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ULTRASTRUCTURE OF LOCALIZED MYXEDEMA

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Abstract. Five biopsies from three patients with localized myxedema were studied by electron microscopy. The dermis showed an overwhelming accumulation of microfibrils with knobs (acid glycosaminoglycans) and an amorphous material containing glycoprotein. Furthermore, evidence of degradation without new formation of collagen fibrils was found. Considerable numbers of mast cells with various types of granules, fibroblasts with dilated granular endoplasmic reticulum, and macrophages were seen in the mucinous areas.

Key words: Localized myxedema; Glycosaminoglycans; Ground substance; Dermal connective tissue; Mast cell; Fibroblast; Macrophage

Histopathologically, the dermal lesions of localized myxedema are characterized by an accumulation of mucinous substance in the dermis (1, 4). Excess content of hyaluronic acid (36) in the mucinous areas has been demonstrated by mucicarmine (37), colloidal iron (2), alcian blue (25) and by metachromasia with toluidine blue (1, 2). The staining is prevented if the section is influenced by hyaluronidase (1). In the lesion, splitting and degeneration of collagen fibril bundles and degenerated elastic fibres have also been described (4, 27, 28, 37).

Gottron & Korting (11) described special vascular changes, "Sperrarterien", in the lower corium. These authors found swollen smooth muscle cells in the subintimal areas of small arteries. The cells of the dermis produce hyaluronic acid. Asboe-Hansen (2, 4) found numerous mast cells in the lesions and proposed the theory that the mast cells produce the hyaluronic acid. Others (27, 36) have found numerous fibroblasts and few mast cells. They supposed that the fibroblasts are the origin of hyaluronic acid. In the mucinous areas, stellate cells have been found (28). Korting et al. (25) demonstrated alcian blue-positive, metachromatic granules in dermal cells which they distinguished from fibroblasts naming them "mucoblasts".

MATERIAL AND METHODS

Three patients suffering from localized myxedema and hyperthyroidism were studied with the electron microscope.

A 29-year-old woman developed Graves disease with exophthalmos at the age of 23. One year later, she underwent subtotal surgical thyroidectomy. Soon after the operation, a localized pretibial myxedema developed on both legs. Six years later, two biopsies were taken from the lesions.

A 31-year-old woman had suffered from Graves disease with exophthalmos since the age of 30 and received propyl thio-uracil therapy. When her exophthalmos became stable after 1½ years' medication, plaques and nodules developed in both pretibial areas. A biopsy was taken from a one-month-old plaque.

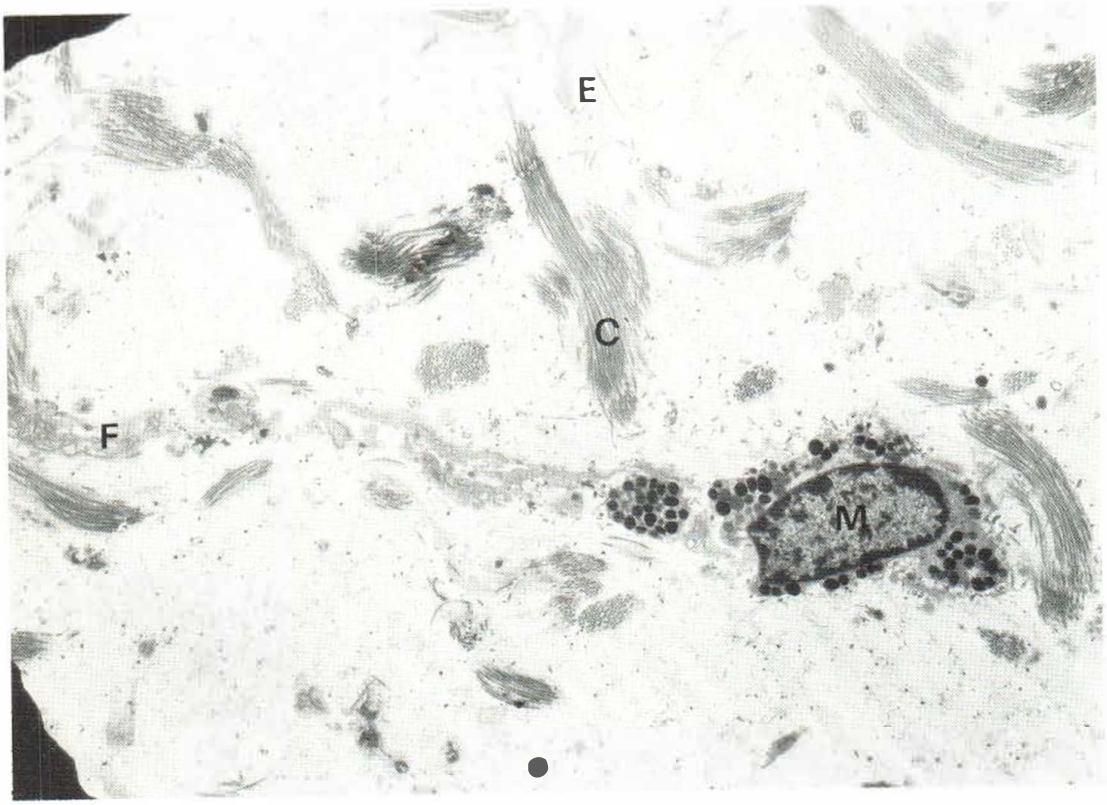
A 74-year-old woman had had Graves disease since the age of 50. When she was 62 years of age, she was thyroidectomized. Pretibial swellings appeared soon after the operation. Twelve years later, one biopsy was taken from the lesion on the left leg, and, after a further half year, one from the lesion on the right leg.

Fig. 1. The corium in a myxedematous area shows wide interfibrous spaces, dispersed collagen fibril bundles (*C*) and elastic fibres (*E*). Amorphous material covers a mast cell (*M*), fibroblast (*F*) and the fibres, and spreads in the interfibrous compartment. Numerous microfibrils with knobs, indicating GAG, are seen in the spaces and in the dispersed bundles of collagen. ×4000.

Fig. 2. Amorphous material (*A*) and microfibrils with knobs (*arrows*). Collagen fibrils (*C*). Elastic fibre (*E*). ×60000.

Fig. 3. Microfibrils with knobs, forming meshes. Amorphous material (*A*) is seen in collagen bundles (*C*) and in the ground substance. ×30000.

Fig. 4. Accumulation of microfibrils with knobs, forming distinct meshes. Collagen fibrils (*C*). Elastic fibrils (*E*). No amorphous material is seen. ×30000.



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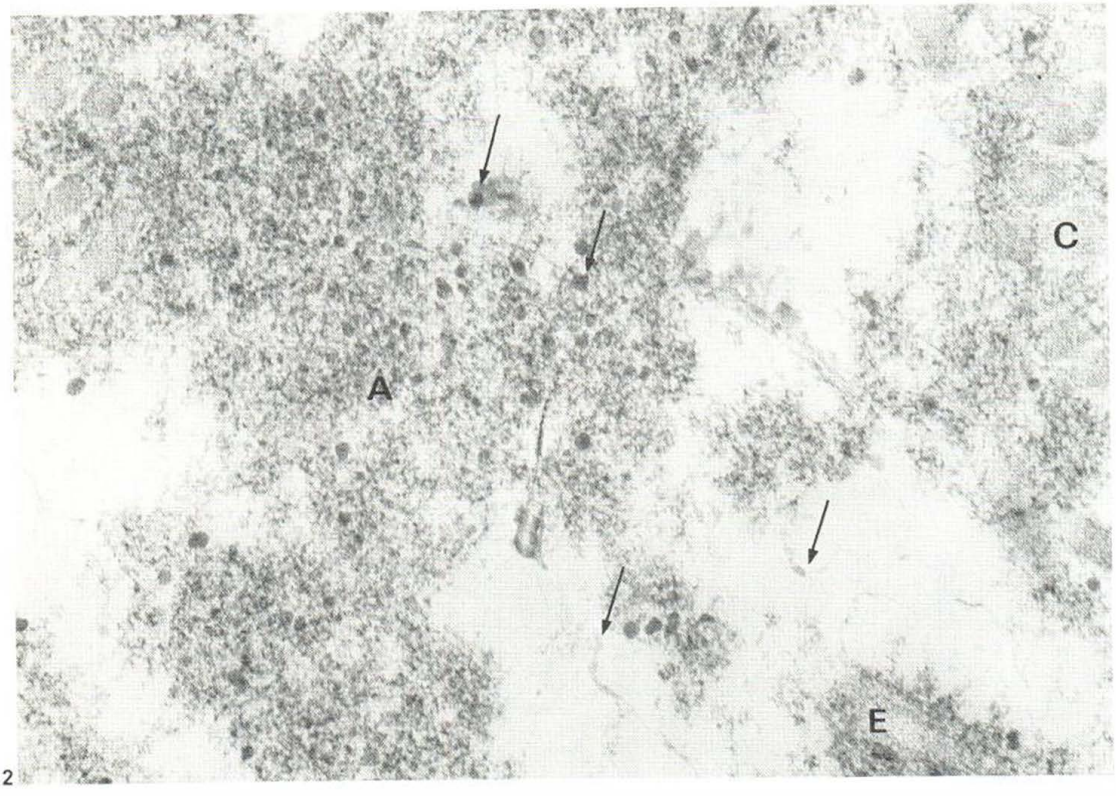

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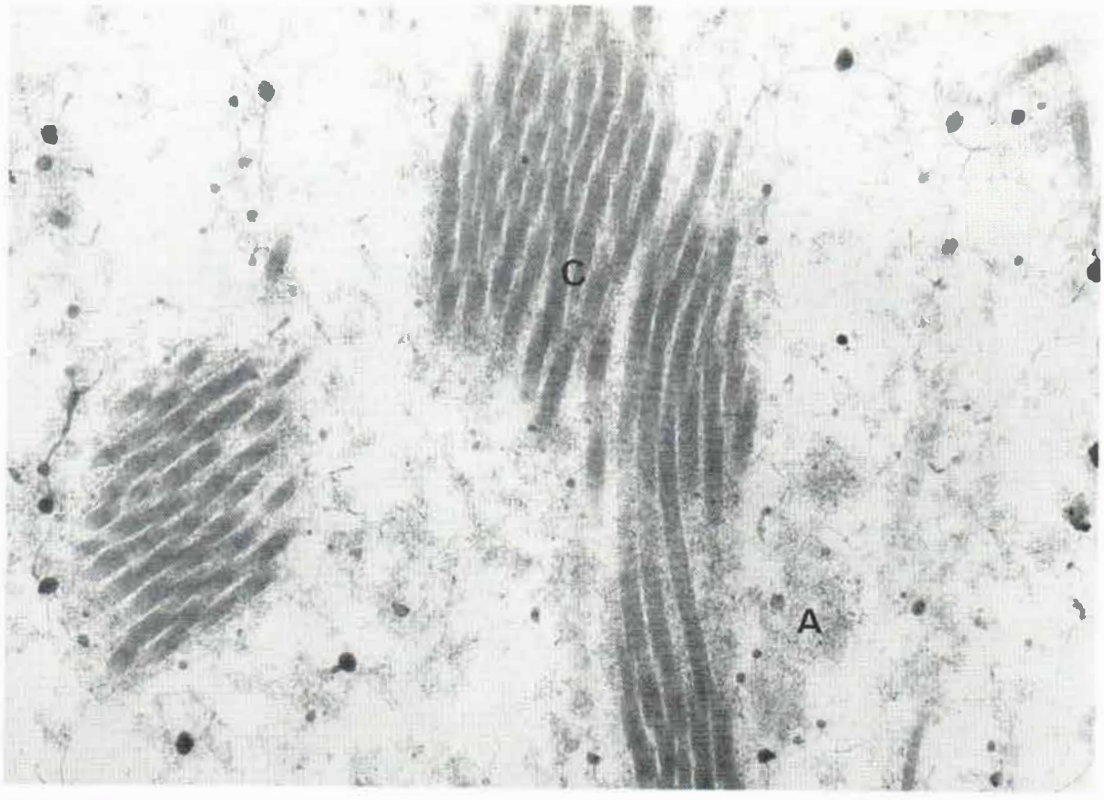

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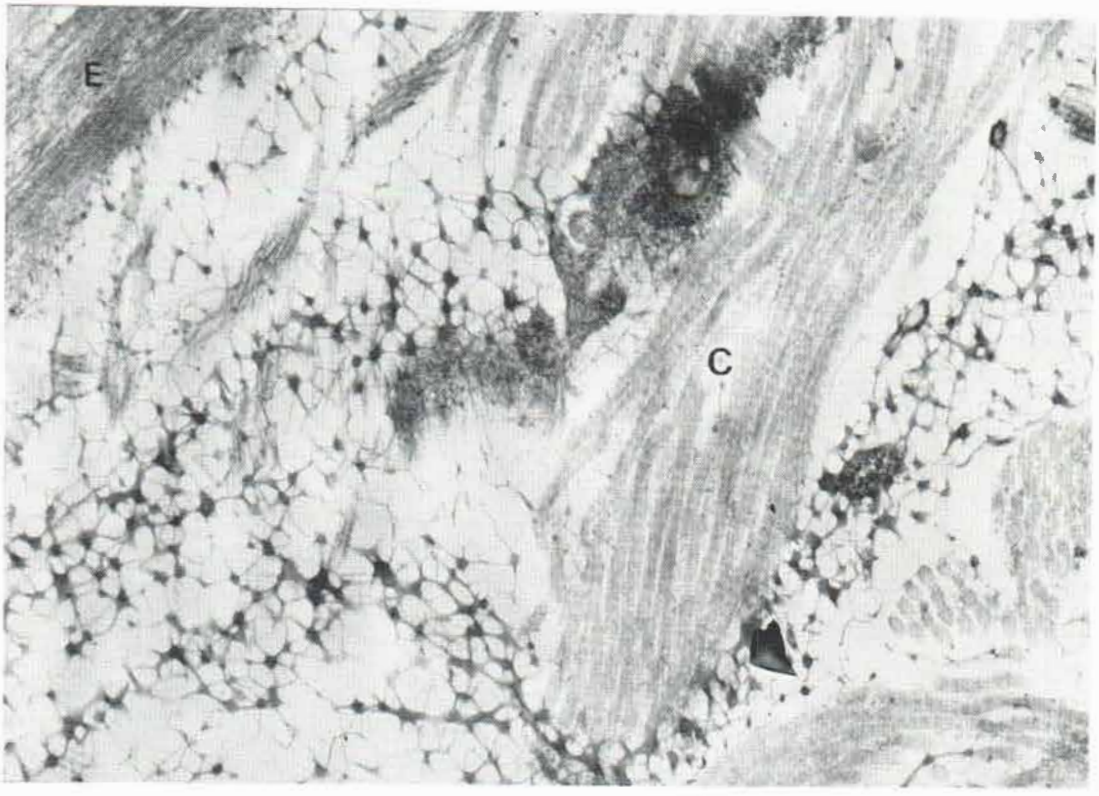

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Fig. 5. Microfibrils with knobs and amorphous material are stained, while collagen fibrils (*C*) remain unstained. 17% aqueous lead subacetate stain. ×60 000.

Light microscopy of skin from all 3 patients showed localized myxedema with intense metachromasia after staining with a 0.1% aqueous toluidine blue solution.

For electron microscopy, tissue specimens were fixed in a 6% glutaraldehyde solution in Veronal acetate buffer, pH 7.2, with 7.5% sucrose. They were osmicated, dehydrated and embedded in Epon 812. Ultrathin sections were cut on LKB and Reichert ultramicrotomes and stained with uranyl acetate–lead citrate, ruthenium red (18), a 17% aqueous solution of lead subacetate (1 h) and periodic acid silver proteinate (PAS) after Thiérry (34). A Siemens Elmiskop IA and a JEOL 6Y electron microscope were operated at 80 kV.

OBSERVATIONS

The corium of all tissue samples showed similar changes, viz. wide interfibrous spaces, dispersed bundles of collagen fibrils, elastic fibres, vessels, nerves and cells (Fig. 1).

In the wide interfibrous spaces, numerous microfibrils with knobs and amorphous material were seen (Figs. 2, 3, 4). The amorphous material was seen to embed the dispersed bundles of collagen and to cover elastic fibres and cells (Fig. 1). The fine microfibrils with small knobs were seen in the amorphous material (Figs. 2, 3, 8). While both materials were present in equal quantities in the first biopsy from the 74-year-old patient, in the second biopsy, the microfibrils with knobs predominated and the amorphous material was scanty (Fig. 4). The microfibrils were branched and joined each other, forming networks in the interfibrous spaces. Both the amorphous material and the microfibrils with knobs became stained with lead subacetate (Fig. 5), while ruthenium red stained only the microfibrils and knobs (Fig. 6). The amorphous material was stained by the PAS method (Fig. 11 (1)).

The collagen fibril bundles were dispersed (Fig. 1). Individual fibrils showed round cut-surfaces with a diameter of about 60 nm. The fibrils were straight and had a normal axial periodicity. In some specimens, thin filaments occasionally showed an arrangement parallel to the fibril axis (Fig. 7). These threads stained with neither ruthenium red nor by PAS.

The elastic fibres were covered by amorphous material as described above. The strips of matrix

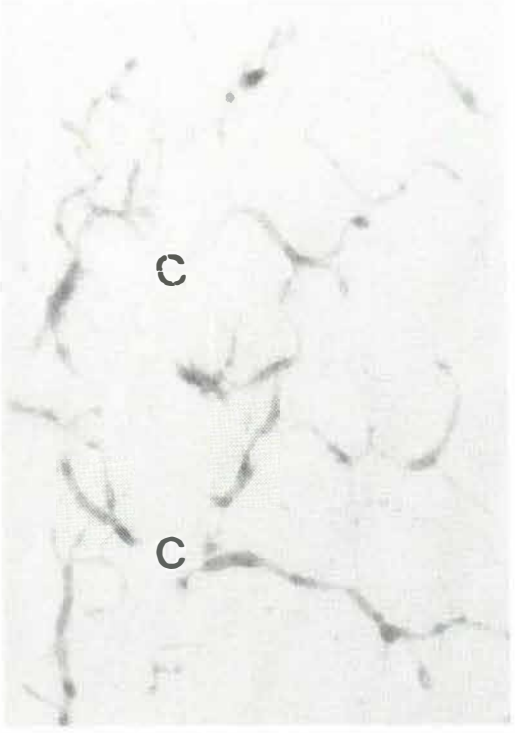


Fig. 6. Specific staining with 0.5% ruthenium red of microfibrils with knobs in the interfibrous compartment. Collagen fibrils (*C*) are unstained. ×60 000.

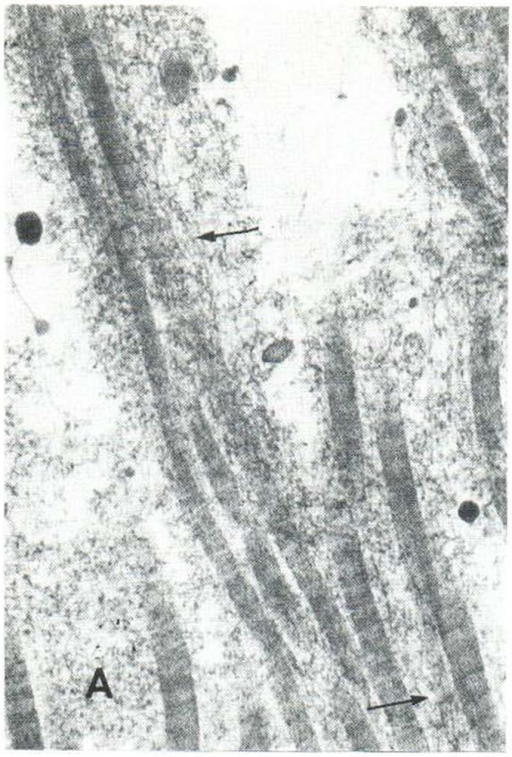


Fig. 7. Collagen fibrils are surrounded by amorphous material (*A*). Arrows indicate filaments parallel to the axes of the collagen fibrils. ×60 000.

were broadened and appeared dense and amorphous. Elastic fibrils emerged irregularly from the matrix. No obvious changes of the fibrils were found (Fig. 8).

In the dermo-epidermal junction (Fig. 9), the basal lamina was seen as an irregular band with branches into the corium. The lamina appeared meshy, with the anchoring filaments arranged parallel and crossing the subepidermal space, or else in a felt-like system. Most of the anchoring fibrils were thin or blurred. Some were of normal size, but PAS-positive cross-bands could not be demonstrated. Elastic fibril bundles were anchored to the basal lamina.

The basal lamina of smooth muscle cells in medium-sized vascular walls had a meshy substructure. Microfibrils with knobs were mostly seen beneath the basal lamina. Some small vessels, however, did not have mucinous deposits under the lamina.

Considerable numbers of cells were found in the mucinous areas. They were mostly mast cells and fibroblasts with few macrophages here and there. However, the second biopsy of the third patient showed few cells in the mucinous areas.

The fibroblasts (Fig. 10) were large and remarkable because of their dilated granular endoplasmic reticulum in a dark ground cytoplasm and by round cytoplasmic protrusions. Intracytoplasmic filaments were few. The content of the endoplasmic reticulum was PAS positive (Fig. 11 (II)) but ruthenium red negative. No filament aggregation was found on the cell surfaces. The nuclei contained round inclusions. Occasionally, large polygonal fibroblasts with irregularly shaped reticula were seen. The content of their reticula was also PAS positive and ruthenium red negative. Macrophages were distinguished from fibroblasts by their vacuolated lysosomes containing microfibrils with knobs and by their phagosomes containing amorphous material (Fig. 12).

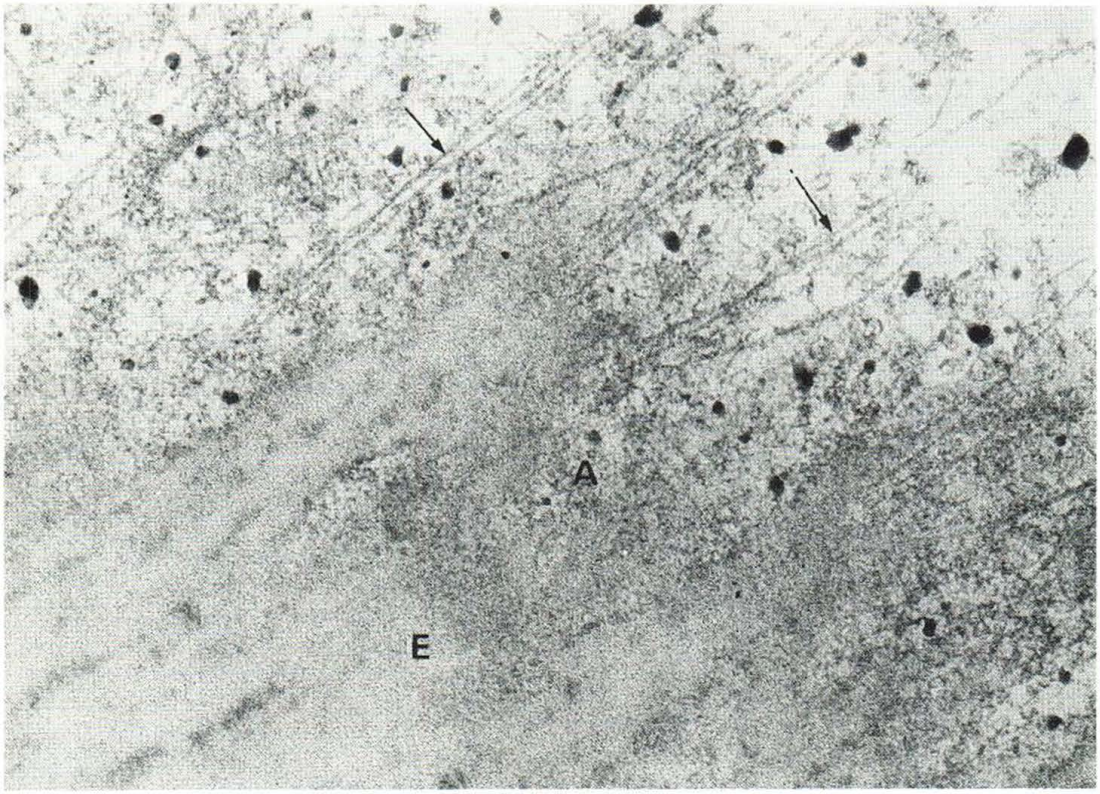
Mast cells were numerous in the mucinous areas, especially perivascularly. Their sizes, shapes and granules varied from cell to cell and from area to area. Villus-like cytoplasmic protrusions were also varying. The cells could be classified into five types according to the substructure of the granules. 1) Mast cells which contained small mature granules showing distinct lamellae and disintegrating granules. They were intermingled with abnormal granules, i.e. dense homogeneous granules lacking lamellae and granules of coarse granular material and a dense core (Fig. 13). 2) Mast cells containing small granules of dense homogeneous material and faint or no lamellae (Fig. 14). 3) Large mast cells containing numerous large granules with dense fine granular material and distinct lamellae (Fig. 15). 4) Large mast cells containing numerous large granules with lucent homogeneous material and lamellae (Fig. 16). 5) Large

Fig. 8. The borders of elastic matrix (*E*) are covered by amorphous material (*A*). Microfibrils with knobs (GAG filaments) are seen in this material. Arrows indicate elastic fibrils. ×60 000.

Fig. 9. Dermo-epidermal junction. The basal lamina (*BL*) appears meshy. Thick arrows indicate anchoring fibrils with smooth surfaces. Anchoring filaments forming a felt-like system are pointed by *thin arrows*. ×60000.

Fig. 10. A fibroblast covered by amorphous material. It contains dilated granular endoplasmic reticulum. No filaments are aggregated on the cell surface. ×20 000.

Fig. 11. (*I*) PAS-stained amorphous material (*arrows with A*) around collagen fibrils (*C*). GAG figures are unstained (*thin arrows*). ×60000. (*II*) A fibroblast after PAS staining. The content of the dilated granular endoplasmic reticulum is PAS positive (*asterisks*). ×60 000.



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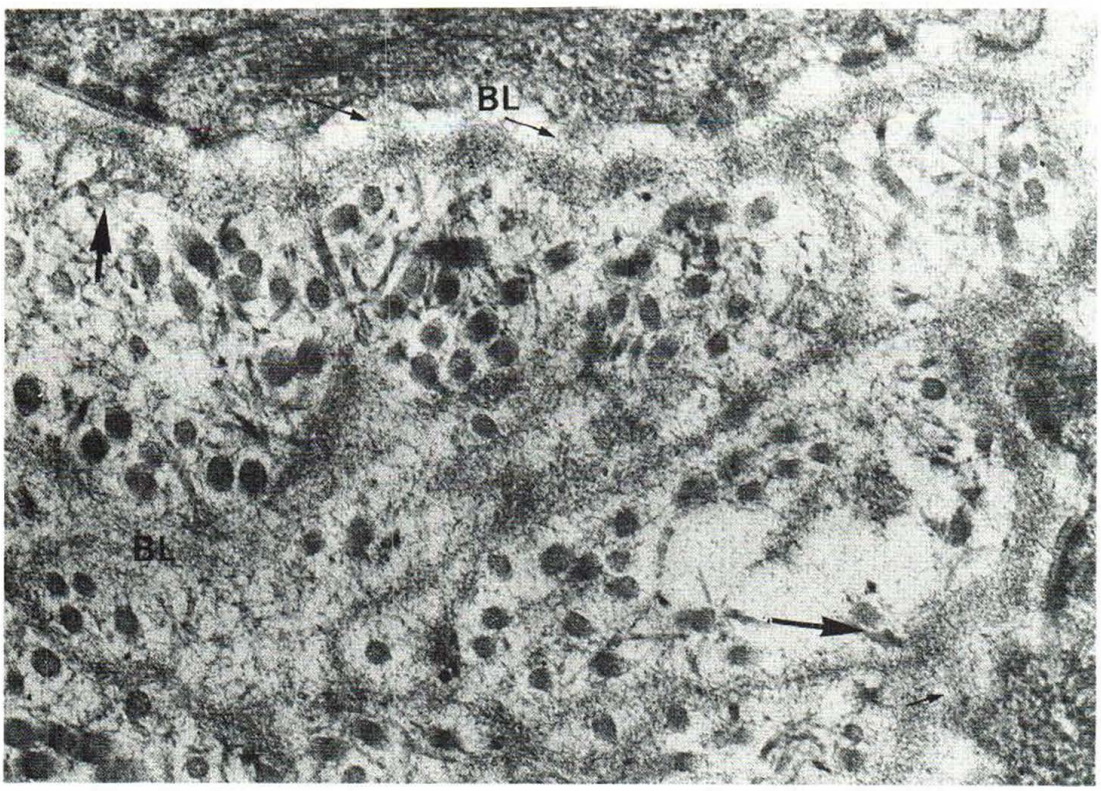

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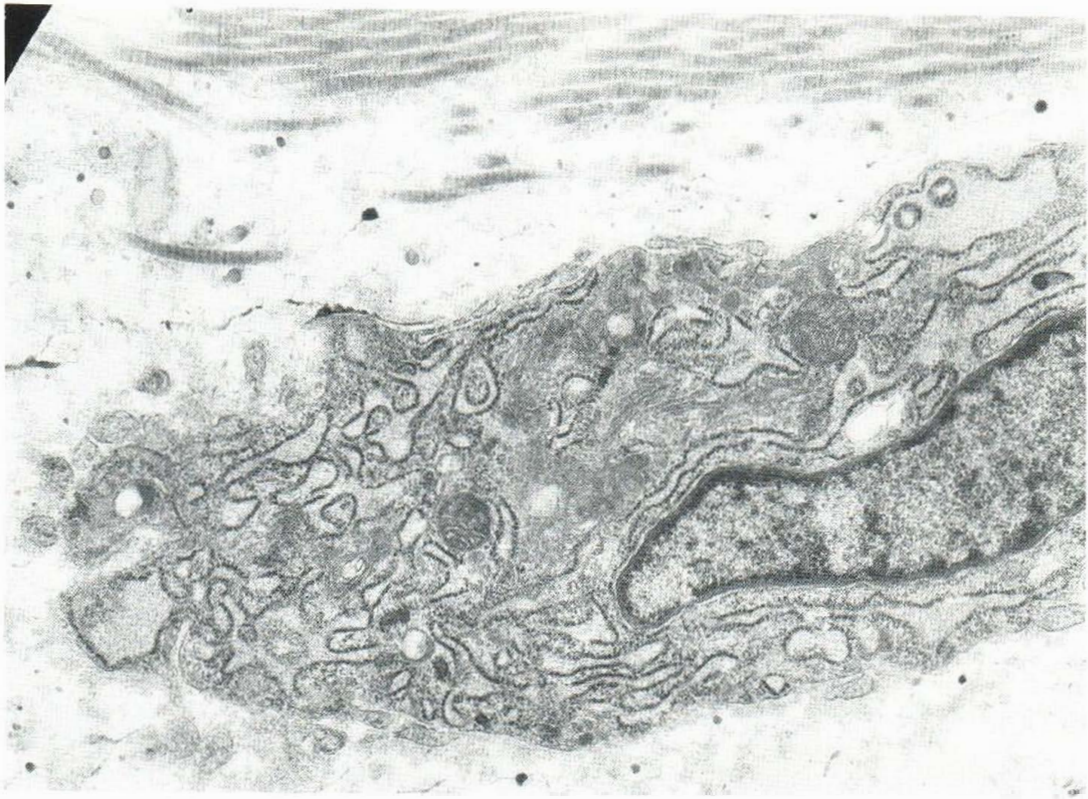

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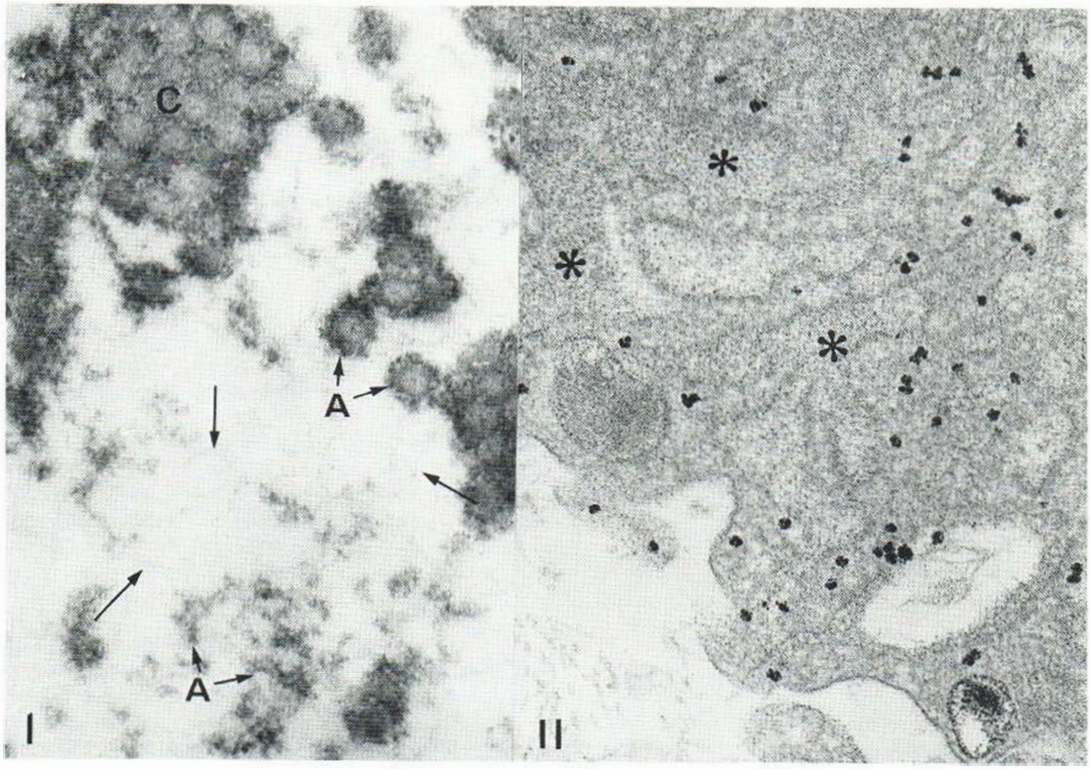

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mast cells containing numerous abnormal granules showing fine granular material in the periphery and coarse granular material in the centre. No lamellae could be seen (Fig. 17). Evidence of extrusion of granules was also found. Types 1 and 2 were mainly located around vessels while types 3, 4 and 5 were found scattered in the mucinous areas.

Cells with honey-comb like cytoplasm (Fig. 18), probably degranulated mast cells, were found in the mucinous areas. The cytoplasmic figures were very much like those of macrophages (Fig. 12). The cells were filled with large vacuoles containing microfibrils with knobs and dense homogeneous masses. A few phagosomes with amorphous material and non-dilated granular endoplasmic reticulum were seen among the vacuoles.

DISCUSSION

In ultrathin sections, glycosaminoglycan (GAG) rich ground substance in human skin and umbilical cord (19, 22, 24) displays characteristic microfibrils with knobs. Ruthenium red and lead subacetate enhance their contrast (22, 24). The numerous microfibrils with knobs represent interfibrous deposits of GAG, as known from previous histochemical (4) and biochemical (36) studies.

The amorphous material contains proteoglycans (glycoproteins) giving a positive PAS reaction and an affinity to lead compounds. The inverse relationship of the occurrence of the amorphous material and microfibrils with knobs may raise the question—can GAG develop from the amorphous material? The predominant accumulation of microfibrils in the second biopsy of the 74-year-old patient, in contrast to the findings in the first biopsy, supports the concept. The splitting of the bundles of collagen fibrils and the decrease in the total amount of fibrils indicate that a degradation of collagen fibrils is probably taking place. However, degradation might not be due to cellular collagenase, because the characteristic figure of this type of degradation, i.e. thread and filament bundles with cross bands (12, 23), is scarcely seen in the collagen bundles.

Dilated endoplasmic reticulum and filament aggregation on the surface of fibroblasts are evidence of collagen fibril formation by these cells (14, 21, 30, 32, 38). The thin collagen fibrils grow in the extracellular space (9, 21, 31). In the present study, neither filament aggregation nor thinned collagen fibrils were found, while the dilated endoplasmic reticulum suggests increased production of collagen precursor material (30). It is therefore proposed that the formation of collagen is presumably interrupted. No evidence of hyaluronic acid secretion from fibroblasts was disclosed by the present investigation. The lack of normal configuration in the dermo-epidermal junction, especially anchoring fibrils (15) represents degenerative changes. The present study has failed to demonstrate the "Sperrarterien" of Gottron & Korting (11). The changes in the vascular basal lamina were identical with those in the epidermal basal lamina.

Mast cells are constantly seen in the mucinous areas, as described in previous histopathological studies (3, 4). They can divide by mitosis in the non-granular fibroblast-like stage of development (5, 6). The granules develop in the Golgi zone and, in human mast cells, typical lamellae appear later on (8, 16). Human mast-cell granules without lamellae are atypical (20). On these grounds, the mast cells in the mucinous areas can be identified as follows: Types 1 and 2 represent young mast cells, while type 3 is mature. The granules of type 4 mast cells seem to be swollen. Identical granules have been found in the mucinous dermis of basalioma (17). Type 5 consists of abnormal mast-cell granules. Disintegration and extrusion of granules are evidence of active degranulation and release of GAG and proteases to the extracellular space (13, 33, 35). These granular contents vary, depending upon the maturation of the granules (29). Mastocytoma mast cells, for instance, contain considerable amounts of hyaluronic acid and proteases (3, 7, 26, 35). Fujita et al. (10) described cells in the mucinous areas of localized

Fig. 12. A macrophage containing lysosomes with GAG figures (*arrows*). ×20 000.

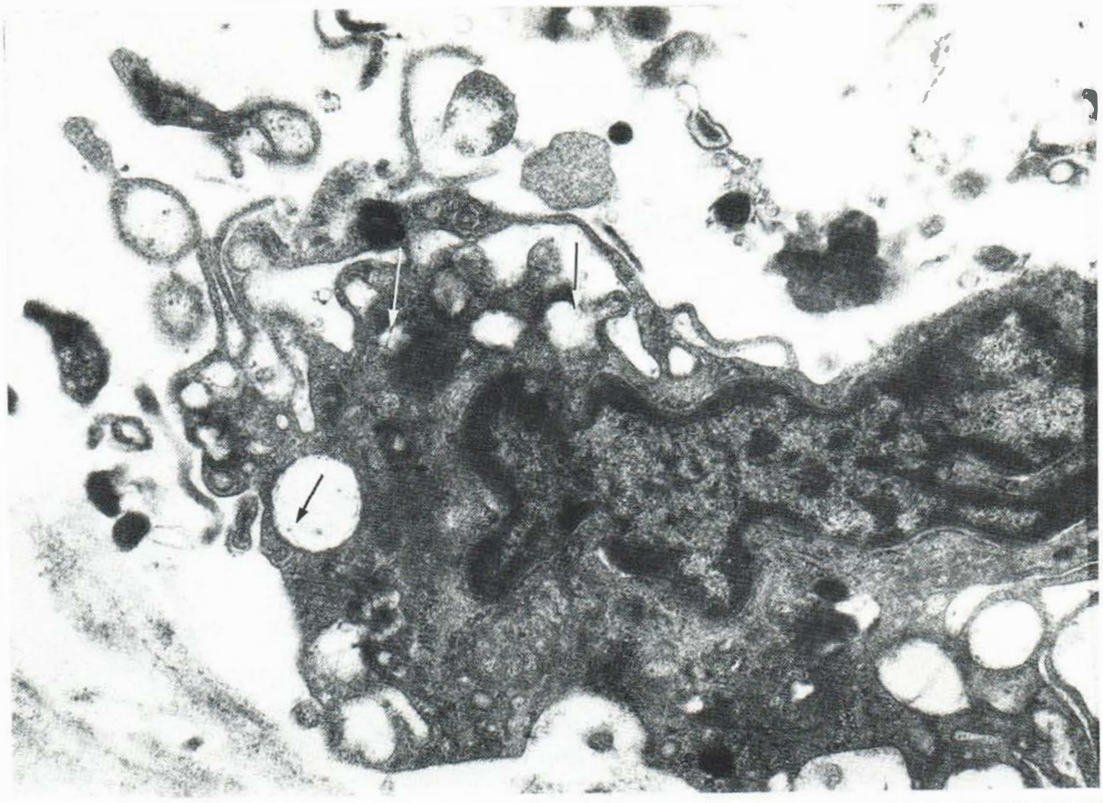
Fig. 13. (*I*). A mast cell containing small granules. ×20 000. (*II*) A mast cell containing mature (1), abnormal, lysosome-like (2) granules, and other granules with dense central cores (3). ×40 000.

Fig. 14. A mast cell containing homogeneous granules, close to a blood vessel (*V*). ×20 000.

Fig. 15. (*I*) A mast cell containing numerous mature granules. ×14 000. (*II*) Granules with scrolls and dense, fine granular material. ×40000.

Fig. 16. (I) A mast cell filled with large granules. ×10 000. (II) Granules containing lucent material with scrolls. ×40 000.

Fig. 17. (I) A mast cell filled with abnormal granules. Arrows indicate microfibrils with knobs. ×20000. (II) Granules showing dense, coarse granular material without lamellae. Arrows indicate granules without plasma membrane, during extrusion from the cell. ×80000.



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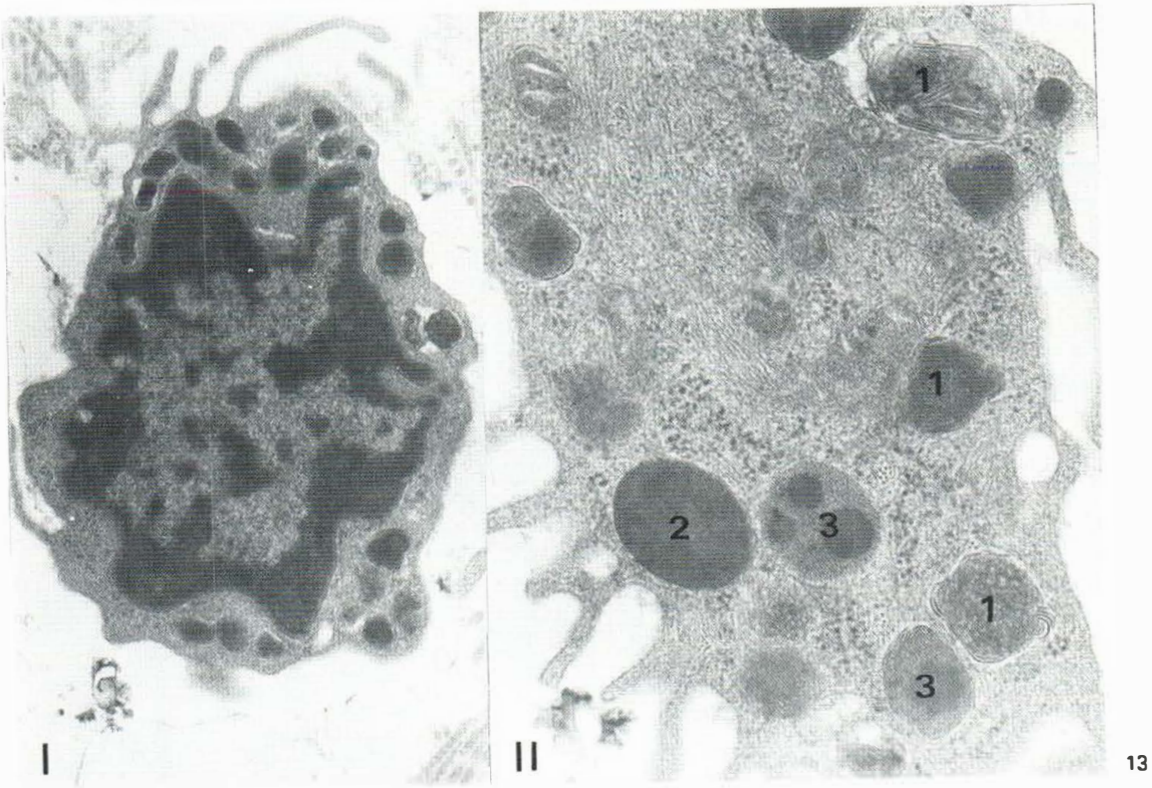

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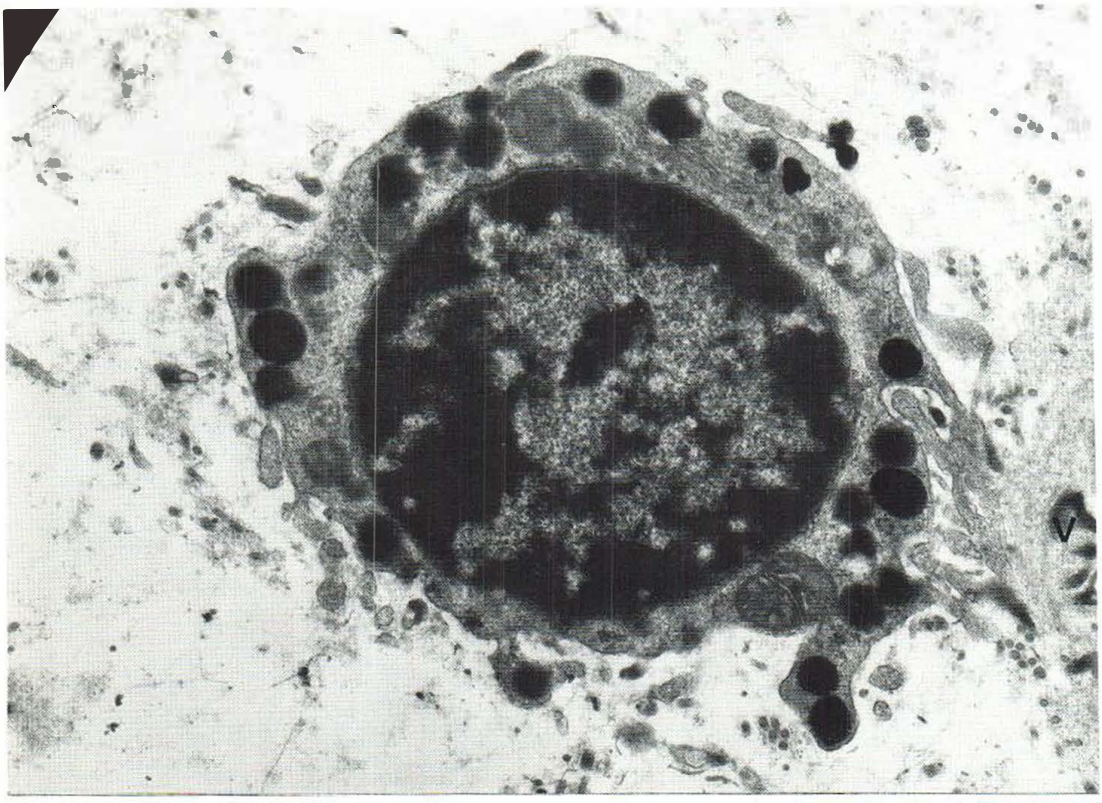

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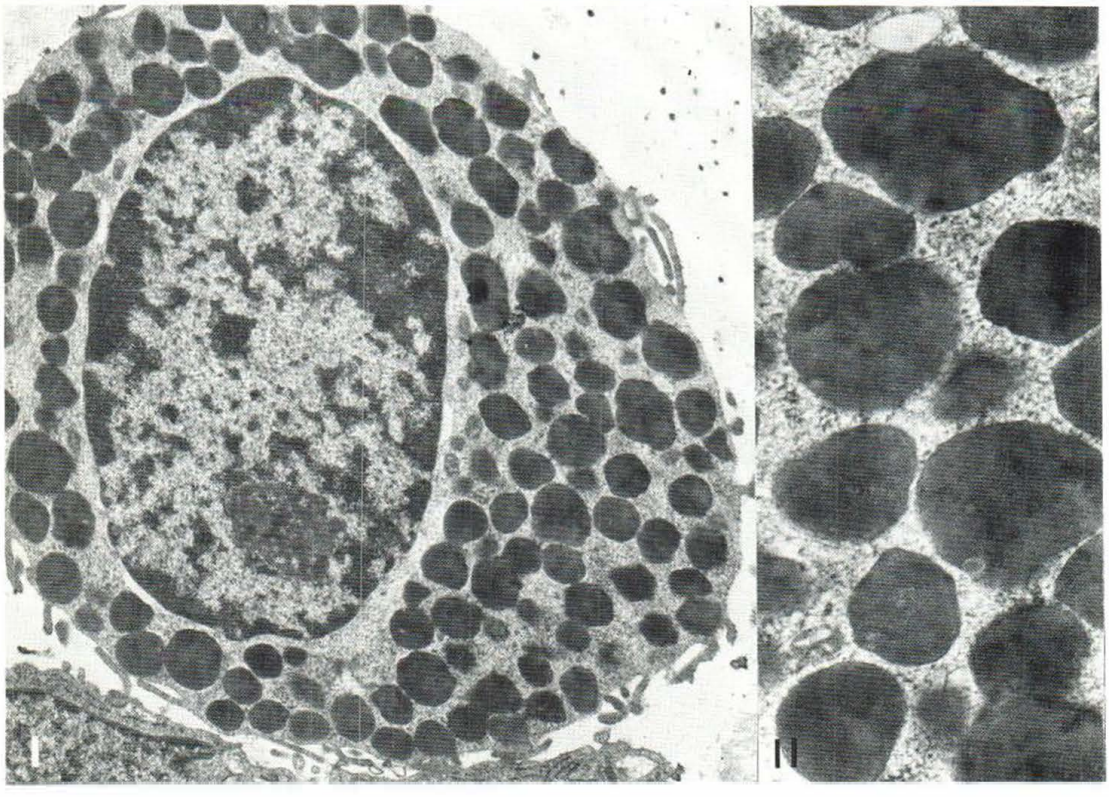

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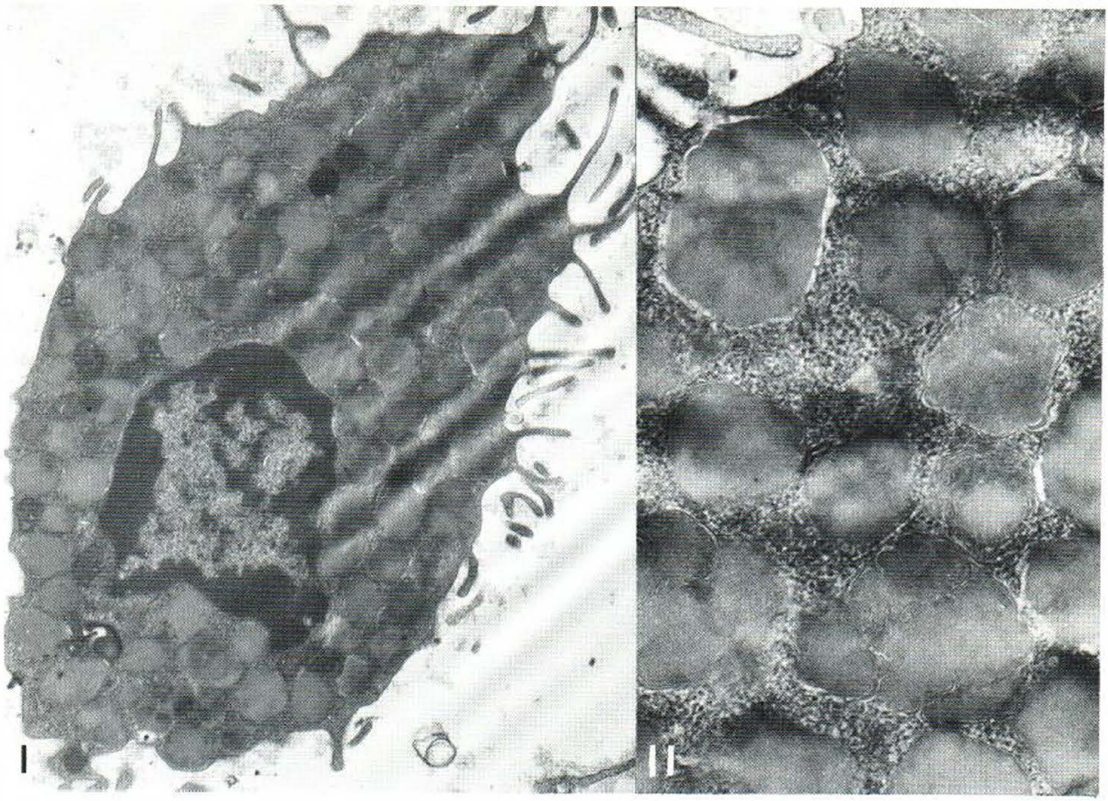

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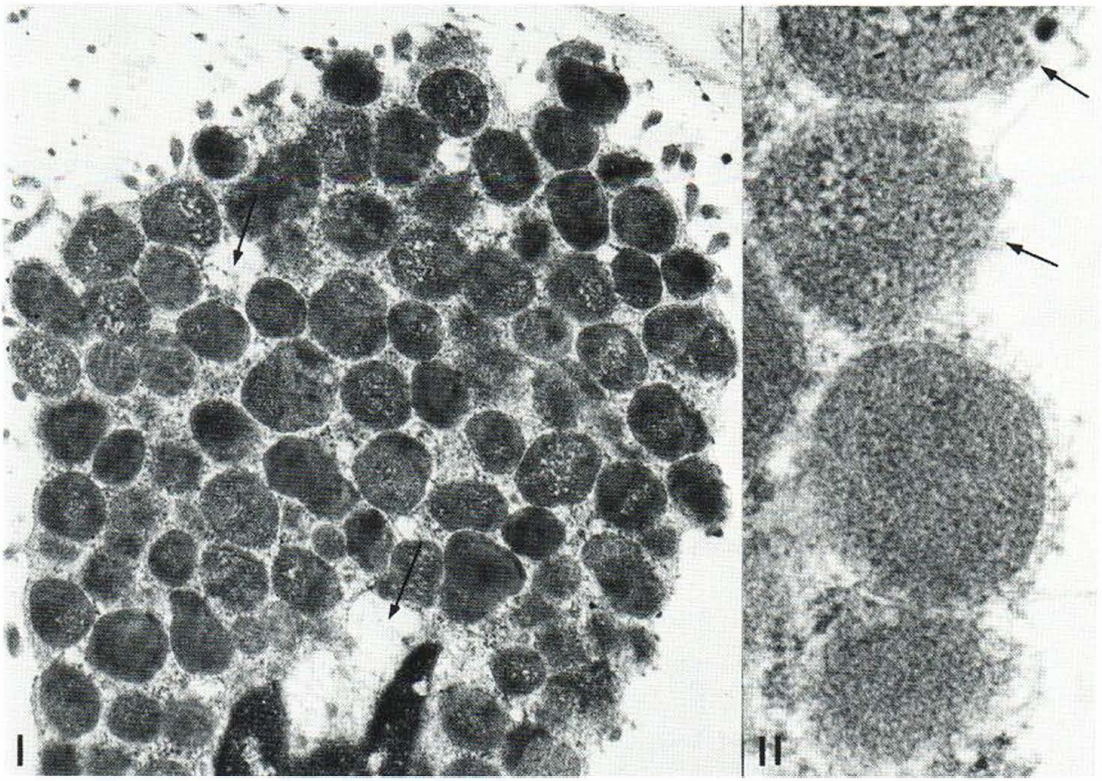

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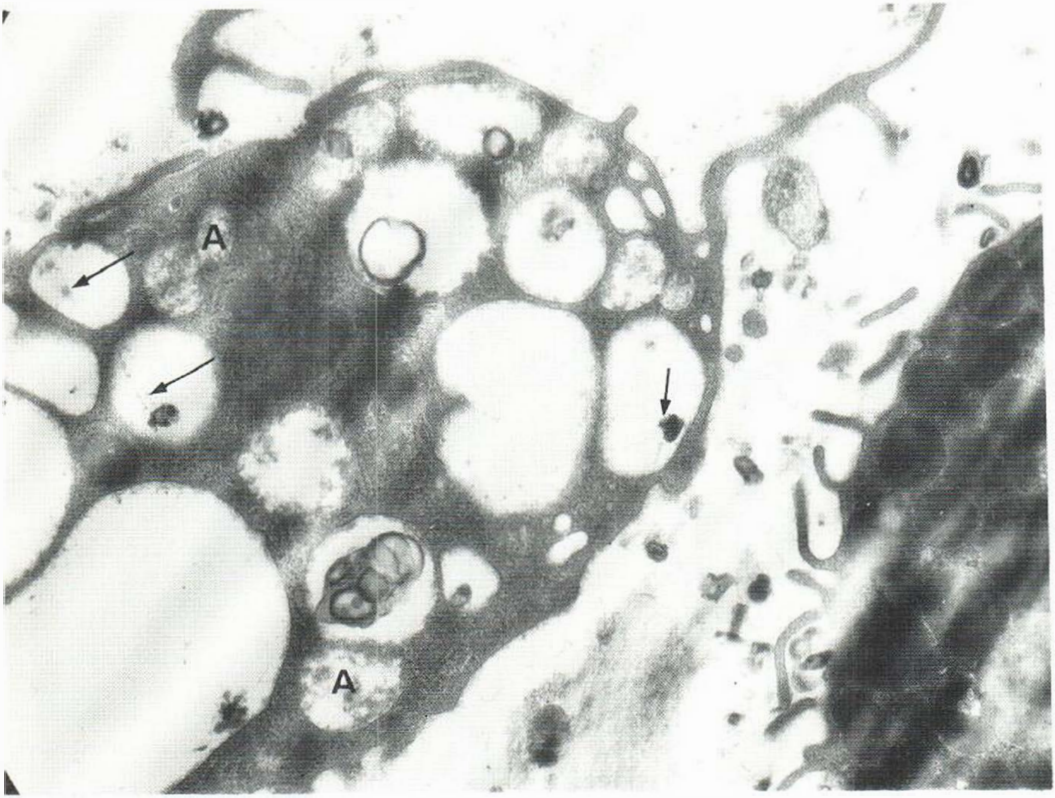

Fig. 18. Honey-comb like cytoplasm of a cell which is probably a degranulated mast cell. Vacuoles contain microfibrils with knobs (*thin arrows*) and amorphous material (A). ×20 000.

myxedema the cytoplasm of which was filled with large homogeneous lysosome-like granules with GAG microfibrils. They assumed that these cells were atypical mast cells. Stellate cells containing dilated granular endoplasmic reticulum have also been thought to produce GAG (25). However, in the present study the content of such reticula was PAS positive and the "stellate cells" are therefore believed to be fibroblasts.

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REFERENCES

1. Asboe-Hansen, G.: The intercellular substance of the connective tissue in myxedema. J Invest Dermatol *15:* 25, 1950.
2. — Om bindevævets mucinøse substanser. Rosenkilde og Bagger, Copenhagen, 1951.
3. Asboe-Hansen, G. & Clausen, J.: Urinary excretion of acid mucopolysaccharides in twelve cases of mastocytosis (urticaria pigmentosa). J Invest Dermatol *43:* 81, 1964.
4. Asboe-Hansen, G. & Wegelius, O.: On the pathogenesis of circumscribed myxedema. Acta endocr (Kbh) *33:* 287, 1960.
5. Asboe-Hansen, G. & Levi, H.: Mitotic activity of tissue mast cells as indicated by the uptake of tritiated thymidine. Acta Path Microbiol Scand *56:* 241, 1962.
6. Asboe-Hansen, G., Levi, H., Nielsen, A. & Weis Benzon, M.: Premitotic uptake of tritiated thymidine by mast cells. Acta Path Microbiol Scand *63:* 533, 1965.
7. Brunish, R. & Asboe-Hansen, G.: Acid mucopolysaccharides in the Rask-Nielsen transplantable mouse mastocytoma. Acta Path Microbiol Scand *65:* 185, 1965.
8. Combs, J. W.: Maturation of rat mast cells. J Cell Biol *31:* 563, 1966.
9. Daems, W. Th., Gebhardt, D. O. E. & Smiths, G.: On the procollagens during development of the skin. The Fourth Intern Conference on Electron Microscopy, Berlin, 1958. Springer, Berlin, 1960; p. 335.

10. Fujita, H., Asagami, C., Suetomi, Y., Uchida, T., Kinoshita, K. & Araki, I.: Study on the ultrastructure and mucin production in pretibial myxedema. Acta Path Jap *21:* 207, 1971.
11. Gottron, H. A. & Korting, G.: Zur Pathogenese des Myxoedema circumscriptum tuberosum. Arch Derm Syph (Berl) *195:* 625, 1952/53.
12. Hentzer, B & Kobayasi, T.: Ultrastructural changes of human skin in organ culture. IRCS J Med Sci *3:* 211, 1975.
13. Horsfield, G. I. & Summerly, R.: Mucopolysaccharides in mast cells. Brit J Dermatol *78:* 476, 1960.
14. Kobayasi, T.: Development of fibrillar structures in fetal human skin. Acta Morphol Neerl Scand *3:* 257, 1966.
15. — Electron microscopy of the elastic fibres and the dermal membrane in normal human skin. Acta Dermatovener (Stockholm) *48:* 303, 1968.
16. Kobayasi, T., Midtgaard, K. & Asboe-Hansen, G.: Ultrastructure of human mast-cell granules. J Ultrastruct Res *23:* 153, 1968.
17. Kobayasi, T. & Asboe-Hansen, G.: Ultrastructure of mast-cell granules in basal cell carcinoma. Acta Dermatovener (Stockholm) *49:* 111, 1969.
18. — Ruthenium red staining of ultrathin sections of human mast-cell granules. J Microsc (Oxf) *93:* 55, 1970.
19. Kobayasi, T., Danielsen, L. & Asboe-Hansen, G.: Hyaluronate (?) microfibrils in human dermis. Acta Dermatovener (Stockholm) *51:* 27, 1971.
20. Kobayasi, T., Helin, P. & Asboe-Hansen, G.: Biochemistry and ultrastructure of mast-cell granules. 14th Intern Congr Dermat, Venice, 1972.
21. Kobayasi, T. & Asboe-Hansen, G.: Ultrastructure of generalized scleroderma. Acta Dermatovener (Stockholm) *52:* 81, 1972.
22. — Dermal ground substance in ultrathin sections. J Ultrastruct Res *42:* 403, 1973.
23. Kobayasi, T. & Hentzer, B.: Influence of collagenase, elastase and dithioerythritol on dermal fibres. 2nd Meeting of Electron Microscopy Applied in Dermatology. Milan, 1975.
24. Kobayasi, T. & Asboe-Hansen, G.: Electron microscopy of connective tissue ground substance. 1st Meeting of Electron Microscopy Applied in Dermatology. Lyon, 1973.
25. Korting, G. W., Nürnberger, F. & Müller, G.: Zur Ultrastruktur der Bindegewebszellen beim Myxoedema circumscriptum praetibiale. Arch Klin Exp Derm *229:* 381, 1967.
26. Mariano, M.: Mucosubstances in normal and in neoplastic mast cells in the dog. Acta Histochem *36:* 14, 1970.
27. Montgomery, H.: Cutaneous myxedematosis. *In* Dermatopathology, vol. 2, p. 830. Hoeber Medical Division, New York and London, 1967.
28. Pillsbury, D. M. & Stokes, J. H.: Circumscribed myxedema of the skin. Arch Derm Syph (Chic) *24:* 255, 1931.
29. Pretlow II, T. G. & Cassady, I. M.: Separation of mast cells in successive stages of differentiation using programmed gradient sedimentation. Am J Pathol *61:* 323, 1970.
30. Ross, R. & Benditt, E. P.: Wound healing and collagen formation. III. A quantitative radioautographic study of the utilization of proline H^3 in wounds from normal and scorbutic guinea pigs. J Cell Biol *15:* 99, 1962.
31. Rupec, M. & Braun-Falco, O.: Elektronenmikroskopische Untersuchungen über das Verhalten der Kollagenfibrillen der Haut bei Sklerodermie. Arch Klin Exp Derm *218:* 543, 1964.
32. Schwarz, W., Merker, H. J. & Kutzsche, A.: Elektronenmikroskopische Untersuchungen über die Fibrogenese in Fibroblastenkulturen. Zschr Zellforsch *56:* 107, 1962.
33. Taylor, A. C.: Collagenolysis in tissue culture. II. Role of mast cells. J Dent Res *50:* 1301, 1971.
34. Thiérry, P.: Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. J Microsc *6:* 987, 1967.
35. Vensel, W. H., Komender, J. & Barnard, E. A.: Non pancreatic protease of the chymotrypsin family. II. Two proteases from a mouse mast cell tumor. Biochim Biophys Acta *250:* 395, 1971.
36. Watson, E. M. & Pearce, R. H.: The biochemistry of the skin. A review with particular reference to the mucopolysaccharide. Br J Derm Syph *59:* 327, 1947.
37. Wodiansky, P.: Über die Aetiologie und Pathogenese des Myxoedema circumscriptum praetibiale symmetricum. Arch Klin Exp Derm *205:* 22, 1957.
38. Zelickson, A. S.: Fibroblast development and fibrogenesis. Arch Dermatol *88:* 497, 1963.

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