Acta Dermatovener (Stockholm) 52: 179–195, 1972

HYALINOSIS CUTIS ET MUCOSAE

An Electron Microscopic Study

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Abstract. A case of the non-light-sensitive type of hyalinosis cutis et mucosae was examined with a high resolution electron microscope. Hyalin substance was present, forming various-sized islands similar to those of normal collagen. Fibroblasts showing signs of active fibrillogenesis such as dilated rough-surfaced endoplasmic reticulum and pinching off of peripheral cytoplasm often separated these hyalin islands. Typical islands were composed of normal collagen, abnormally large collagen, unit filaments of collagen, filaments (50–100 Å), protofilaments (15–20 Å) and a large amount of amorphous substance embedding these fibrous components. Degenerating collagen fibrils and pinched off peripheral cytoplasm of fibroblasts were often mineralized. No osmiophilic lipid substances were found either intracellularly or extracellularly. It was postulated that the major portion of hyalin is produced locally by abnormal fibroblasts.

Non-light-sensitive type of hyalinosis cutis et mucosae, also known as lipoid proteinosis (Urbach-Wiethe) (30–32), is an autosomal recessive genodermatosis. Early onset of symptoms such as hoarseness, fragility of the skin and distribution over non-exposed areas is characteristic of this type. Affected areas show hyperkeratotic nodules. When healed, they leave pitted scars. Spontaneous scars can also occur (10). The cause of the hoarseness is firm infiltration of the larynx (10). Convulsive seizures can occur (18, 24). The condition is rare; only about 150 cases (10) are on record. Some of them seem to belong to the light-sensitive type, i.e., erythropoietic protoporphyria and, therefore, may be different conditions.

Histochemical and biochemical studies done by Fleischmajer et al. (6) suggested that PAS-positive hyalin of this disease consists of neutral polysaccharides bound to a protein, most probably of non-collagenous origin, since it exhibited a positive reaction for tryptophan and was susceptible to pepsin digestion. Sasai (28), on the basis of histochemical studies, however, suggested that hyalin contains a sialic acid-containing mucoprotein.

A preliminary electron microscopic study reported by Falkmer et al. (5) and Grosfeld (10) and a rather extensive investigation done by Rodermund & Klingmüller (25) revealed a non-organized mass of fine filaments or fine-grained substance. An observation that a close association of hyalin islands with fibroblasts containing dilated endoplasmic reticulum induced Rodermund & Klingmüller (25) to postulate that the hyalin in this condition was produced by the fibroblasts in the lesion. They also described perivascular deposition of hyalin, particularly upon the multiplicated basal lamina, and electron-dense particles which were so hard that they caused scratch marks on the section. Grosfeld et al. (10) also noticed hard particles. These findings of previous workers were all confirmed by the present investigation.

One of us (K. H.) has performed a series of histochemical and ultrastructural studies on lichen amyloidosus (11, 15) and colloid milium (16). It is particularly interesting that the histochemical reaction pattern observed in these studies as well as of others (9) is very similar to that of hyalin in hyalinosis cutis et mucosae (6) (Table I). Re-examination of the case originally studied by Rodermund & Klingmüller (24, 25) was undertaken to further elucidate the fine structure of hyalin at a higher resolution level.

Table 1. *Histochemical properties of amyloid, colloid and hyalin*

	Amyloid (11)	Colloid (9)	Hyalin (6)
Congo red	+	+	+
Dichroism (with Congo red)	+	+	?
Metachromasia	+	+	+
PAS (post-diastase)	+	+	+
Thioflavin T	+	+	?

MATERIALS AND METHODS

Case history

The details of the clinical history of this patient were presented elsewhere (24) with clinical pictures. The following is, therefore, a brief resumé of the original description.

A 34-year-old white male presented himself with papulonodular infiltrations of yellowish-brown color distributed over the neck, face, trunk, hands and genitalia including scrotum, penis and anus. Oral, laryngeal and pharyngeal mucous membranes were similarly involved. Whitish, atrophic scars were intermingled with these infiltrations. In the eye-grounds a change was found which appeared to be hyalin deposit in the choriocapillaries. Electroencephalography showed a marked paroxysmal dysrhythmia suggestive of convulsive seizures. This seemed to be related to intracerebral calcification in the vicinity of sella turcica as demonstrated by X-ray.

Past history revealed that he began to develop blisters on the face, hands and lower extremities during the first year of his life. His skin was so fragile that he had ulcerations on his legs, and subsequently over other areas. Sometime later the bullous lesions decreased, and nodular or plaque types of lesions became predominant. At the age of 20 he was examined for a neurasthenic, depressive state which in recent years developed into epileptic delirium with episodic loss of consciousness. Since birth he was hoarse. At the age of 29 a respiratory difficulty due to pharyngeal infiltration required tracheotomy.

Lab. data. Ultraviolet irradiation did not elicit any pathological reactions. Coproporphyrin was found to be increased in the urine in 1953 and 1959, but no porphyrins were present in the urine in 1966. Porphyrins in serum and erythrocytes were not increased. Blood picture was normal, with hemoglobin 12.7 g% and normal differential of white cells. Serological tests for syphilis were negative. Except for a slight elevation of γ-globulin, no abnormal serum proteins (paraproteins) were detected by immunoelectrophoresis and ultracentrifugation. Serum lipids were normal. Cold agglutinin of non-diluted serum was weakly positive against his own blood cells but negative against others. In spite of a history of blistering lesions on sun-exposed areas early in life, the later development of non-blistering lesions on non-exposed skin and negative porphyrin studies speak in favor of classifying this case into the non-light-sensitive type.

Biopsy material. Specimen was taken under 2% novocaine local anesthesia from the right shoulder and cut into 1 mm^3 blocks. These were immediately fixed in 1.3% osmic acid in collidine buffer (pH 7.4) for 1 hour at 4°C. After dehydration through graded concentrations of ethanol, all tissue blocks were embedded in Epon 812. Thin sections, 400–600 Å, were cut on an ultramicrotome, placed on uncoated copper grids, stained for 30 to 60 min in a saturated solution of uranyl acetate in 50% ethanol and then, before completely dried, restained for 10 min in Reynolds lead citrate solution (23). The stained sections were examined in an Hitachi HU-11C electron microscope operated at an accelerating voltage of 100 kV.

In the following, the terms "fibrils," "filaments (50–100 Å)," and "protofilaments (15–20 Å)" will be used whenever possible, according to the customary usage of the terms in the studies of normal collagen (1) and keratin (19).

RESULTS

Hyalin deposits were seen in various forms of islands (Fig. 1). Collagen fibrils and elastic fibrils could be admixed with hyalin at varying ratios (Fig. 1). Most of the hyalin islands were separated by either the bodies or processes of fibroblasts (Fig. 2). Although hyalin islands could be detached from these cellular components, probably due to a condensation of loosely knit hyalin during dehydration, most of the islands were tightly surrounded by these fibroblasts (Fig. 2).

Fibroblasts

Fibroblasts in the lesion showed a number of dilated cisternae of rough-surfaced endoplasmic reticulum (Fig. 3). These cisternae contained amorphous as well as protofilament-like materials (Fig. 3). When compared with similar materials comprising hyalin (see below) all corresponding materials except for very large fibrils could be found (Fig. 3). Many of the fibroblasts in the lesion, particularly those showing dilated cisternae, exhibited pinching off of numerous cytoplasmic buddings and balloonings (Figs. 3, 4). Part of the peripheral cytoplasm was thus segregated, and its contents upon disintegration seemed to be incorporated into hyalin. (Figs. 1–6, 8, 9). A number of half-desmosome-like structures were produced between these fibroblasts and hyalin or extra-cellular amorphous material (Figs. 2, 5). Some fibroblasts contained numerous filaments with an average diameter of 100 Å (Fig. 4). These filaments resembled unit filaments (19) of collagen fibrils in diameter and in other characteristics: for example, they were long, rather straight or curved only gradually, and well-defined with a uniform diameter throughout their length (Fig. 4).

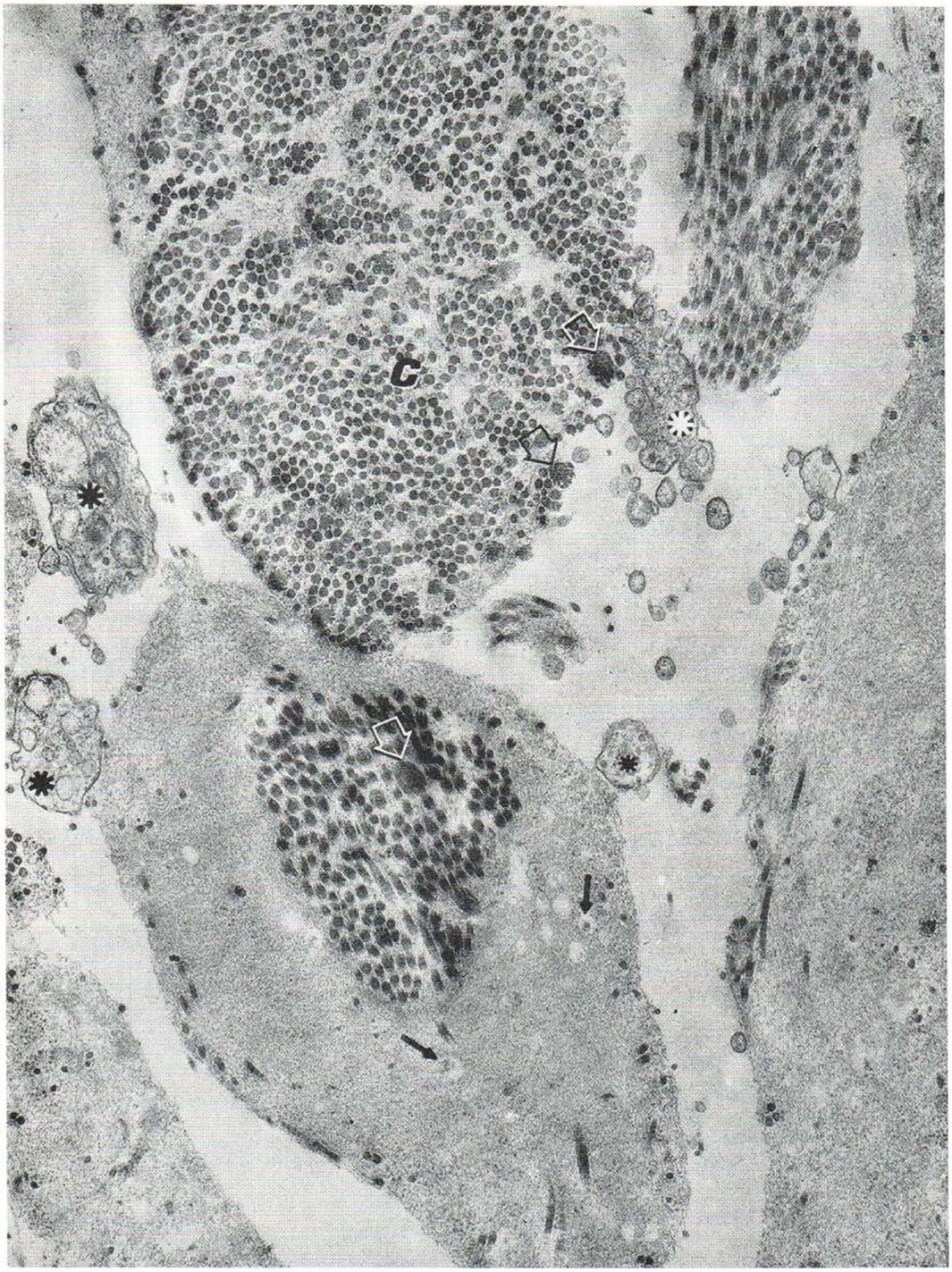

Fig. 1. Various forms of hyalin islands are composed of different ratios of admixture of normal collagen (*C*), abnormally large collagen (*hollow arrows*) and fine filamentous materials. ✱, Cellular fragments of fibroblasts. *Solid arrows:* degenerating collagen fibrils with halos. × 11 500.

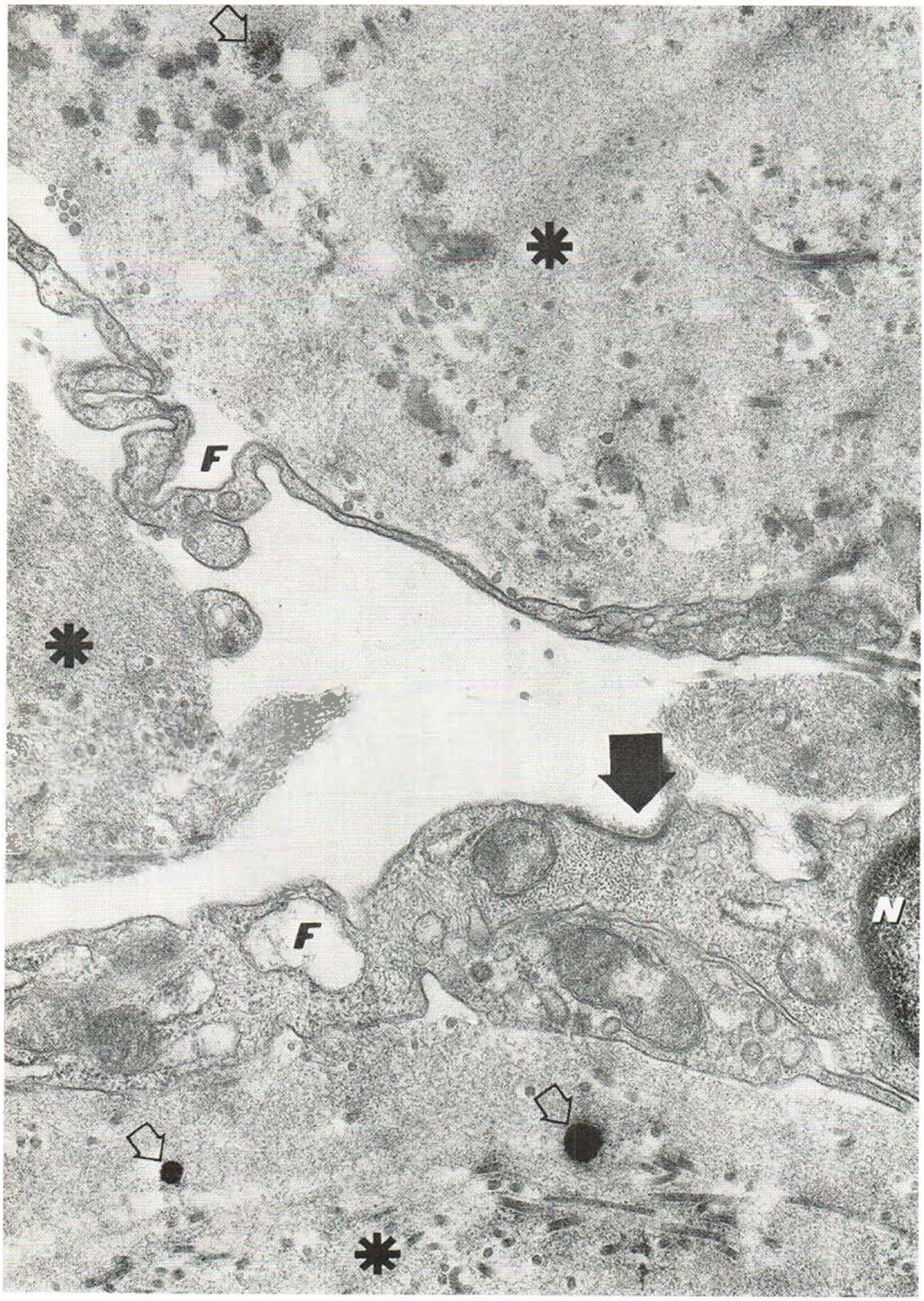

Fig. 2. The body and processes of fibroblasts (*F*) separate hyaline mass into islands (*). *N*, Nucleus of a fibroblast. *Solid arrow:* half-desmosome-like structure. *Hollow arrows:* electron-dense mineral deposits. × 28 000.

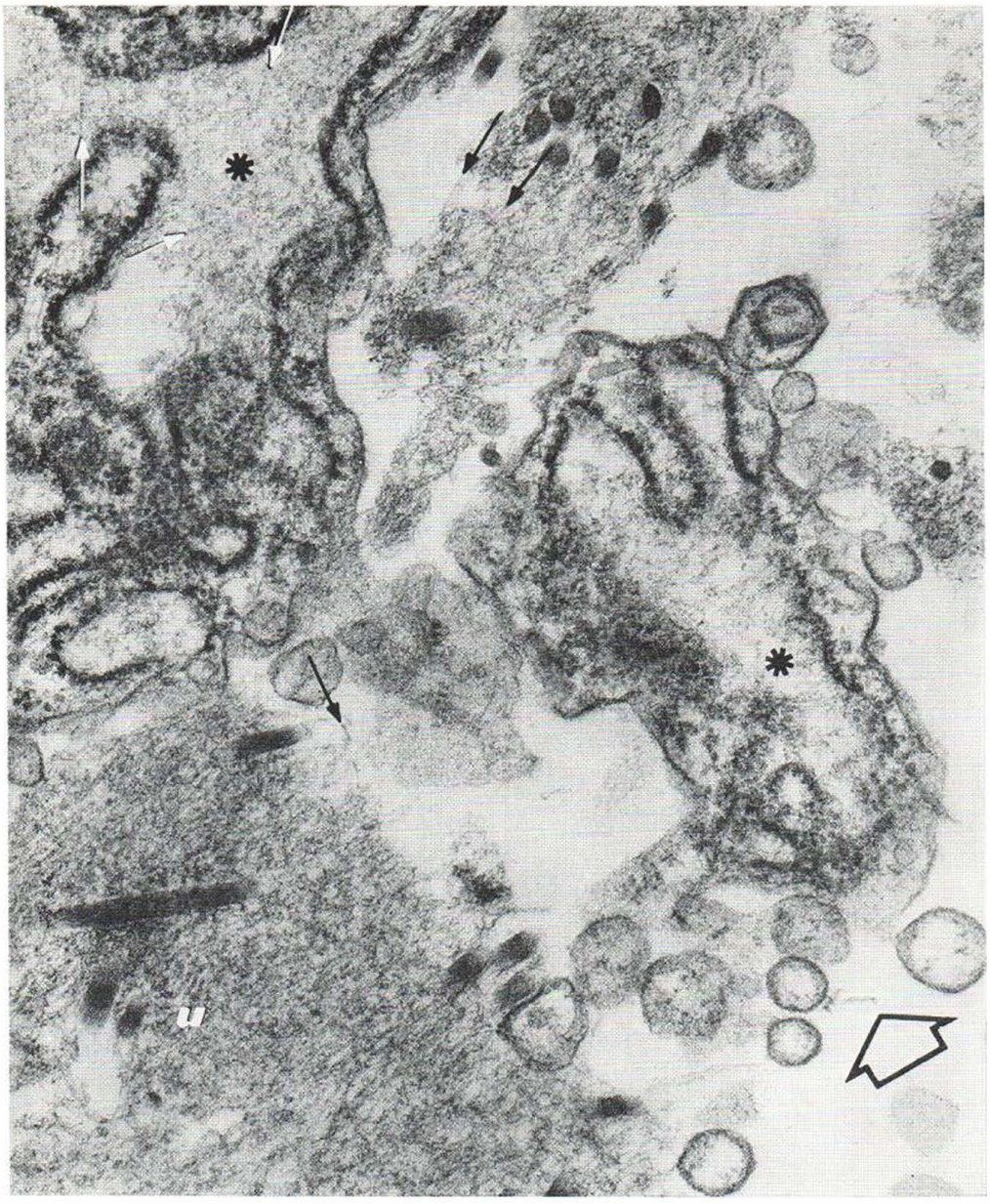

Fig. 3. A fibroblast in the lesion shows dilated cisternae of rough-surfaced endoplasmic reticulum (*). These cisternae contain filaments (*white arrows*) and amorphous material which appear similar to the protofilaments (*black arrows*) and amorphous material of extracellular hyalin. There are a number of ballooned or pinched vesicles of peripheral cytoplasm (*hollow arrow*). *u*, Unit filaments of collagen. × 57 000.

Hyalin island

A typical hyalin island was composed of a peripheral rim with an admixture of normal collagen, unit filaments of collagen (19) and other thin filaments and a central mass of hyalin. It was found that this central mass of hyalin is not an accumulation of pure substance. There were at least four different components:

1. *Fibrous components.* Normal collagen fibrils (Fig. 1), abnormally large collagen fibrils (Figs. 6, 7), unit filaments of collagen (21) (Fig. 8) and protofilaments (Fig. 4) could be differentiated.

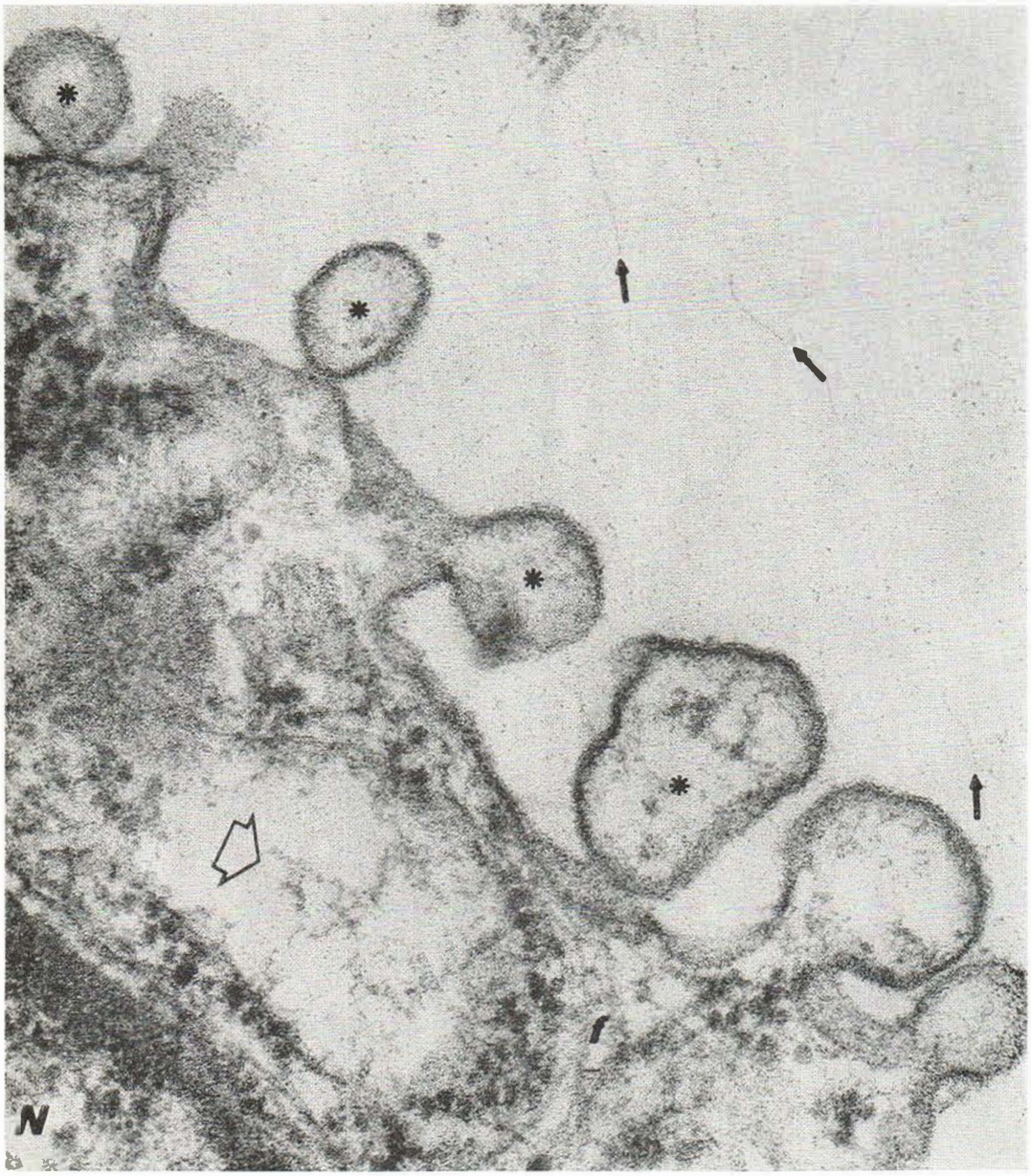

Fig. 4. Ballooning (*) of the plasma membrane of a fibroblast. The peripheral cytoplasm seems to be segregated into the extracellular spaces. A dilated cisterna of rough-surfaced endoplasmic reticulum contains thin filaments (*hollow arrow*) slightly larger than the protofilaments (*solid arrows*) of extracellular hyalin. *N*, Nucleus. × 112 500.

Abnormally large collagen fibrils had diameters up to 1 800 Å and were often split into unit filaments and others further into protofilaments (Figs. 6, 7). Normal-sized collagen fibrils were similarly split (Figs. 8, 9). *Protofilaments* 15–20 Å were rather straight and usually short and non-anastomosing (Figs. 4, 8). As mentioned above, these protofilaments resembled those found in the dilated endoplasmic reticulum of active fibroblasts surrounding hyalin islands (Figs. 3, 4). The fibrous components of the range of *filaments* (50–100 Å) were rather ill-defined, often curved and branched (Figs. 8–10). *Unit filaments* of collagen fibrils had diameters similar to the larger

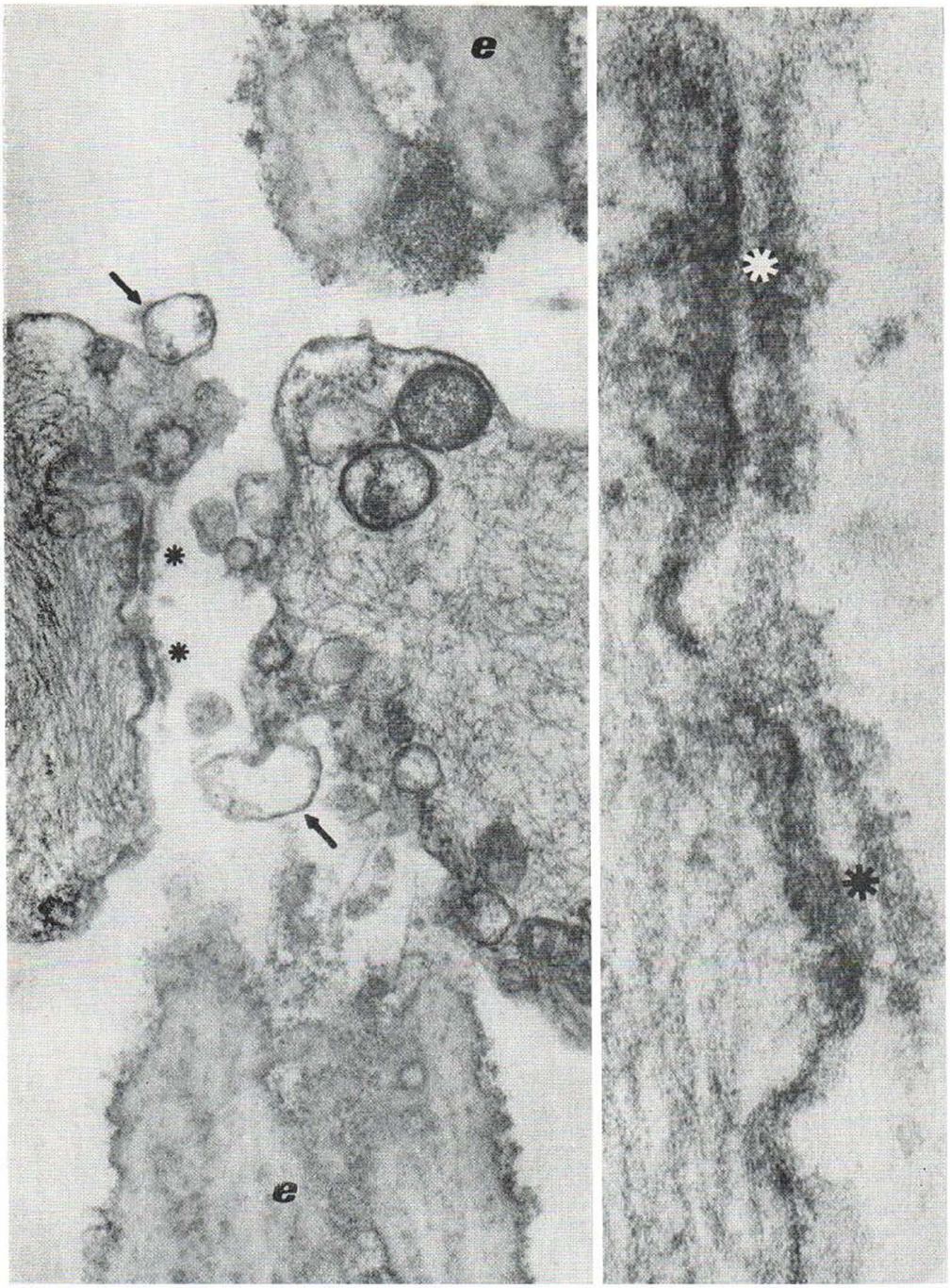

Fig. 5. Half-desmosome-like structures (*) of fibroblasts, normal elastic fibers (*e*) and peripheral budding (*arrows*) of a fibroblast. Left: × 39 500. Right: × 199 250.

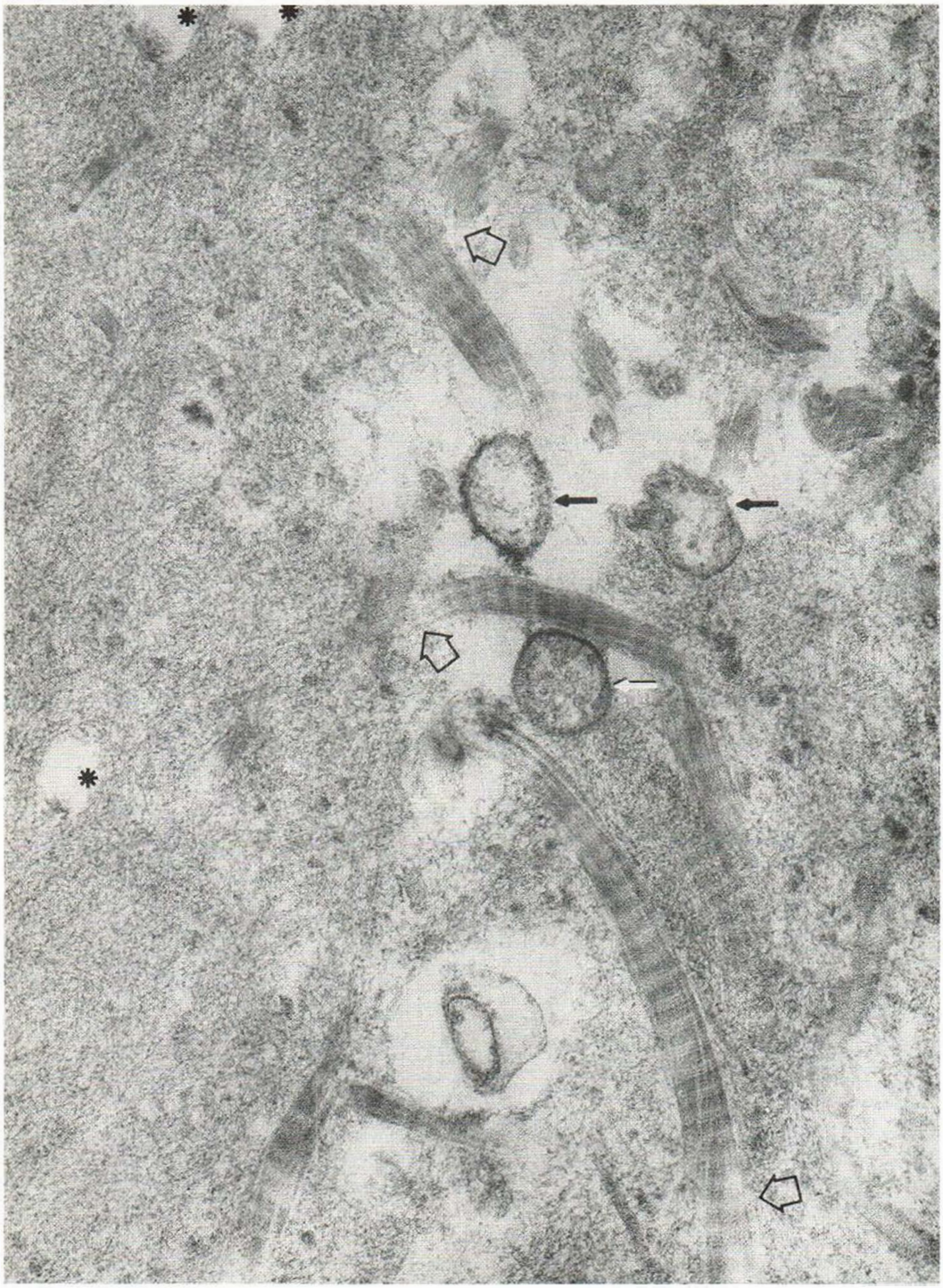

Fig. 6. Abnormally large collagen fibrils are longitudinally split into smaller unit filaments (*hollow arrows*). Segregated buds of fibroblast are undergoing degeneration and mineralization (*solid arrows*). *, Degenerating collagen fibril with clear halo. × 30 250.

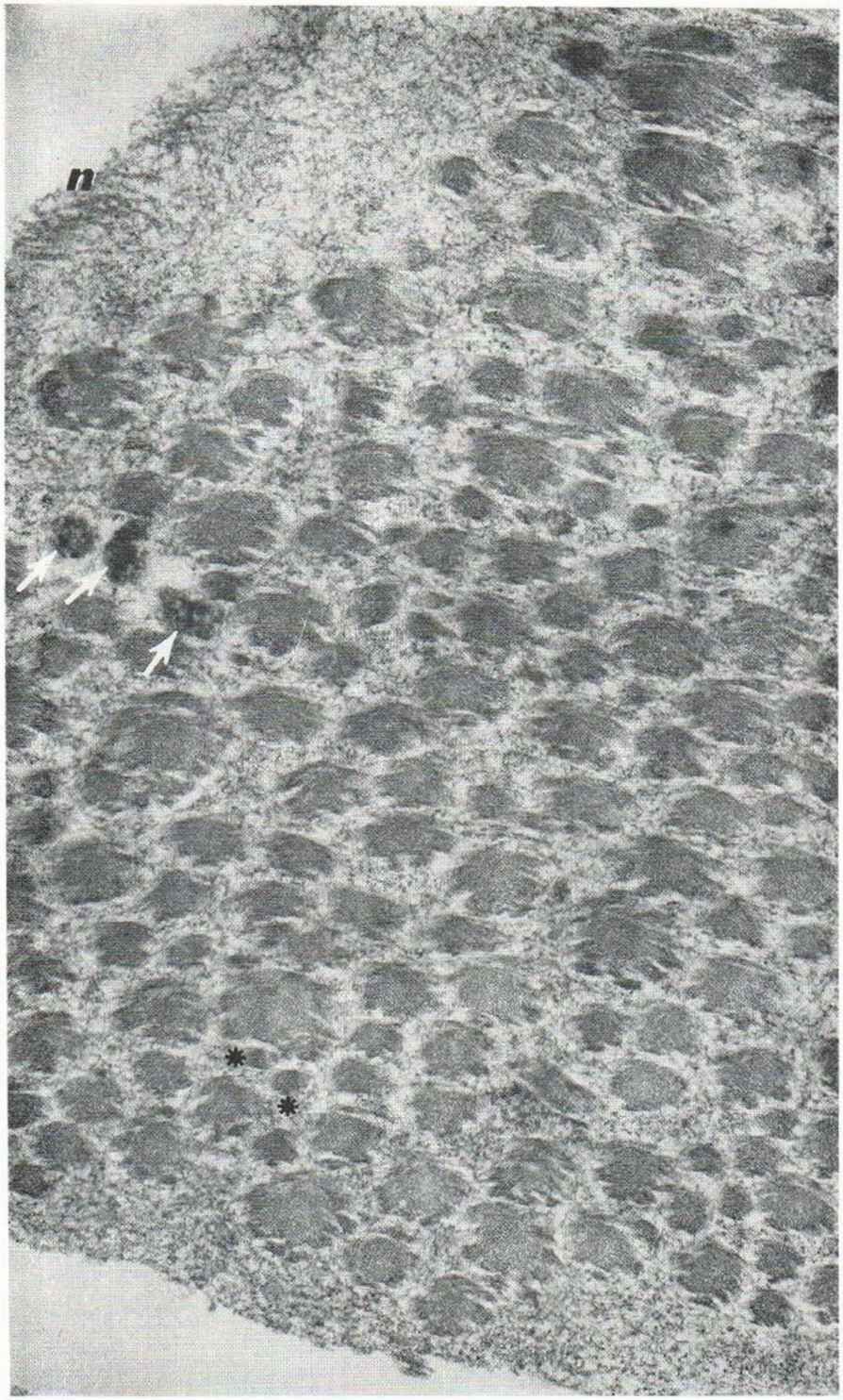

Fig. 7. Cross-section of abnormally large collagen fibrils. Some of these exhibit splitting into smaller subunits similar to unit filaments of collagen (*n*). Smaller filaments and amorphous substance fill the space between them. *Arrows:* mineralized collagen fibrils. *, Normal-sized collagen fibrils. × 57 000.

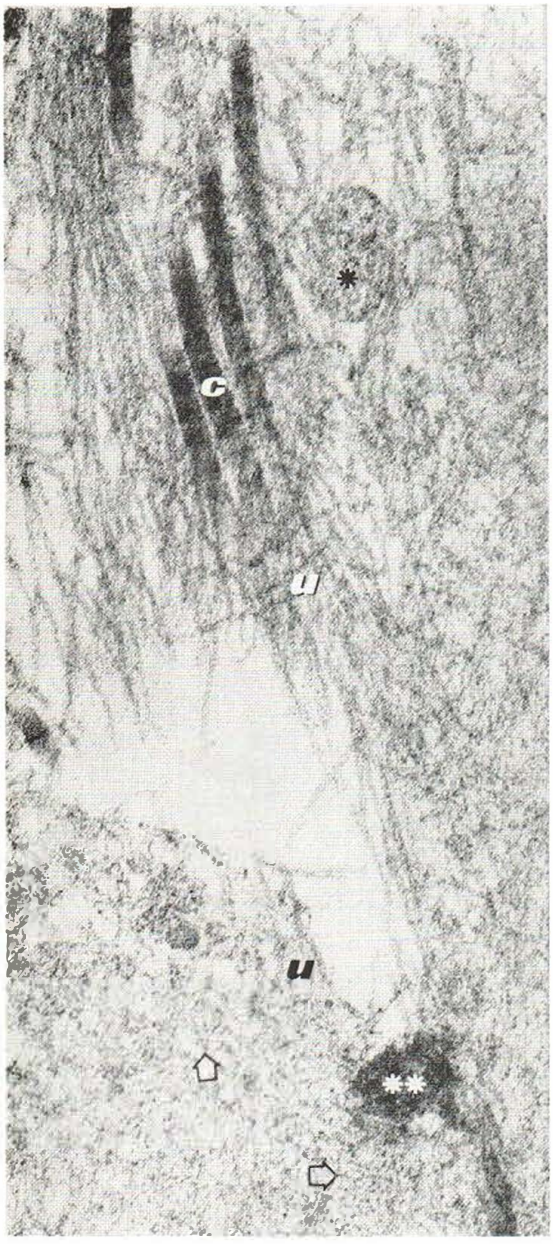

Fig. 8. This picture and Fig. 9 could be interpreted in two ways: Splitting of collagen fibrils (*c*) into unit filaments (*u*) and further into smaller filaments and protofilaments (*arrows*), or defective polymerization of protofilaments into larger collagenous fibrils. Mineralized (**) and non-mineralized (*) fragments of fibroblast. ×68 300.

variety of filaments (about 100 Å), but were uniform throughout their length, well-defined, rather straight and only occasionally branched (Figs. 8, 10).

2. *Amorphous components.* Amorphous to finely granular materials with moderate electron density filled the spaces between fibrous components or were present as aggregated masses (Figs. 9–11). Some of the amorphous substances appeared to have derived from degraded fibrous components including degenerated collagen fibrils (see below) (Figs. 9–11), while others came from pinched-off cytoplasmic processes (see below) (Figs. 3, 6).

3. *Degenerated collagen, mineralized collagen and disintegrated processes of fibroblasts.* Normal (Figs. 9–11) as well as abnormal (Fig. 7) collagen fibrils in hyalin islands became swollen, split and/or frazzled. They were usually surrounded by a clear halo (Figs. 7, 9–11). Degenerated collagen showed amorphous and filamentous components which were similar to amorphous, filamentous and protofilamentous materials of hyalin as described above. Many of these degenerated collagen fibrils surrounded by halos showed electron-dense material compatible with mineral deposition (Fig. 11). Pinched-off processes of fibroblasts admixed within hyalin islands also showed apparent mineralization of plasma membranes (Fig. 6) and the contents (Fig. 11). Dense deposits were also found without relation to any of these structures (Fig. 2).

4. *Elastic fibers.* Normal elastic fibers were occasionally found within hyalin islands. They were present in normal numbers outside the hyalin islands (Fig. 5).

Dermo-epidermal, perivascular, perifollicular and periglandular areas

Hyalin mass infiltrated these areas rather heavily. Basal lamina of the epidermis often became obscure when hyalin infiltrated this structure (Fig. 12). Anchoring fibrils and small varieties of collagen, i.e., reticulum fibrils which are normally present in this area, were often absent (Fig. 12). Perivascular basal laminae showed multiplication (Fig. 13) and were often masked by infiltrating hyalin.

Others

Neither mast cells nor plasma cells were encountered in this survey. There were no detectable lipid substances, either intracellularly or extracellularly, although it was possible that lipids were extracted during fixation and dehydration.

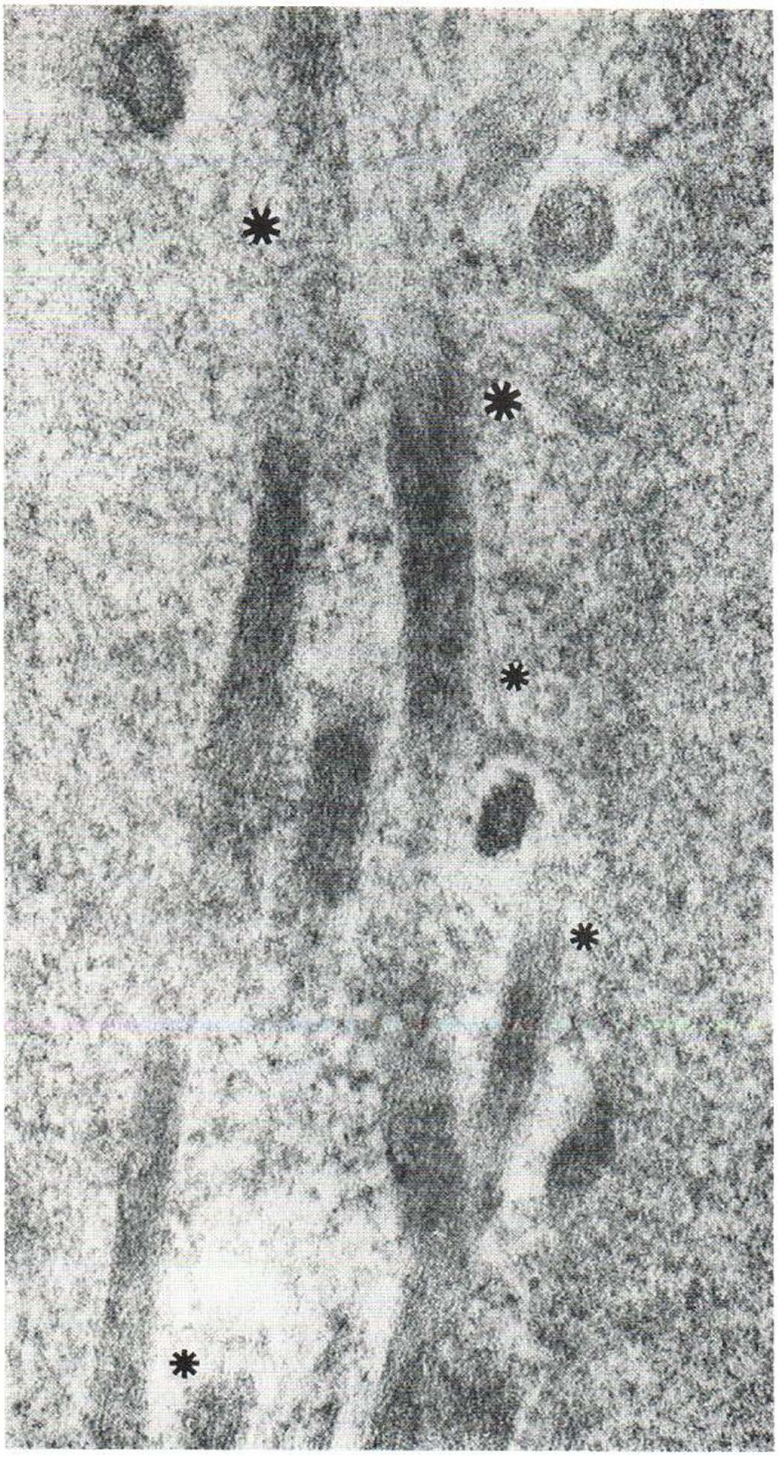

Fig. 9. High magnification view of the same phenomenon as demonstrated in Fig. 8. At several points (*) frazzling of collagen fibrils into smaller subunits is seen. Alternatively, this may represent a faulty polymerization. × 159 750.

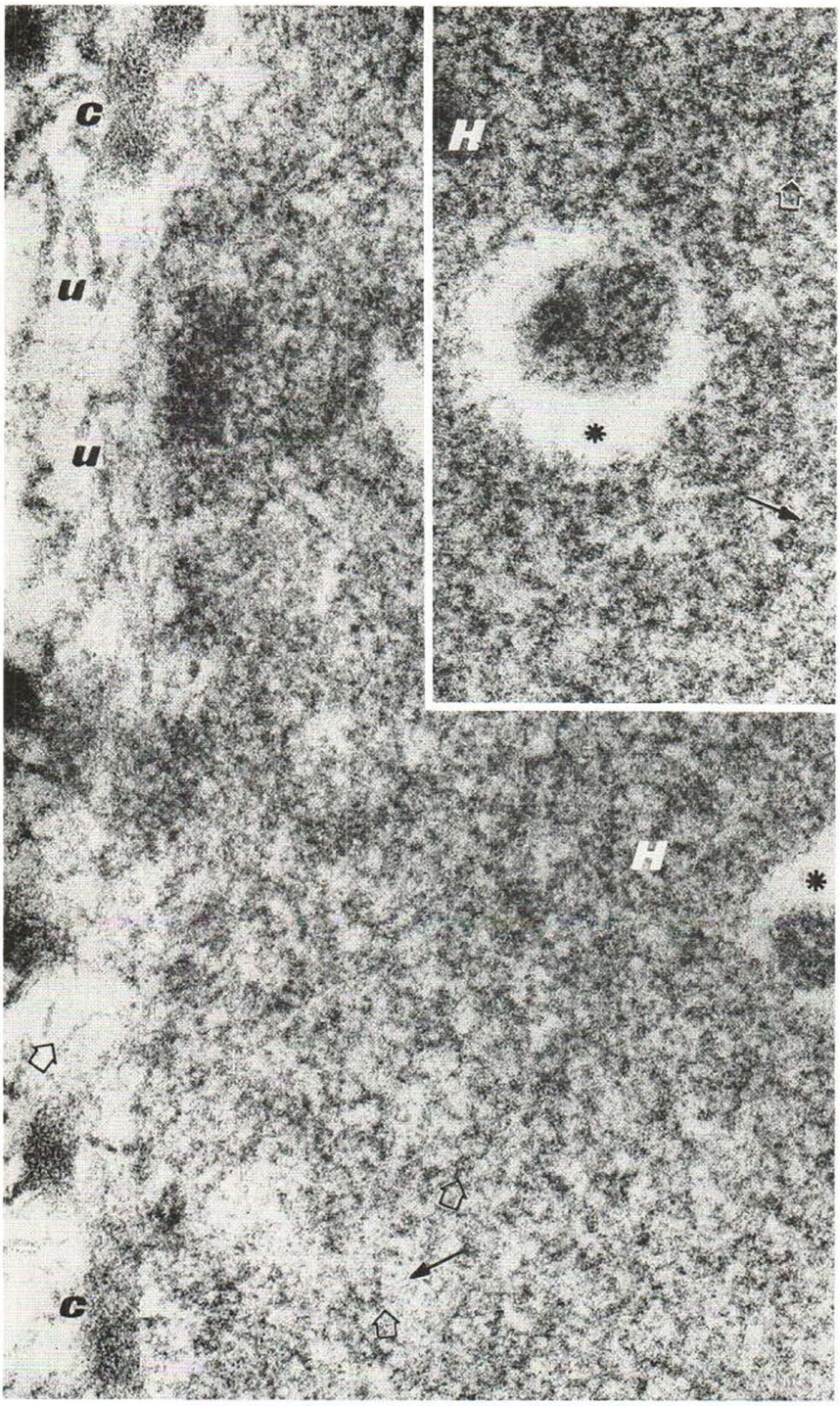

Fig. 10. High magnification view of collagen fibrils (*c*), unit filaments of collagen (*u*), filaments (*hollow arrows*) and protofilaments (*solid arrows*). Degenerating collagen surrounded with clear halo (*) shows amorphous as well as filamentous components, both of which are similar to those surrounding hyalin (*H*). × 147 250.

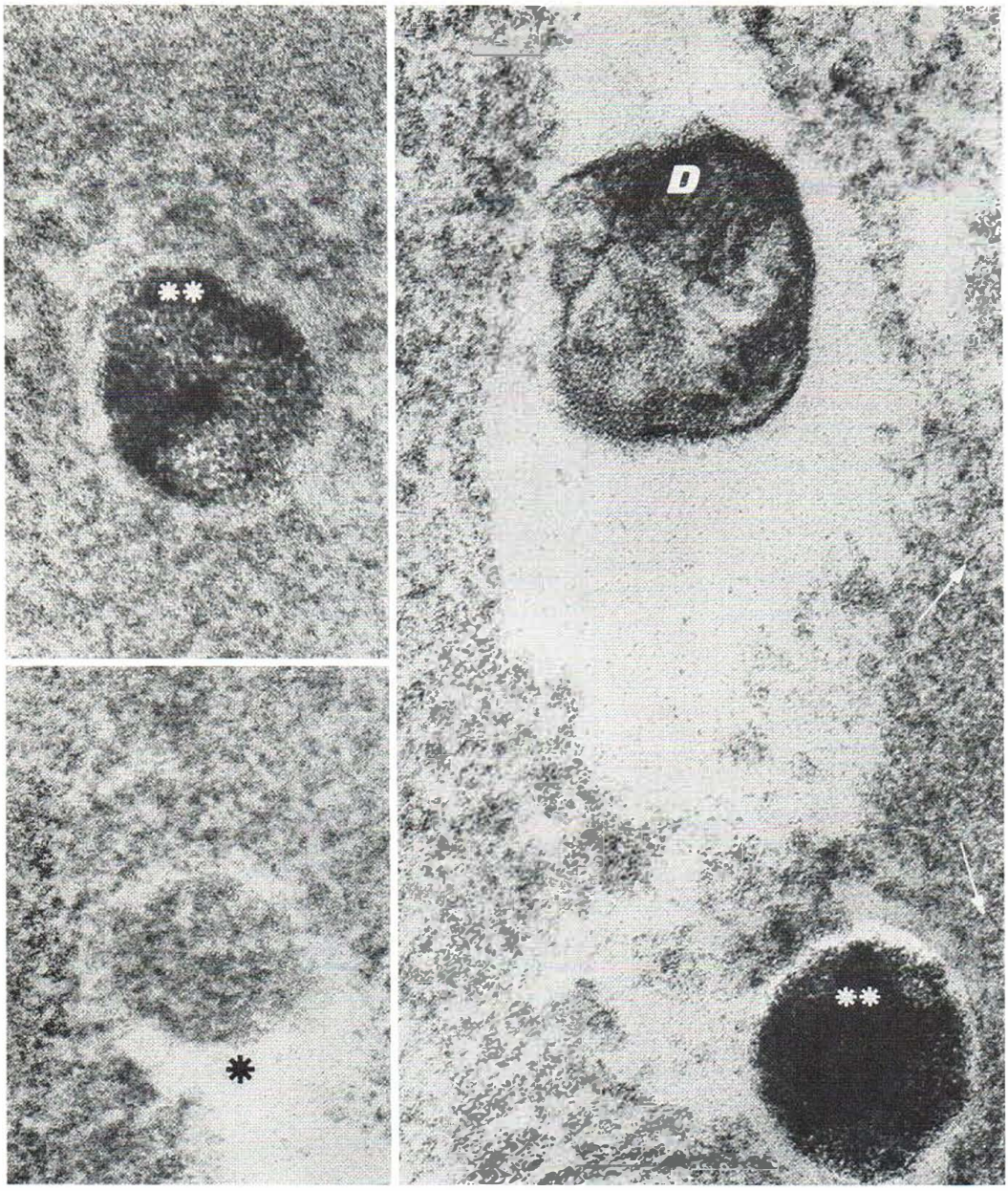

Fig. 11. Half-degenerated collagen (*), mineralized collagen (**) and mineralized cellular debris (*D*) are surrounded with halos. *Arrows:* protofilaments of hyalin. Left upper and lower: × 180 000. Right: × 147 250.

DISCUSSION

Since Waldeyer (33) and von Recklinghausen (22) introduced the term "hyalin" around 1882–1883, it has been used to describe miscellaneous glassy substances including keratohyalin (33). It is therefore understandable that under the powerful resolving power of the electron microscope various hyalins were revealed to be quite different. For example, keratohyalin was found to be mainly amorphous, whereas hyalin comprising thickened pleura and spleen capsule in Boeck's sarcoid was found to consist of a fine network of finest collagen protofilaments (8). In hyalinosis cutis et mucosae, hyalin was resolved into amorphous

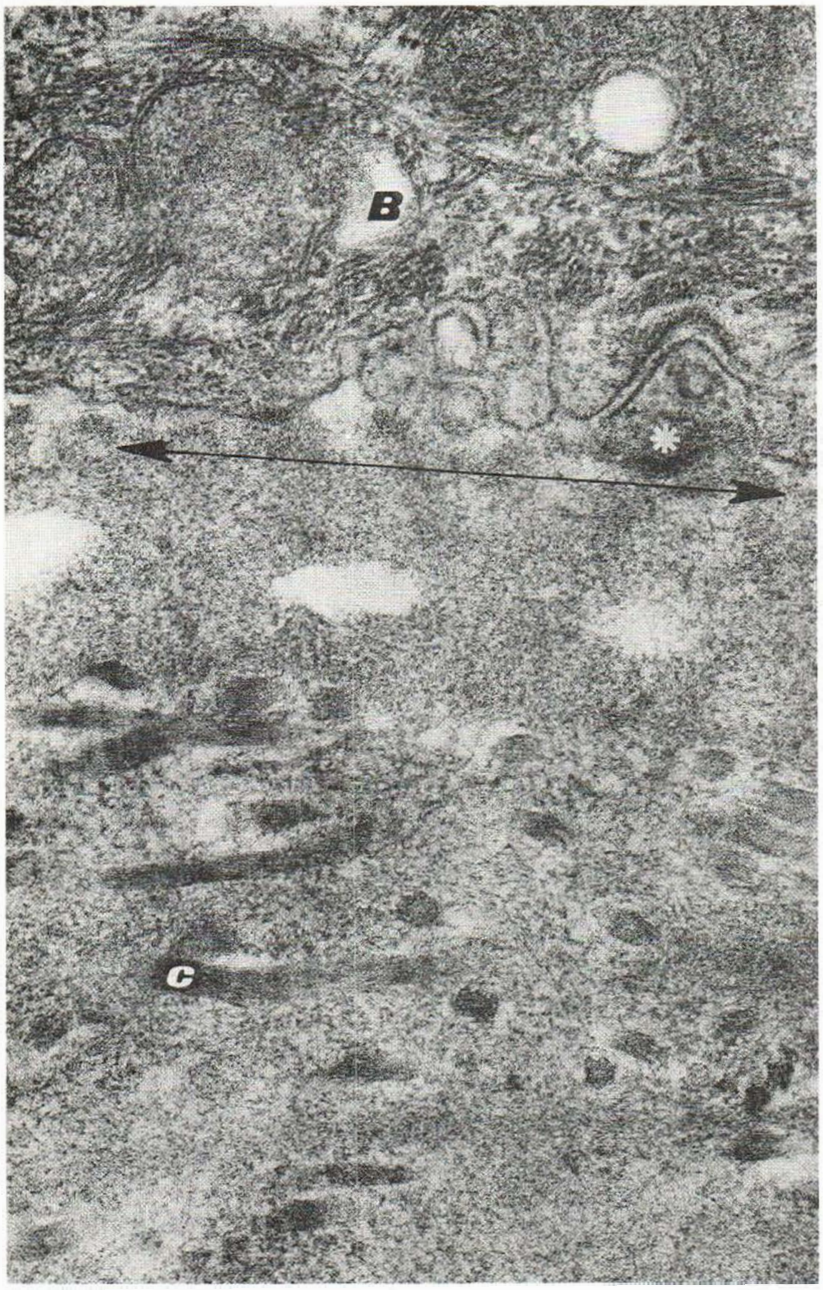

Fig. 12. Hyalin material infiltrated the subepidermal area and obliterated the basal lamina (arrows). *B*, Basal cell of the epidermis; *C*, collagen; *, half-desmosome. × 72 500.

and filamentous components. The filamentous component was briefly described by Grosfeld (10) and in more detail by Rodermund & Klingmüller (25). It is of interest that hyalin membranes and hyalin droplets of cylindroma contain similar amorphous material and fine filaments (13) and occur at the epithelio-dermal junctions as in hyalinosis cutis et mucosae. It is conceivable that epithelio-dermal interaction plays some role in the development of "hyalin" in both diseases.

Hyalin, colloid and amyloid

Although hyalin shares certain histochemical characteristics with colloid (9, 16) and amyloid (2, 11, 26, 27) (Table I), amyloid is ultrastructurally different from the other two because of the pre-

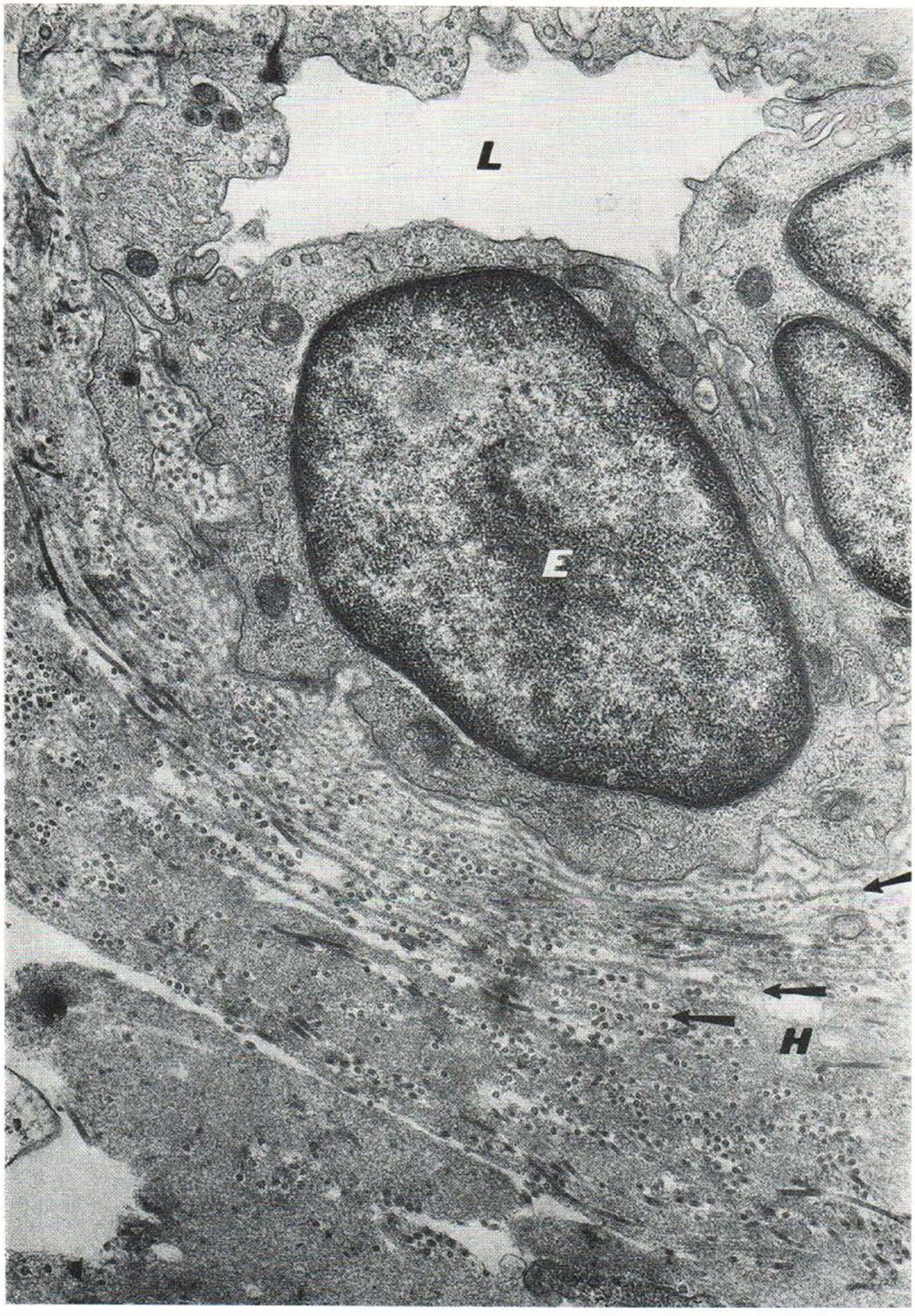

Fig. 13. Perivascular infiltration of hyalin (*H*) obscures the multiplied basal lamina (*arrows*). *E*, Endothelial cell; *L*, lumen. × 15 250.

dominance of long, rigid, non-branching filaments. The ultrastructure of hyalin is very similar to that of colloid (16). Both have in common protofilaments, filaments and unit filaments, amorphous material, and degenerating collagen with surrounding clear halos. Mineralization of such collagen was, however, only found in hyalin.

In the lesions of hyalinosis cutis et mucosae, colloid milium (16) and lichen amyloidosus (11, 15), fibroblasts show signs of active fibrillogenesis such as dilatation of rough-surfaced endoplasmic reticulum and pinching off of peripheral cytoplasm. Hyalin, colloid and amyloid are deposited in various forms of islands just as collagen is laid down in the normal skin. These observations led us to a conclusion that hyalin could be one of the abnormal products of fibroblasts as colloid in colloid milium (16) and amyloids in lichen amyloidosus (11, 15, 26, 27, 29) and in other forms of amyloidosis (3, 7, 17, 34) were thought to be the local products of fibroblasts or reticuloendothelial cells. It may be that the fibroblasts of the patient with this disease have a defect of genes which regulate the synthesis of fibrous proteins. Abnormal building materials thus produced may not be polymerized into normal collagen molecules, although they form islands similar to those of collagen, or, alternatively, they may first be built into collagen but may soon be broken down.

The ground substance in which extracellular polymerization of collagen molecules is supposed to take place may be abnormal as has been postulated in amyloidoses (3).

The nature of the electron-dense material deposited on the degenerated collagen and pinched off cytoplasmic processes is not clear. It does not appear needle- or spicule-like as one common form of calcium in the tissue, i.e., hydroxyapatite, does, for example, in calcifying epithelioma of Malherbe (12). Histochemical detection of calcium with Kossa's method was reported to be negative (10), although this method does not stain calcium but carbonates and phosphates (20) with which not only calcium but other minerals have affinity. Intracranial "calcification" of our patient as well as others (10) and "calcification" of the larynx (4) seem to have something to do with this electron-dense material which could represent any material.

Half-desmosome-like structures were observed between connective tissue cells and ground substances regularly in hair germ mesenchymal cells at a certain stage of embryonic development (14) and also in tissue-cultured fibroblasts (unpublished data). Although in the normal postnatal skin these structures are definitely rare, they could be found very commonly in the fibroblasts in the lesion of lichen amyloidosus (15) and colloid milium (16). It seems that these structures are a concomitant of active fibrillogenesis, either normal or abnormal.

ACKNOWLEDGEMENTS

This work was supported in part by the Part I Designated Research Grant and Medical Investigatorship Award from the Veterans Administration.

REFERENCES

1. Baer, R. S.: The structure of collagen fibrils. Adv Prot Chem *7:* 69, 1952.
2. Brownstein, M. H. & Helwig, E. B.: The cutaneous amyloidoses. I. Localized forms. Arch Derm (Chicago) *102:* 8, 1970.
3. Cohen, A. S.: Amyloidosus. New Engl J Med *277:* 522, 1967.
4. DeSouza, A. R. & Patricio, L. D.: Fisiopathologica a "Lipoido-Proteinose de Urbach-Wiethe". An da Fac Med S Paulo *24:* 291, 1948–1949.
5. Falkmer, S., Hofer, P.-A. & Hollström, E.: Lipoglycoproteinosis (Urbach-Wiethe). A preliminary report. Acta Dermatovener (Stockholm), Suppl. Hellerström, p. 47, 1966.
6. Fleischmajer, R., Nedwich, A. & Ramos e Silva, J.: Hyalinosis cutis et mucosae. A histochemical staining and analytical biochemical study. J Invest Derm *52:* 495, 1969.
7. Gafni, J., Merker, H.-J., Shibolet, S., Sohar, E. & Heller, H.: On the origin of amyloid. Study of an amyloid tumor in multiple myeloma. Ann Intern Med *65:* 1031, 1966.
8. Gieseking, R.: Die hyaline Umwandlung des kollagenen Fasergewebes im elektronenoptischen Bild. Zbl allg path Anat *107:* 579, 1965.
9. Graham, J. H. & Marques, A. S.: Colloid milium: A histochemical study. J Invest Derm *49:* 497, 1967.
10. Grosfeld, J. C. M., Spass, J., Van de Staak, W. J. B. & Stadhouders, A. M.: Hyalinosis cutis et mucosae. Dermatologica *130:* 239, 1965.
11. Hashimoto, K., Gross, B. G. & Lever, W. F.: Lichen amyloidosus. Histochemical and electron microscopic studies. J Invest Derm *45:* 204, 1965.
12. Hashimoto, K., Nelson, R. G. & Lever, W. F.: Calcifying epithelioma of Malherbe. Histochemical and electron microscopic studies. J Invest Derm *46:* 391, 1966.

13. Hashimoto, K. & Lever, W. F.: Histogenesis of skin appendage tumors. Arch Derm (Chicago) *100:* 356, 1969.
14. Hashimoto, K.: The ultrastructure of the skin of human embryos. V. The hair germ and perifollicular mesenchymal cells. Brit J Derm *83:* 167, 1970.
15. Hashimoto, K. & Onn, L. L. Y.: Lichen amyloidosus. Electron microscopic study of a typical case and a review. Arch Derm, to be published.
16. Hashimoto, K., Miller, F. & Bereston, E. S.: Colloid milium. Histochemical and electron microscopic studies. Arch. Derm. (Chicago). In press.
17. Heller, H., Gafni, J. & Sohar, E.: The Inherited Systemic Amyloidoses. *In* The Metabolic Basis of Inherited Disease, 2nd ed. (ed. J. B. Stanbury, J. B. Wyngaarden & D. S. Fredrickson), p. 995. McGraw-Hill Book Company, New York, 1966.
18. Laymon, C. W. & Hill, E. M.: An appraisal of hyalinosis cutis et mucosae. Arch Derm (Chicago) *75:* 55, 1957.
19. Mercer, E. H., Munger, B. L., Rogers, G. E. & Roth, S. I.: A suggested nomenclature for fine structural components of keratin and keratin-like products of cell. Nature *201:* 367, 1964.
20. Pearse, A. G. E.: Histochemistry, Theoretical and Applied. Little & Brown Co., Boston, 1960.
21. Porter, K. R.: Cell Fine Structure and Biosynthesis of Intercellular Macromolecules. *In* Connective Tissue: Intercellular Macromolecule (ed. The New York Heart Assoc.), pp. 167–196. Little, Brown & Co., Boston, 1964.
22. Recklinghausen, F. D. v.: Allgemeine Pathologie. G. Thieme Verlag, Stuttgart, 1883.
23. Reynolds, E. S.: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol *17:* 208, 1963.
24. Rodermund, O.-E.: Zur Hyalinosis cutis et mucosae (Urbach-Wiethe). Z Haut Geschlechtskr *43:* 493, 1968.
25. Rodermund, O.-E. & Klingmüller, G.: Elektronenmikroskopische Befunde des Hyalins bei Hyalinosis cutis et mucosae. Gleichzeitig ein Beitrag zur Frage der Entstehung des Hyalins. Arch Klin Exp Derm *236:* 238, 1970.
26. — Zur elektronenmikroskopischen Struktur des Lichen amyloidosus. Vortr. 28. Tgg. Dtsch. Dermat. Ges. Tübingen Sept. 1968. und Arch Klin Exp Derm *237:* 110, 1970.
27. — Zur submikroskopischen Struktur des Amyloid. Arch Klin Exp Derm *236:* 147, 1970.
28. Sasai, Y.: Hyalinosis cutis et mucosae: Histochemical study of hyaline material. Tohoku J Exp Med *100:* 305, 1970.
29. Shapiro, L., Kurban, A. K. & Azar, H. A.: Lichen amyloidosus. Arch Path *90:* 499, 1970.
30. Urbach, E. & Wiethe, C.: Lipoidosis cutis et mucosae. Virchow Arch Path Anat *273:* 285, 1929.
31. Urbach, E.: Über eine familiäre lokale Lipoidose der Haut und Schleimhäute auf Grandlage einer diabetischen Stoffwechselstörung. Arch Derm Syph (Berlin) *157:* 451, 1929.
32. — Lipoidstoffwechselerkrankungen der Haut. *In* Handbuch der Haut- und Geschl.-Kr., Band XII/2 (ed. J. Jadassohn). Springer, Berlin, 1932.
33. Waldeyer, W.: Untersuchungen über die Histogenese der Horngebilde, besonders der Haare und Federn. Beitr Anat Embryol (Festschrift für Henle), 141, 1882.
34. Zucker-Franklin, D. & Franklin, E. C.: Intracellular localization of human amyloid by fluorescence and electron microscopy. Am J Path *59:* 23, 1970.

Received September 13, 1971

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