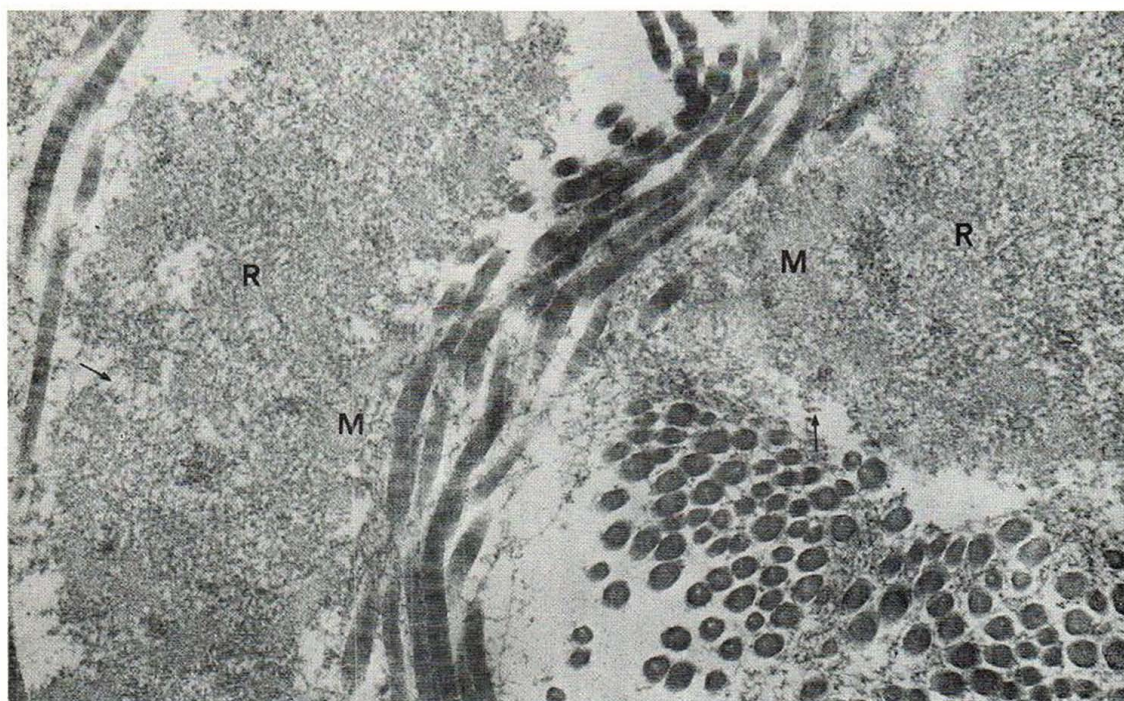


Fig. 5. Two elastic fibres with reticular matrix (R) and remnants of homogeneous matrix (M). Elastic fibrils are

indistinct and sparse (arrows). The thickness of collagen fibrils varies. $\times 45\ 000$.

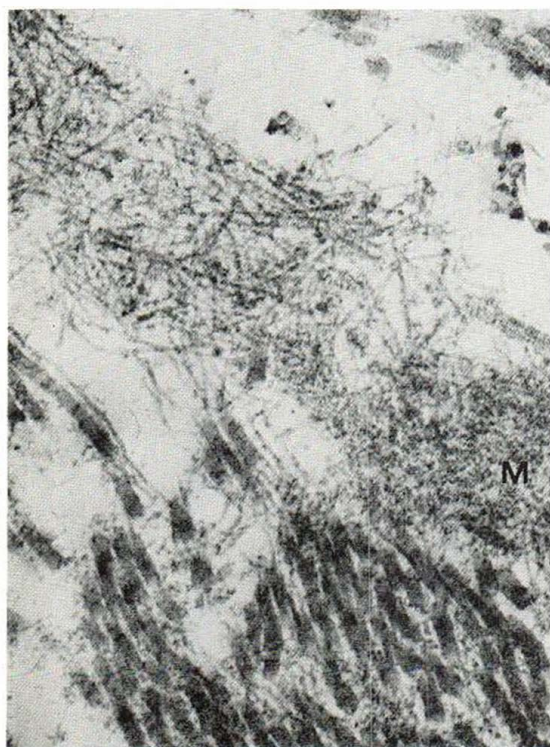


Fig. 6. Straight, dense, degenerate elastic fibrils in random arrangement. Matrix (M) appears granular. $\times 46\ 800$.



Fig. 7. Bundle of loosely arranged normal collagen fibrils. Microfibrils with knobs (arrows) in the interfibrillar compartment. $\times 94\ 000$.

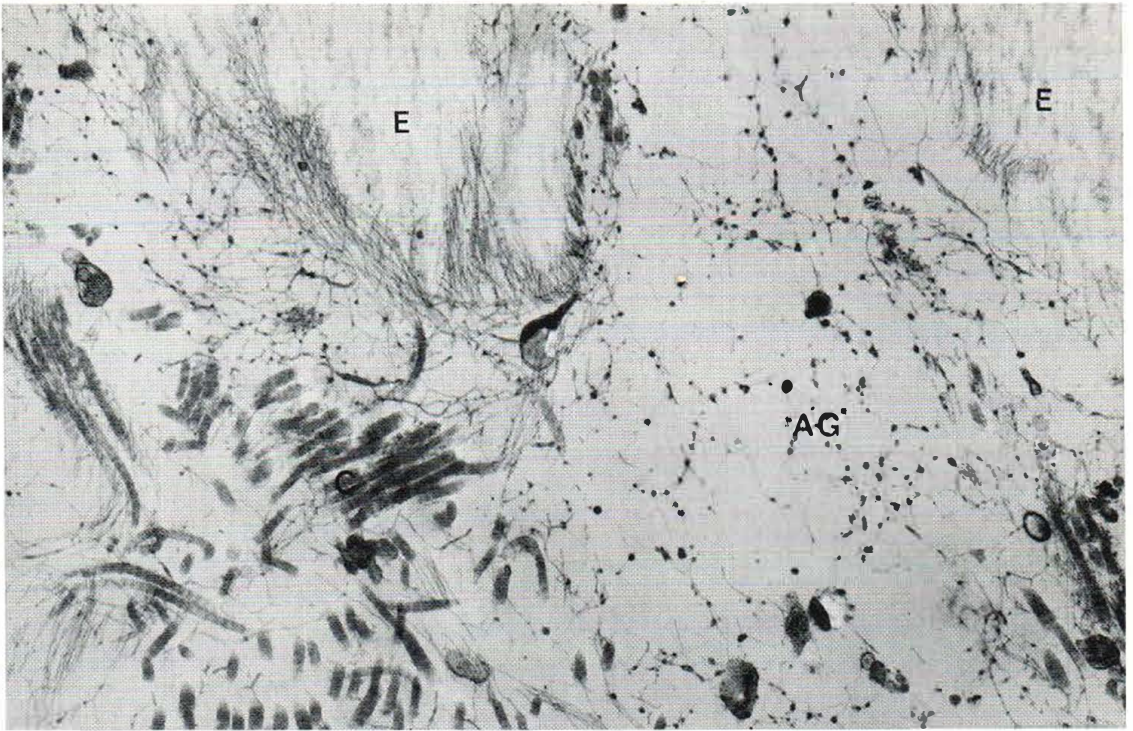


Fig. 8. Mucinous change in patient No. 1. Elastic (E) and fibrils, branched microfibrils with knobs representing acid glycosaminoglycans (AG) are numerous. $\times 22\,500$.

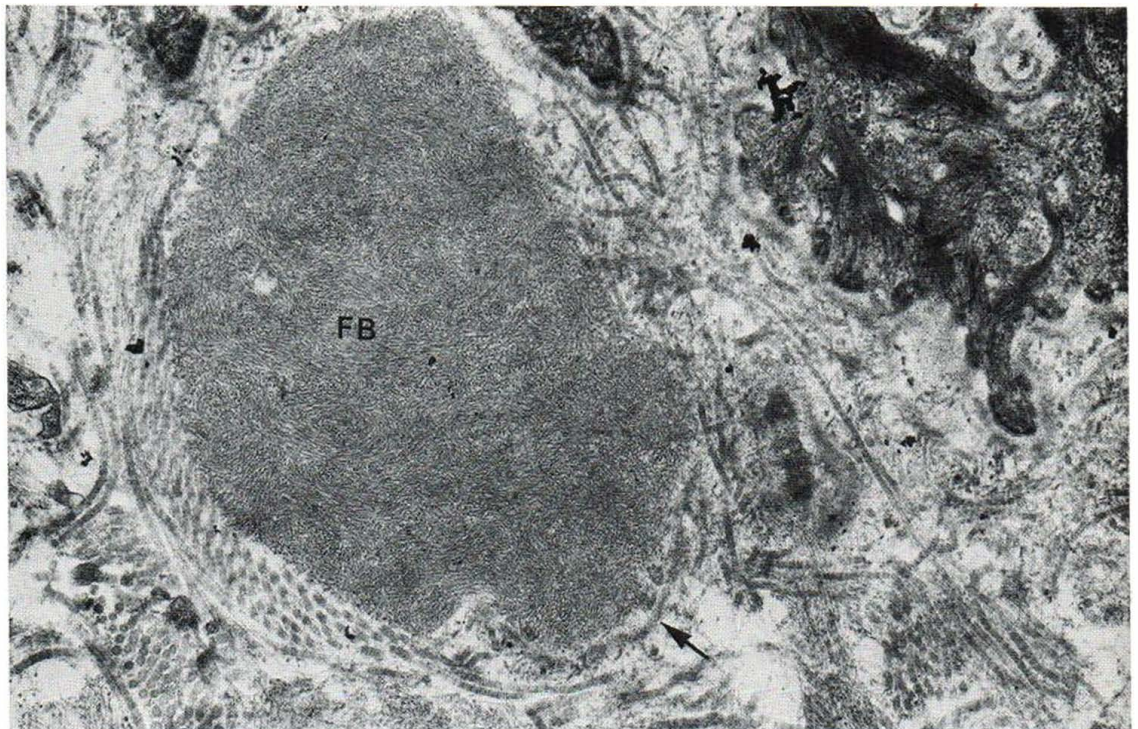


Fig. 9. Filamentous body (FB) in the outermost corium, partially surrounded by basal lamina material (arrow). $\times 22\,500$.



Fig. 10. Dermo-epidermal junction. Basal lamina (BL) is folded. The numerous anchoring fibrils (A) are normal. The subepidermal space is filled with felt-like fabric (F) and dense granules (G). No anchoring filaments, distinct

basal-cell membrane or half-desmosomes can be seen. Arrow indicates an indistinct half-desmosome. Tonofilaments (T) in basal cells. $\times 45\ 000$.

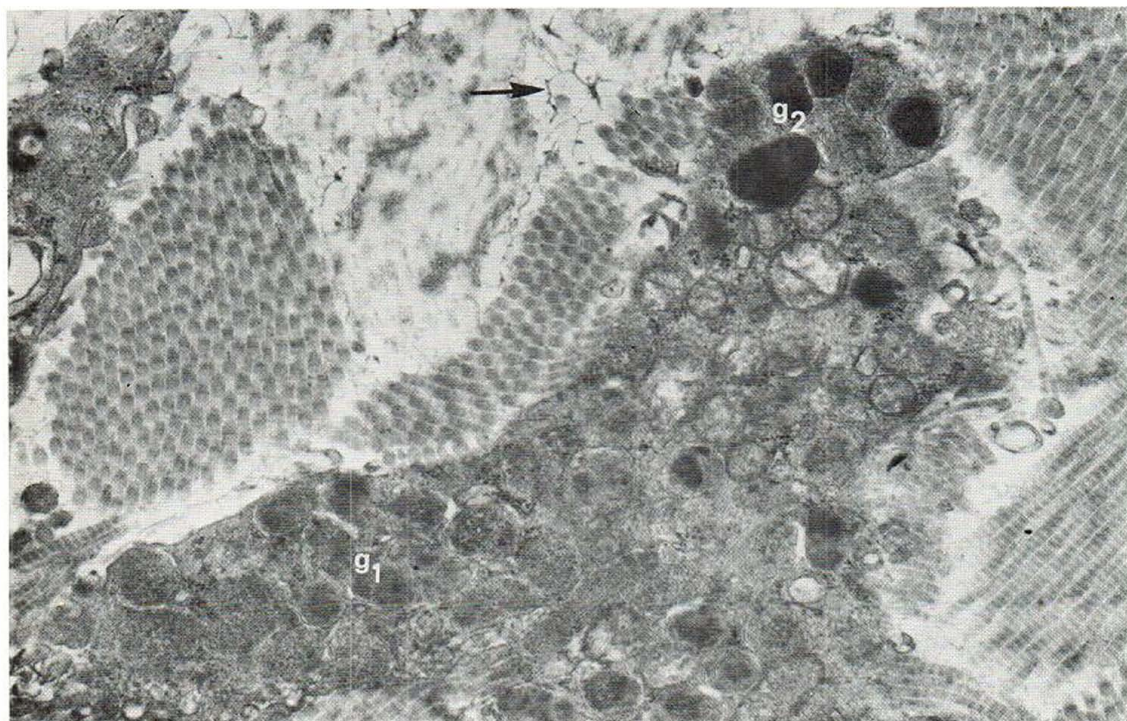


Fig. 11. A mast cell in a mucinous area. The mast cell contains disintegrating (g_1) as well as mature (g_2) granules.

An arrow indicates acid glycosaminoglycan microfibrils. Collagen fibrils and elastic fibres are normal. $\times 22\ 500$.

Table 1. *Dermal changes in the inflammatory zone of morphea*

Patients								
No.	Sex	Age (y.)	Elastic fibres	Collagen fibrils	GAG micro-fibrils	Cells	Dermo-epidermal junction	Filam. bodies
1	♀	9	Granular coat; distinct elastic fibrils	Normal; thick compact bundles; felt-like material	Numerous	<i>M: degranulated; honey-comb cytopl.</i> F: normal H: normal	Normal	+
2	♂	69	Granular coat; bands of matrix; lack of fibrils	Normal; occasionally abnormal; thick bundles	Few	M: mature granules	Slightly pathological	-
3	♂	14	Reticular matrix Slightly coated; dense straight fibrils	Normal <i>Abnormal</i>	Moderate number	<i>M: degranulated</i> F: normal	<i>Pathological</i>	+
4	♂	57	Reticular matrix Granular coat; lack of fibrils	<i>Slightly abnormal</i> <i>Severely abnormal</i>	Few	Scarce	<i>Pathological</i>	

GAG = Acid glycosaminoglycans. M = Mast cells. F = Fibroblasts. H = Histiocytes. Changes inherent in the sclerodermic process are italicised.

in the elastic fibres appeared inversely with the collagen fibril changes, while changes in the dermo-epidermal junction coincided with changes in the collagen fibres. Mucin accumulation occurred independently of changes in fibres, but was regularly accompanied by degranulation phenomena in the mast cells. Filamentous bodies seemed unrelated to other dermal changes.

DISCUSSION

Extraordinarily thick collagen bundles have been found in shagreen patches of the adenoma sebaceum syndrome (9) and in skin of generalized scleroderma (6). The bundles in shagreen patches contained mostly straight and curled fibrils. The latter had irregular cut-surfaces and were embedded in a thready material. In generalized scleroderma the thickness of the collagen fibrils varied considerably, as in the present findings in the inflammatory zone of localized scleroderma. No abnormalities in the individual fibrils were

found, however. Bundles of protocollagen-like filaments were also found in generalized scleroderma (6).

Transformation of filament bundles into thin collagen fibrils represents evidence of fibril formation. The nature of the felt-like material covering collagen bundles remains obscure.

A granular coating of elastic fibres, a band-shaped matrix, and a lack of fibrils may occur in the skin of normal individuals even in the early twenties, depending upon internal and external influences (1). A reticular matrix has also been seen in generalized scleroderma, indicating disintegration of elastic fibres (6). Ten-nm thick fibrils have previously been found in localized scleroderma skin, said to be the thinnest collagen fibrils known (12). These fibrils had no distinct axial periodicity, however. We consider them to be elastic fibrils as also described by the present authors in generalized scleroderma (6). Abnormal, dense, straight elastic fibrils have been found in systemic lupus erythematosus skin (8).

Numerous microfibrils with knobs in the ground substance indicate acid glycosaminoglycan accumulation (5). Previous reports on localized scleroderma (12) and scleromyxedema (3) interpreted the amorphous material adsorbing colloidal iron as representing acid mucopolysaccharides.

A thickened basal lamina of the dermo-epidermal junction, vessels and nerves has been found previously in fibrotic lesions of localized scleroderma (4). However, the changes of the dermo-epidermal junction found in this study were not identical with those described in that paper though both suggest basal-cell participation in the sclerodermic disease process.

Filamentous bodies are similar to those found in the subepidermal space (7) and in the outer corium (2, 8) of systemic lupus erythematosus skin. In previous immunofluorescence and histochemical studies, hyalin bodies have been found in the dermis of lupus erythematosus, dermatomyositis, acrosclerotic scleroderma, lichen planus, fixed drug eruption and mycosis fungoides (11). The bodies, which contain immunoglobulins and occasionally fibrin, were supposed to originate from basal epidermal cells (11). The filamentous bodies are probably identical with "hyalin bodies" (8).

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G. Asboe-Hansen, M.D.
Department of Dermatology
Rigshospitalet
Blegdamsvej 9
DK-2100 Copenhagen
Denmark