

HISTOGENESIS OF MUCIN IN FOLLICULAR MUCINOSIS

An Electron Microscopic Study

Akira Ishibashi

*From the Department of Dermatology, Kyorin University School of Medicine,
Shinkawa, Mitaka, Tokyo, Japan*

Abstract. Many dilated cystic ergastoplasms, including their granular, filamentous contents, the developed Golgi apparatuses, and vesicles were observed in the cytoplasm of affected root sheath cells, which were accompanied by much amorphous agglutination of fine granular or flocculent materials in the interstices. These extracellular substances were stained with ruthenium red and seemed to correspond to increased metachromatic materials, probably composed of hyaluronic acid. Possible structures for hyaluronic acid were discussed. Mucin in follicular mucinosis seemed to be produced by secretory function of follicle cells.

Key words: Mucinosis follicularis; Alopecia mucinosa; Ultrastructure; Histogenesis; Mucin; Hyaluronic acid

The pathogenesis of follicular mucinosis is still obscure, although some autoradiographic (14) and electron microscopic studies (5, 6, 18) have been reported, in addition to many excellent studies on its histochemistry. While electron microscopic studies have dealt with changes in the sebaceous glands (5), in the stratum spinosum (18), and in the dermis surrounding the affected follicles (6), hair follicles may play a significant role in the development of this condition.

This paper reports some new data concerning the histogenesis of follicular mucinosis on the basis of an electron microscopic study of affected follicles.

MATERIALS AND METHODS

The patient was a 52-year-old woman who had depilated erythematous lesions, with or without follicular papules, on the scalp, face, and cervical region, and had plaques of grouped follicular papules without redness on the breast and the back. Onset of present illness was said to be 6 or 7 years ago.

Specimens biopsied from the papular lesions with almost no redness were fixed with formaldehyde-glutaraldehyde fixative (11) and postfixed with 1% osmium tetroxide in Millonig's phosphate buffer. The specimens were dehydrated with graded ethanol and embedded in epoxy resin. Sections were contrasted with uranyl acetate and lead hydroxide, while thick sections were stained with toluidine blue for light microscopy and for trimming. A JEM-100B electron microscope was used.

Ruthenium red staining was done by single fixation with 1.0% osmium tetroxide in cacodylate buffer containing 500 ppm of purified ruthenium red dye (15).

RESULTS

Light microscopy (Fig. 1 a, b, c):

Thick sections from Epon-embedded tissues with toluidine blue staining revealed an intercellular edema of the basalioma-like proliferative, swollen follicles (Fig. 1 a, b), and also destruction of the follicle cells and formation of small cystic spaces in some parts, while intracellular edema was not observed although some pressure on the follicle cells by intercellular edema was suggested in most of the area (Fig. 1 c).

Metachromatic materials, which were amorphous, granular, and sometimes conglomerated, were present in the intercellular spaces, small cystic spaces, and the lumen of the follicles. Metachromatic materials were occasionally intermingled with orthochromatic cytoplasmic debris, and usually adhered to cell walls of the root sheath cells or the infiltrating cells in the follicles (Fig. 1 c). Invasion of inflammatory, small, round cells accompanied by macrophages was observed in some parts, especially in the peripheral parts of this follicle. A few mast cells were also observed, but only in the peripheral, severely destroyed parts of the follicle, while in-



Fig. 1 a, b, c. A basalioma-like proliferative, swollen follicle is seen. Intercellular edema is prominent in the center of the follicle, while invasion of inflammatory cells is observed chiefly in the peripheral parts. Hair root sheath cells are pressed by an intercellular edema and destruction of sheath cells followed by formation of cystic spaces is noted in some parts. Metachromatic substances intermingled with orthochromatic cytoplasmic debris are present in the interstices and the lumen of the follicle. Macrophages in the lumen contain round, vacuole-like inclusions (arrow) in the cytoplasm. Epon-embedded tissue. Toluidine blue stain.

flammatory infiltration in the surrounding dermis was intermingled with a considerable number of mast cells.

Macrophages seen in the follicular lumen often had vacuole-like inclusion bodies in the cytoplasm (Fig. 1c), and small round cells in the follicle and dermis sometimes showed remarkable lobulation and indentation of the nucleus.

No sebaceous gland was detected in any section of

biopsied specimens from this patient who had symptoms of long duration.

Electron microscopy

Root sheath cells in the area (Fig. 1) where only a few infiltrating cells were observed, often had a number of dilated, cystic, rough-surfaced endoplasmic reticula containing fine granular, filamentous materials, considerable ribosomes, and in some parts Golgi

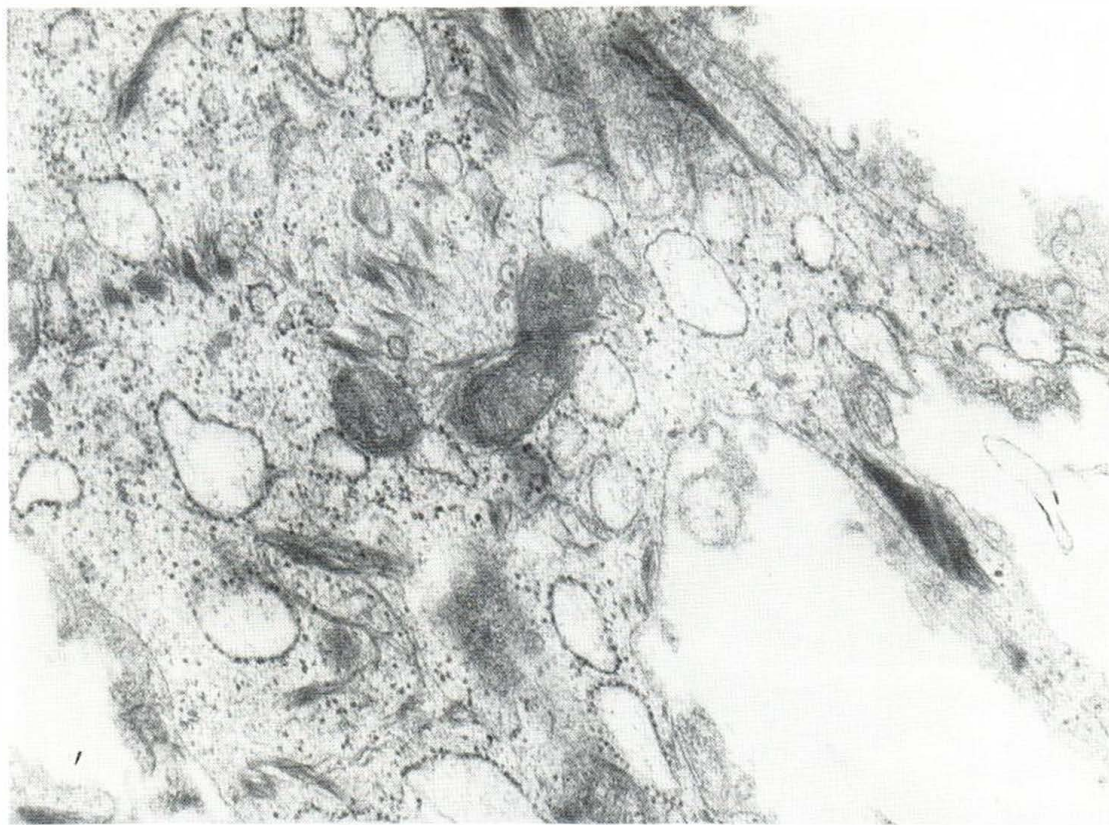


Fig. 2. Many dilated, cystic, rough-surfaced endoplasmic reticula containing fibrillar contents, ribosomes, tonofilaments, and desmosomes are observed in the follicle cells.

Conglomerates of granular amorphous materials of considerable electron density are seen in the intercellular spaces near the cell walls. $\times 35\,000$.

apparatus and many vesicles (Figs. 2, 3, 4). Although destructive changes were conspicuous in some parts, intracellular edema was not detected anywhere. Glycogen granules were sometimes found in the cytoplasm in addition to tonofilaments, desmosomes, mitochondria, etc.

Amorphous agglutination of fine granular or dust-like materials (Fig. 5), sometimes forming globular masses in the follicular lumen and the intercellular spaces, seemed to correspond to the metachromatic substances seen with light microscopy. These materials were apt to adhere to cell surfaces and were sometimes intermingled with cytoplasmic debris. Fine filamentous structures (Figs. 5, 7), dust-like amorphous materials which twined themselves around finer filamentous structures (Fig. 5), and sometimes threads with knobs (12) (Fig. 5) were also observed, usually in connection with conglomerates of granular substances in the follicular lumen.

The macrophages within the follicular lumen having vacuole-like inclusion bodies (Fig. 1c) revealed many lysosomal membrane structures including various dense materials, larger round vacuoles involving nubecula-like contents (Fig. 6) which might be related to intercellular fine granular substances, and tubular rough-surfaced endoplasmic reticula (Fig. 6).

Infiltrating small round cells sometimes contained severely lobulated and indented nuclei, and cytoplasm and cell organelles were scarce, except for some mitochondria and Golgi apparatus.

The increased ruthenium red-positive materials at the cell surface of the external root sheath cells were observed in the upper part of the follicle where baggy swelling, edematous or degenerative changes, and inflammatory cell infiltration were not found by light microscopy of the thick sections. These ruthenium red-positive intercellular materials were amorphous,

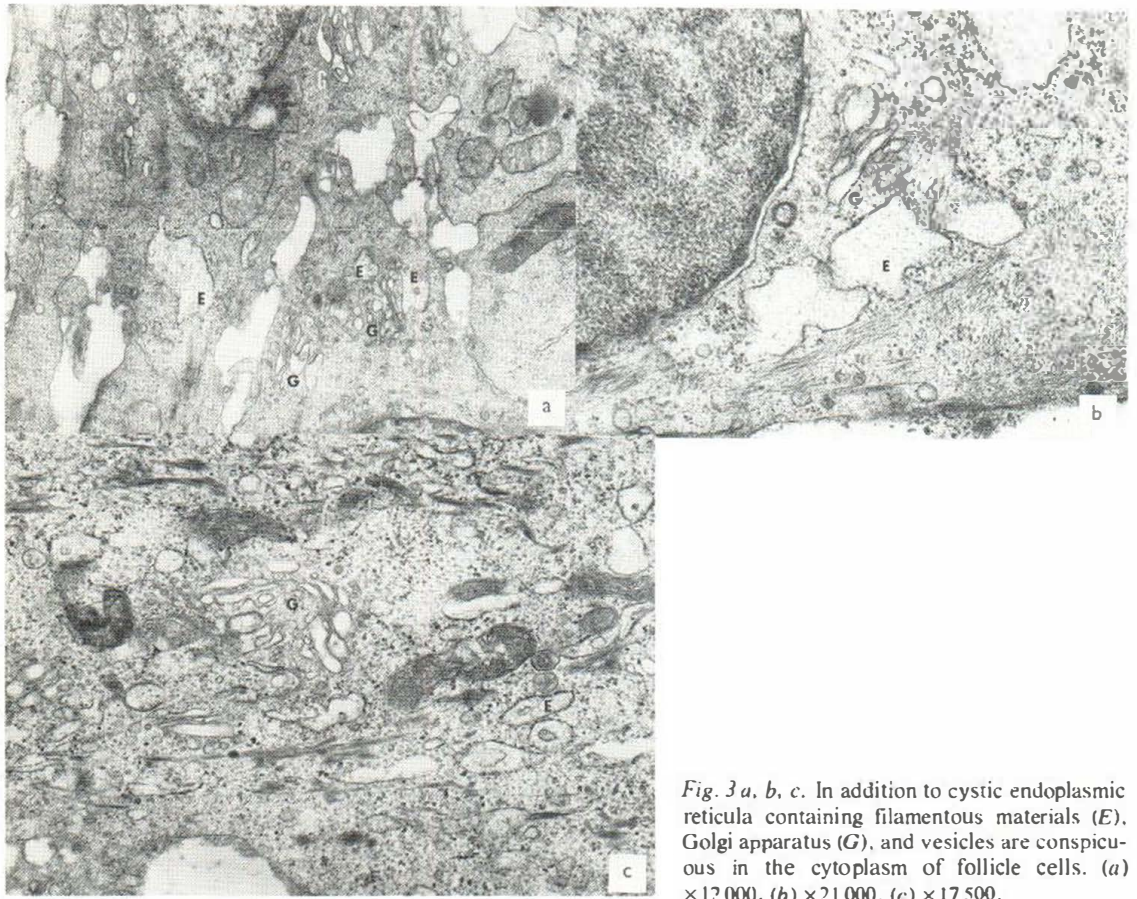


Fig. 3 *a, b, c.* In addition to cystic endoplasmic reticula containing filamentous materials (*E*), Golgi apparatus (*G*), and vesicles are conspicuous in the cytoplasm of follicle cells. (*a*) $\times 12\,000$. (*b*) $\times 21\,000$. (*c*) $\times 17\,500$.

granular, or dust-like in nature, and were often connected with the various above-mentioned filamentous structures having less electron density by ruthenium red staining (Fig. 7).

DISCUSSION

Filamentous structures with a length of several thousand Å and a width of less than 30 Å (7), or unbranched straight-chain polymer with a considerable range of chain length (2) have been reported as hyaluronic acid molecules. The ruthenium red-positive filaments, which were 3.5 nm in diameter and consisted of discrete granules, have been demonstrated as glycosaminoglycans in close association with synovial collagen (16, 17). Similar beaded filaments forming a network, connected with ruthenium red-stained material, 20–25 nm in size, have been found in the interfibrillar matrix of myxofibroma (9) and of pretibial myxedema (3), and

aggregates of ruthenium red-positive flocculent or granular material have been observed in the subcutaneous tissue of a newborn rat (9), in relation to hyaluronic acid.

Therefore, amorphous agglutination of fine granular or dust-like substances, which seemed to be positive for ruthenium red (Figs. 5, 7), and dust-like or granular materials, which could twine themselves around finer filamentous structures and existed in the vicinity of the amorphous agglutination of granular substances (Fig. 5), are likely to correspond to hyaluronic acid, since there has been no evidence of sulfated mucin in this condition (8). Filamentous granular materials within ergastoplasms of follicle cells seem to be comparable to such extracellular, granular or dust-like substances, and may be a precursor of hyaluronic acid.

Various extracellular filaments with smooth profile which seemed to be negative for ruthenium red stain, and the threads with knobs, might also be

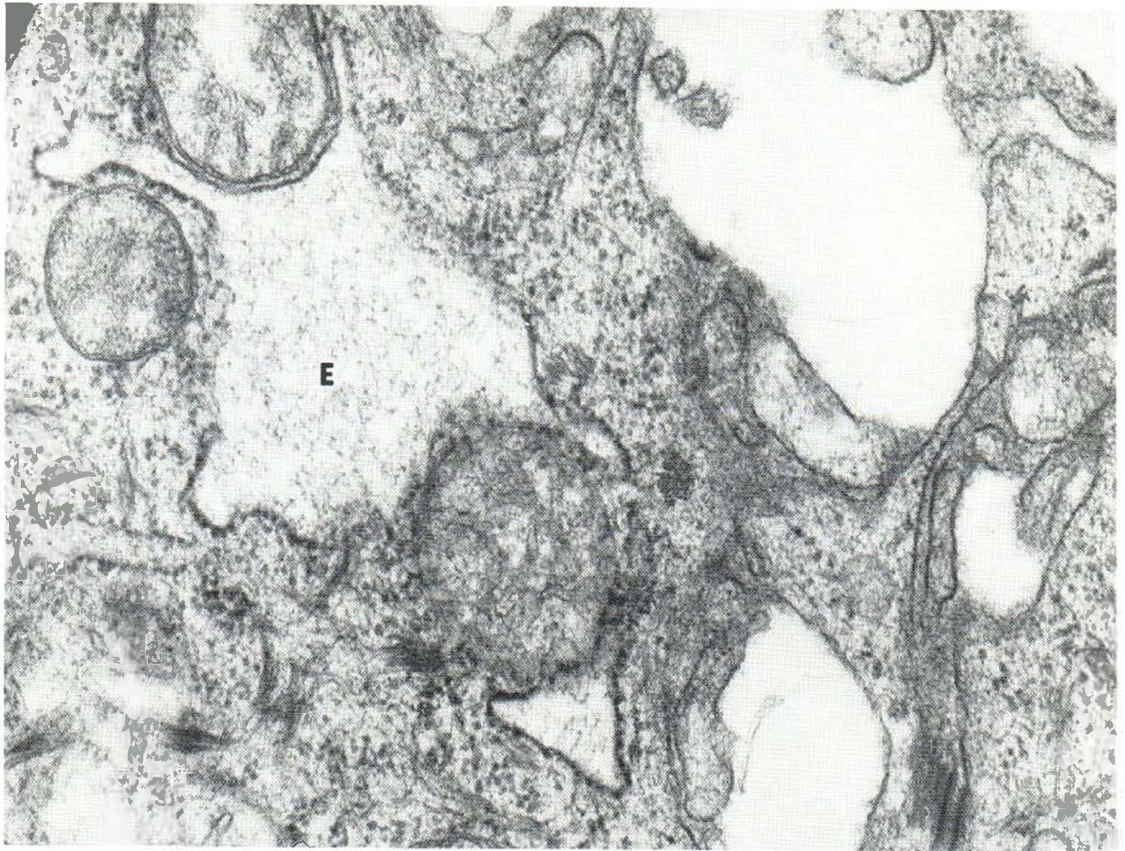


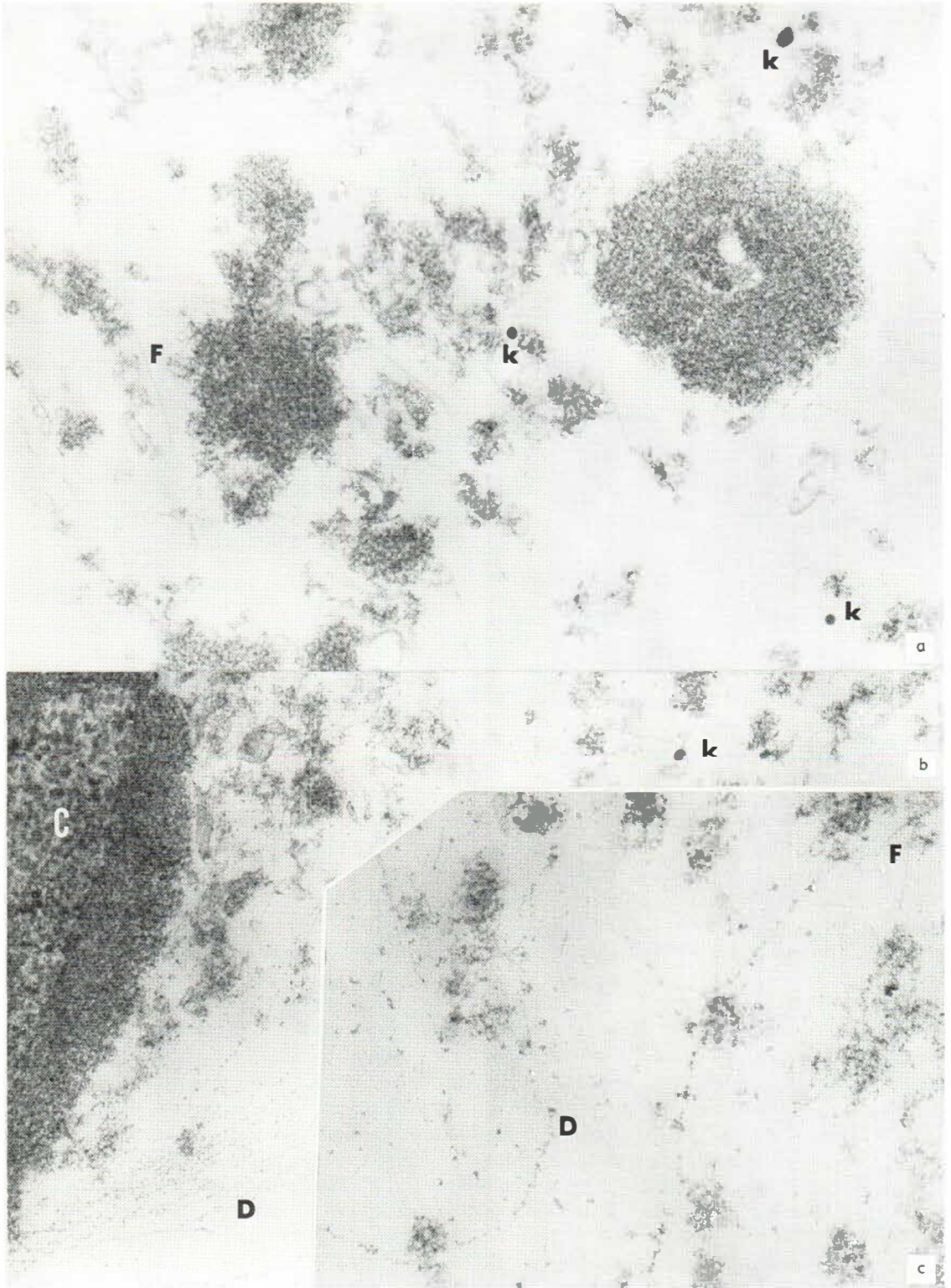
Fig. 4. Close-up of rough-surfaced endoplasmic reticulum containing filamentous contents. $\times 45\,000$.

related to glycosaminoglycans, since various filamentous structures which ranged in thickness from 3 to 10 nm (13, 17) and from 15 to 25 nm (9, 13, 16) and were connected with or without dense granules, and the threads with knobs (12) have been reported as the possible structure for glycosaminoglycans.

Braun-Falco (1) thought that the complexes of mucopolysaccharides with proteins are broken up in follicular mucinosis, which enables the freed compounds to be detected by histochemical methods. Langner et al. (14) indicated that there was no increased synthesis of sulfated acid mucopolysaccharides by means of autoradiographic studies using ^{35}S -sulfate, and they supported Braun-Falco's hypothesis. However, acid mucopolysaccharides in the affected follicles of this disease have been established as non-sulfated acid mucopolysaccharides, i.e., hyaluronic acid, in association with a small amount of protein (8), and hyaluronic acid can

be visualized by histochemical stainings, although this acid is known to be present in the dermis, bound to protein.

The presence of many dilated, cystic, rough-surfaced endoplasmic reticula containing fine granular, filamentous materials, the occasional appearance of developed Golgi apparatus and many vesicles near the ergastoplasms in the root sheath cells, and also the increase in fine granular, ruthenium red-positive materials corresponding to metachromatic materials in the intercellular spaces of affected follicles, even in the part with scarce cell infiltration and degenerative changes, all suggest that mucopolysaccharides in the affected follicles were synthesized by the secretion process of hair root sheath cells. Destructive changes of sheath cells might follow severe intercellular edema due to the accumulation of hyaluronic acid which has a chemical property of retaining a great deal of water, i.e., secretion of hyaluronic acid from sheath cells into



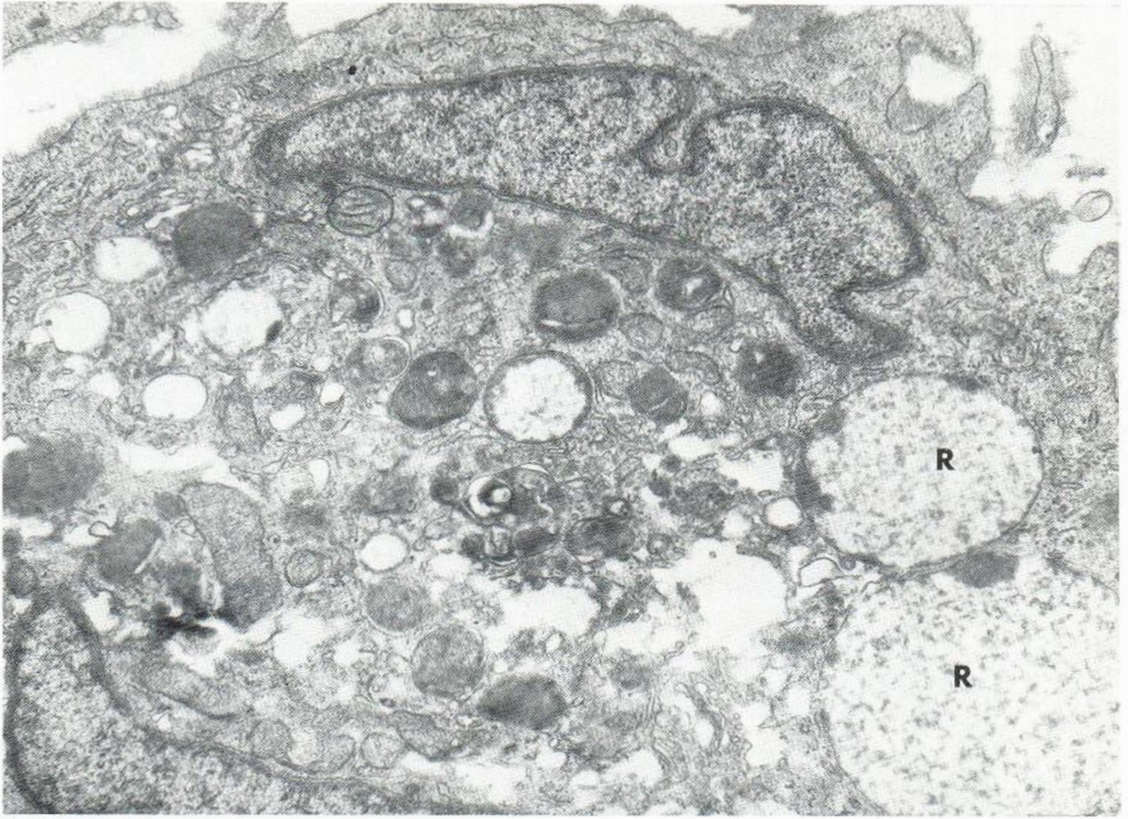


Fig. 6. Many lysosomal membrane structures and larger, round vacuoles (R) involving nubecula-like contents, which seem to be related to extracellular fine granular

substances, are prominent in the macrophage having vacuole-like inclusion bodies (Fig. 1c) as seen by light microscopy. $\times 19000$.

intercellular spaces. Such an interpretation is possible, since hyaluronidase-labile non-sulfated acid mucopolysaccharide (hyaluronic acid) is present in the interstices of normal epidermal cells (8), probably as intercellular cement substances and in the normal and altered follicles (4); the activity of uridine diphosphoglucose dehydrogenase has been indicated in the epidermal cells, external hair root sheath cells, and immature sebaceous cells (10); and Pinkus (10) proposed the hypothesis that the metabolism of follicular sheath cells was affected by a virus in follicular mucinosis.

Fig. 5a, b, c. Amorphous agglutinations of fine granular or dust-like materials which are apt to adhere to cell walls and to form globular masses, knobs with threads (k), fine filaments (F), and dust-like materials which twine themselves around finer filaments (D) are observed in the follicular lumen. These materials might correspond to hyaluronic acid, and are intermingled with cytoplasmic debris. C: cytoplasm. (a) $\times 57000$, (b) $\times 57000$, (c) $\times 120000$.

Mast cells were not found in those parts of the hair follicles where the increased intercellular hyaluronic acid was present without inflammatory infiltrating cells, although the severely destroyed parts of the follicles revealed numerous inflammatory infiltrates intermingled with a few mast cells. Metachromatic substance in mast cells was proved to be a sulfated mucin, viz. heparin, but hyaluronic acid could not be demonstrated by a histochemical method (8). Consequently, mast cells and other infiltrating cells are not likely to be involved in producing hyaluronic acid, though they may be concerned with the disposal of pathologic products.

The nubecula-like content within vacuoles of macrophages seems to be a mucinous substance undergoing digestion. Amorphous materials in the secretory vacuoles of the sebaceous cells in this disease, as illustrated by Hollman et al. (5), might be interpreted as a lipid material undergoing degradation.



Fig. 7a, b. Ruthenium red-positive granular or flocculent material adjacent to the cell wall is strikingly increased in the intercellular spaces of hair follicle, and is often con-

nected with various fine filamentous structures (F) which may be negative or faintly stained with ruthenium red. Ruthenium red stain. (a) $\times 47\,000$, (b) $\times 58\,000$.

Sebaceous gland was not found in any of the sections of biopsied specimens from this case. The activity of uridine diphosphoglucose dehydrogenase has been demonstrated in immature sebaceous cells of normal skin, while fully differentiated sebaceous cells have been negative by histochemical means, and Malpighian cells of the lower layer have demonstrated a greater activity than the upper layer (10). It is therefore possible that the undifferentiated pilosebaceous cells, rather than the mature ones, might undergo metabolic changes and sebaceous glands might not always be essential for this disorder of the pilosebaceous organ.

ACKNOWLEDGEMENTS

The author thanks Prof. O. Miura for his encouragement, and Mr M. Fukuda and Miss S. Tada for their technical assistance in the electron microscopic procedures.

REFERENCES

1. Braun-Falco, O.: Mucophanerosis intrafollicularis et seboglandularis. *Derm Wschr* 136: 1289, 1957.
2. Fessler, J. H. & Fessler, L. I.: Electron microscopic visualization of the polysaccharide hyaluronic acid. *Proc Natl Acad Sci (USA)* 56: 141, 1966.
3. Fujita, H., Asagami, C., Suetomi, Y., Uchihira, T., Kinoshita, K. & Araki, I.: Study on the ultrastructure and mucin production in pretibial myxedema. *Acta Pathol Jpn* 21: 207, 1971.
4. Helwig, E. B.: Alopecia mucinosa. *In* *Dermal Pathology* (ed. J. H. Graham, W. C. Johnson & E. B. Helwig), p. 785. Harper & Row, Hagerstown, Maryland, 1972.
5. Hollman, K.-H., Verley, J.-M. & Touraine, R.: La mucinose folliculaire. Étude d'un cas au microscope électronique. *Ann Derm Syph* 97: 161, 1970.
6. Ishibashi, A. & Chujo, T.: Ultrastructure of follicular mucinosis. *J Cutan Pathol* 1: 126, 1974.
7. Jensen, C. E. & Carlsen, F.: Hyaluronic acid. VI. An electron microscope study of potassium hyaluronate. *Acta Chem Scand* 8: 1357, 1954.

8. Johnson, W. C. & Helwig, E. B.: Histochemistry of the acid mucopolysaccharides of skin in normal and in certain pathologic conditions. *Amer J Clin Pathol* 40: 123, 1963.
9. Kajikawa, K., Nakanishi, I., Hori, I., Matsuda, Y. & Kondo, K.: Electron microscopic observations on connective tissues using ruthenium red staining. *J Electron Microsc* (Tokyo) 19: 347, 1970.
10. Kamihata, S.: Histochemical study on uridine diphosphoglucose dehydrogenase in certain dermatological applications. Part I. Reinvestigation of the method and the enzymatic activities in normal animal and human skins, and in experimental granulation tissues and L cells. *Japan J Dermat, Ser A* (in Japanese) 75: 548, 1965.
11. Karnovsky, J. J.: A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J Cell Biol* 27: 137, 1965.
12. Kobayasi, T. & Asboe-Hansen, G.: Ultrastructure of generalized scleroderma. *Acta Dermatovener (Stockholm)* 52: 81, 1972.
13. Kobayasi, T., Danielsen, L. & Asboe-Hansen, G.: Hyaluronate (?) microfibrils in human dermis. *Acta Dermatovener (Stockholm)* 51: 27, 1971.
14. Langner, A., Jabłońska, S. & Darżynkiewicz, Z.: Studies on the origin of the mucin in mucinosis follicularis. *Acta Dermatovener (Stockholm)* 49: 76, 1969.
15. Luft, J. H.: The fine structure of hyaline cartilage matrix following ruthenium red fixative and staining. *J Cell Biol* 27: 61 A, 1965.
16. Myers, D. B., Highton, T. C. & Rayns, D. G.: Acid mucopolysaccharides closely associated with collagen fibrils in normal human synovium. *J Ultrastruct Res* 28: 203, 1969.
17. — Ruthenium red-positive filaments interconnecting collagen fibrils. *J Ultrastruct Res* 42: 87, 1973.
18. Orfanos, C. & Gahlen, W.: Elektronenmikroskopische Befunde bei der Mucinosis follicularis. *Arch Klin Exp Derm* 218: 435, 1964.
19. Pinkus, H.: The relationship of alopecia mucinosa to malignant lymphoma. *Dermatologica* 129: 266, 1964.

Received March 10, 1975

A. Ishibashi, M.D.
 Department of Dermatology
 Kyorin University School of Medicine
 Mitaka
 Tokyo 181
 Japan