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UNUSUAL STRUCTURES IN THE EPIDERMAL LANGERHANS' CELLS OF NORMAL HUMAN SKIN

Masaaki Morohashi and Ken Hashimoto[1]

Department of Dermatology, Toyama Medical and Pharmaceutical University School of Medicine, Toyama, Japan, and [1]Department of Dermatology and Syphilology, Wayne State University School of Medicine, Detroit, Michigan, USA

Abstract. Two unusual structures were found in the epidermal Langerhans' cells of normal human adult skin: (1) an intramitochondrial crystalline lattice structure composed of numerous electron-dense, fine particles approximately 90 Å in diameter, with a lucent spacing of nearly 90 Å; (2) bundles of unusual cytoplasmic tubular structures, approximately 120 Å wide.

Key words: Epidermal Langerhans' cell; Intramitochondrial inclusion bodies; Crystalline lattice structure; Cytoplasmic tubular structure

Intramitochondrial inclusions in epidermal Langerhans' cells (L cells) have been previously demonstrated by Thorne, Motatz and Zelickson (18). They reported electron-dense spherical granules within the mitochondria of L cells and dermal mononuclear cells following application of India ink to stripped human skin. Such granules, however, were not seen in the mitochondria of normal L cells.

In the present study, intramitochondrial crystalline lattice structures which are quite different from those reported by Thorne, Motatz and Zelickson were found in epidermal L cells. These intramitochondrial crystalline lattice inclusions and the cytoplasmic tubular structures in the epidermal L cells of normal human skin are described and compared with intramitochondrial inclusions reported in other organs.

MATERIALS AND METHODS

Biopsy specimens were taken under 1% procaine anesthesia from the left arm of a 21-year-old normal, black male. The tissue was immediately sliced into 0.5–1.0 mm thick flakes and fixed in 1% glutaraldehyde in 0.1 M cacaodylate buffer (pH. 7.4) for 1 hour and then rinsed in the same buffer for 1 hour. The specimen was processed for acid phosphatase staining *ad modum* Gomori (6) and then osmicated with 1% osmic acid in the same buffer for 30 minutes. After dehydration through graded concentrations of ethanol and propylene oxide, the tissues were embedded in Araldite. Thin sections cut at 400–600 Å from acid phosphatase-stained tissues were first observed without staining. After confirmation of lead phosphate deposition, the sections were contrasted with 15% uranyl acetate in 50% methanol and Reynolds' lead citrate (17). Control specimens were incubated in medium which did not contain the substrate (sodium-β-glycerophosphate).

RESULTS

Two unusual structures were found in the epidermal Langerhans' cell (L cell) of the normal human skin.

(A) *Intramitochondrial inclusion bodies.* An unusual, crystalline lattice structure was seen in the mitochondrion of an L cell (Figs. 2, 3 B, 3 C). This L cell contained numerous mitochondria (Fig. 1 A, C) and characteristic L cell granules (Figs. 1 A, 1 B, 2) in its cytoplasm. The inclusion-positive mitochondrion (Figs. 1 C, 2, 3 B, 3 C) was approximately 3 μm long and 0.3 μm wide. Though the cristae of this mitochondrion were markedly decreased, the inner and outer mitochondrial membranes were intact. In the vicinity of the mitochondrion an acid phosphatase-positive lysosome was seen (Figs. 1 C, 2). The bulk of both the middle and lower portion of this mitochondrion was filled by this unusual crystalline lattice structure. The repeating units of the crystalline lattice structure, i.e., alternating electron-dense and electron-translucent planes, were composed of numerous electron-dense, fine particles, approximately 90 Å in diameter with a lucent spacing of nearly 90 Å. The inclusion was invested by a membrane, which was not continuous but interrupted. In the other portion of this mitochondrion, cristae can be recognized.

(B) *Cytoplasmic tubular structures.* In the cytoplasm of an epidermal L cell from the same specimen which had the crystalline lattice structure, there were bundles of unusual tubular structures cut

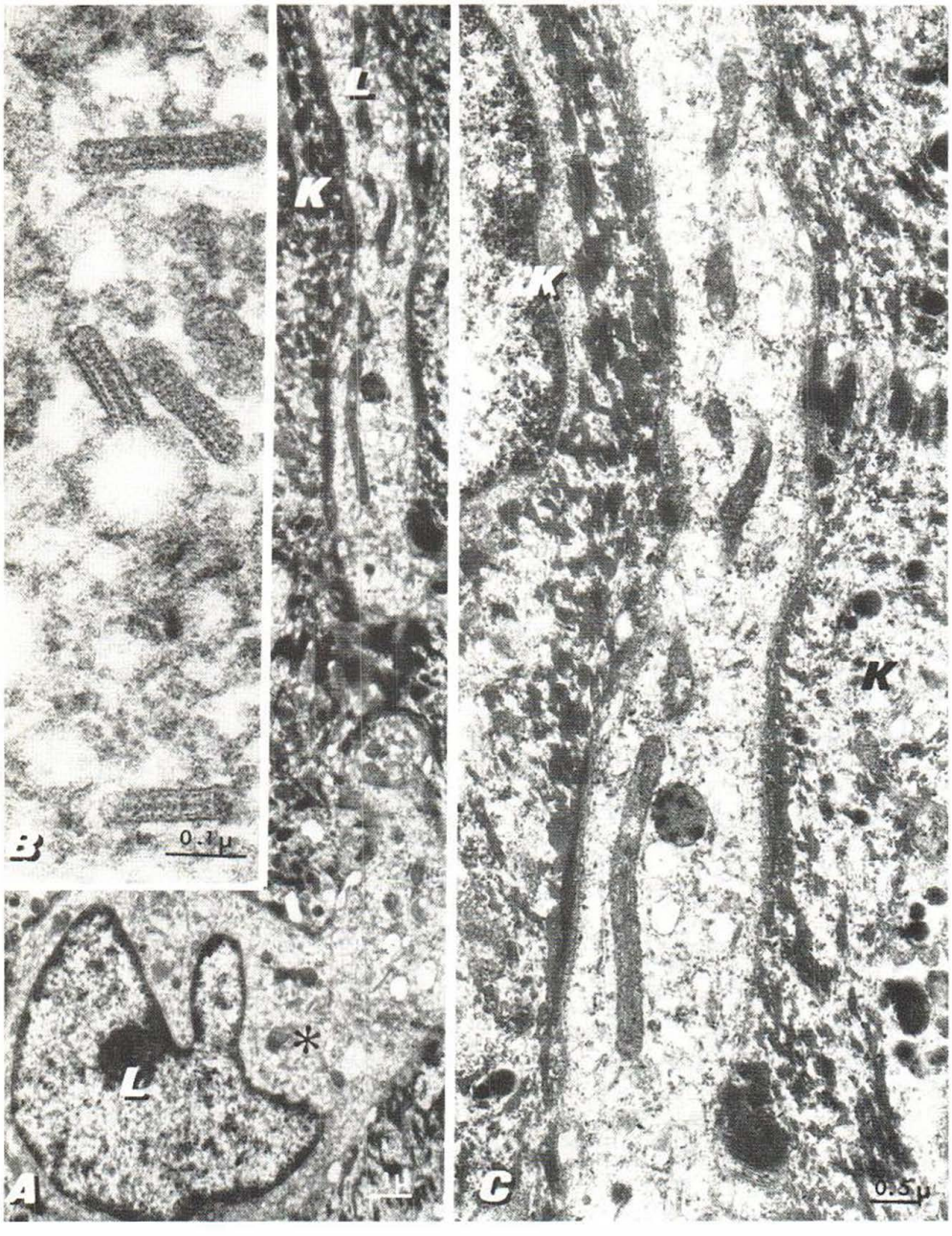

Fig. 1. (A) Low magnification of epidermal Langerhans' cell (L cell). In the upper half of this micrograph, the dendrite of the L cell (*L*) contains a mitochondrion. *K*, keratinocyte. ×6000. (B) Higher magnification of same L cell cytoplasm marked by (*) in (A). Rod-like and bulb-like L cell granules are seen. ×112000. (C) Higher magnification of L cell dendrite shown in (A). ×14000.

transversely, obliquely and longitudinally (Fig. 4). These tubular structures varied in diameter from about 100 to 140 Å and most of them had hollow centers measuring nearly 60 Å in diameter.

DISCUSSION

Intramitochondrial crystalline lattice structure. The presence of intramitochondrial inclusions in the L cell has been demonstrated only by Thorne,

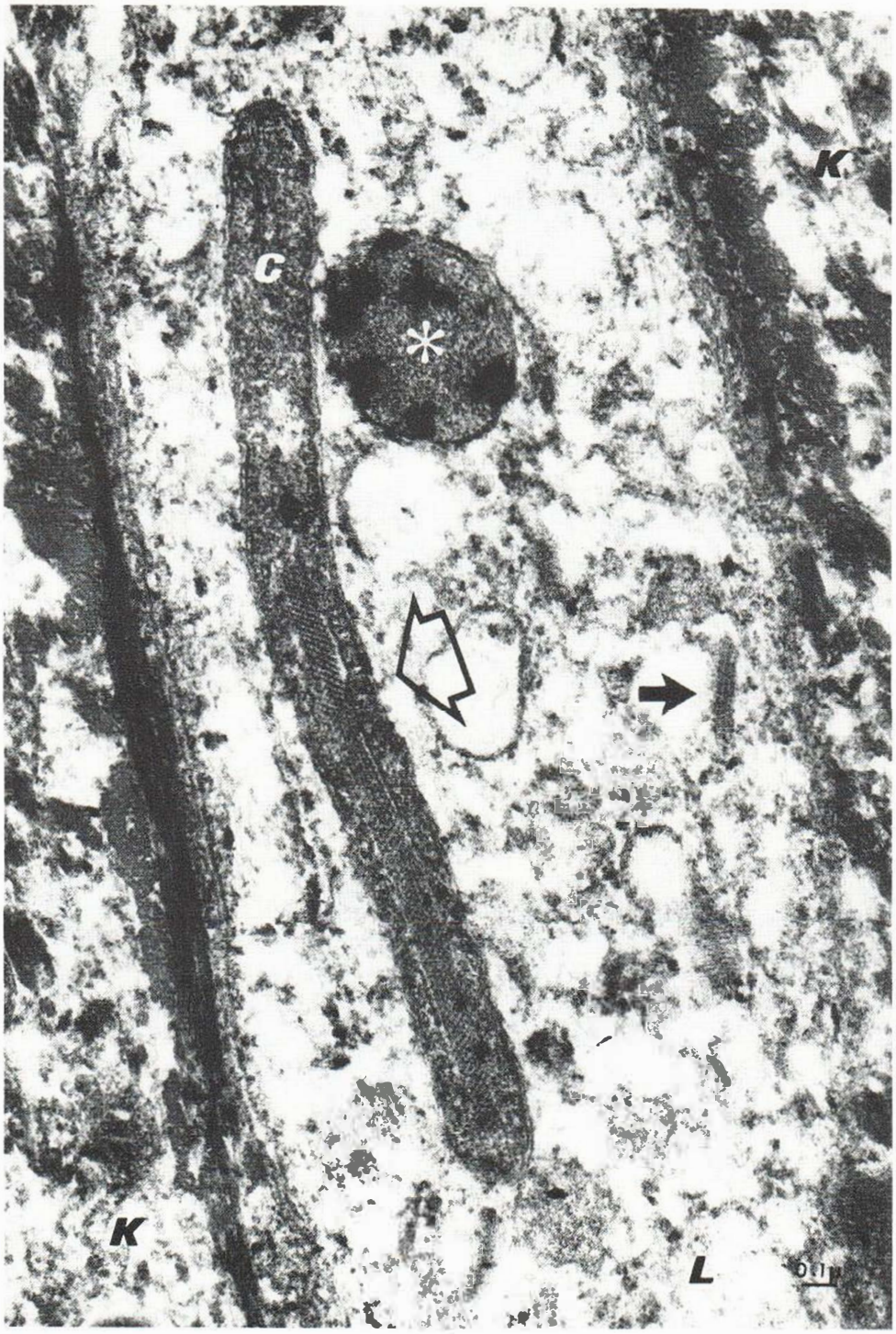

Fig. 2. Higher magnification of Fig. 1 C. An unusual crystalline lattice structure is seen in the mitochondrion (large hollow arrow). A small solid arrow indicates the L cell granule. In the vicinity of the mitochondrion a lysosome (*) is seen. This lysosome is stained positive for acid phosphatase (dense particles of lead phosphate). *C*, cristae; *K*, keratinocyte. ×50 000.

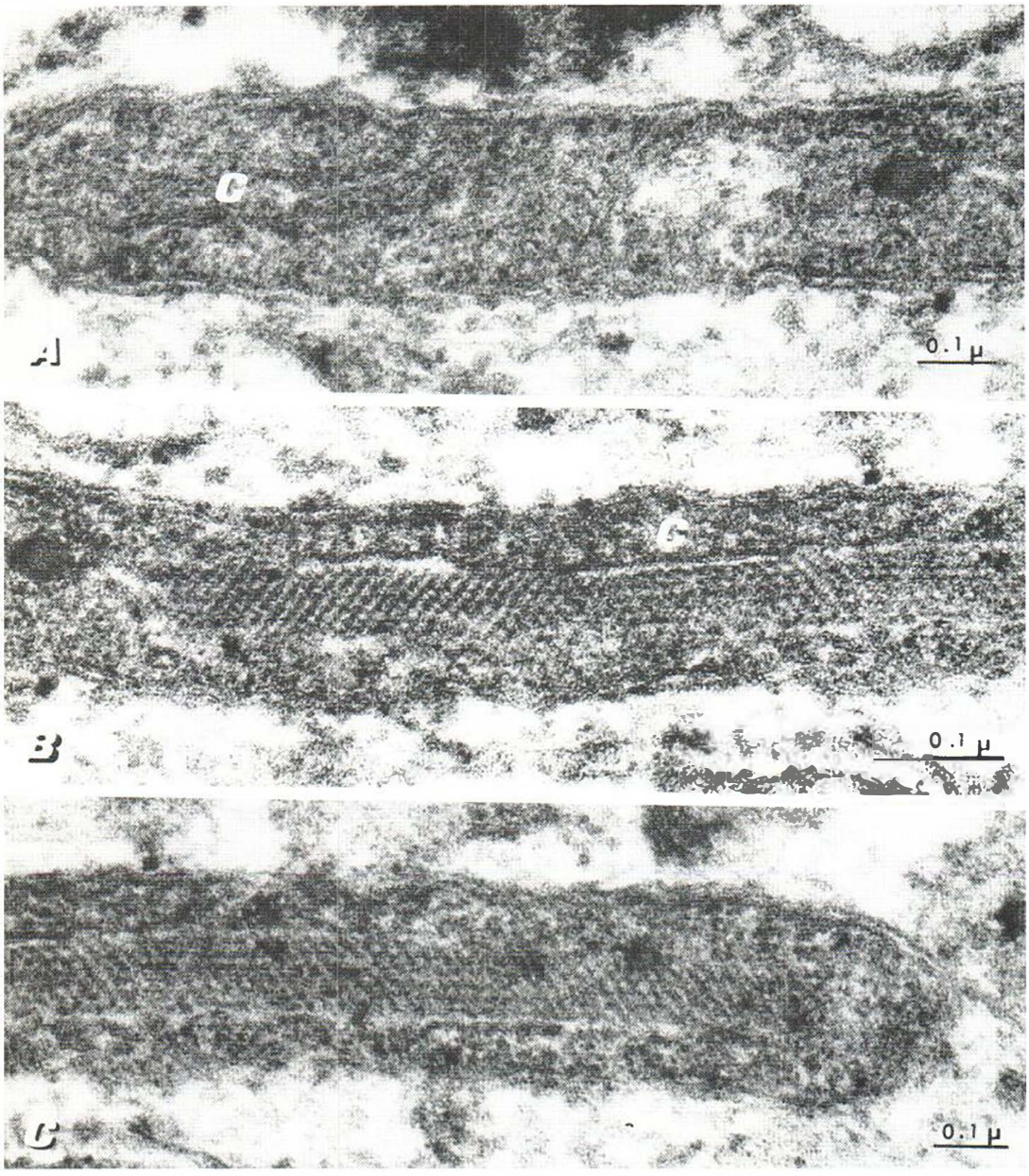

Fig. 3. Higher magnification of mitochondrion in Fig. 2. Though the cristae of this mitochondrion (A) are markedly reduced, one mitochondrial crista (*C*) is recognizable. Most of both the middle (B) and lower portion (C) of the mitochondrion is filled by this unusual crystalline lattice structure which is composed of electron-dense, fine particles (90 Å) in register to form a lattice work with a lucent spacing of about 90 Å. A, B, C: ×115 000.

Motatz, and Zelickson (18). These inclusions, however, appeared to be quite different from those of the present studies. According to their findings, numerous electron-dense, spherical granules (approximately 1000 Å) were found within the mitochondria of both epidermal L cells and dermal mononuclear cells following application of India ink to the stripped human skin. Such granules, however, were not seen in mitochondria of either cell unless ink was applied. Therefore, they postulated

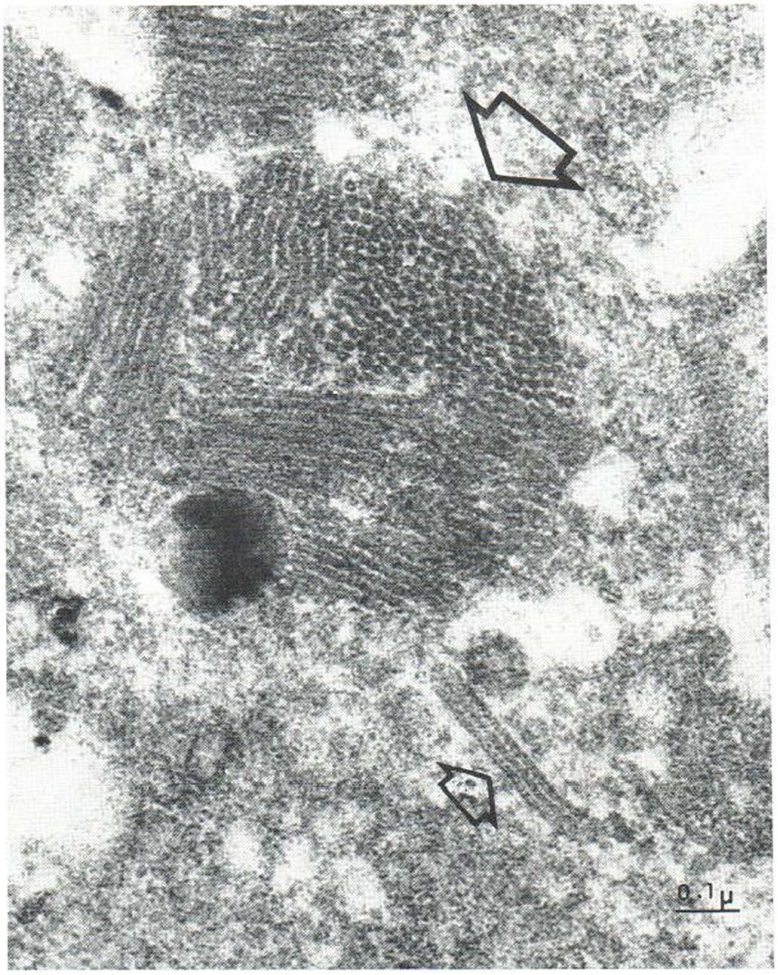

Fig. 4. In the cytoplasm of an epidermal L cell, there are bundles of unusual tubular structures cut transversely, obliquely and longitudinally. In the longitudinal section, the tubular structure is approximately 120 Å wide. ×90 000.

a cytotoxic effect of India ink on the mitochondria. In view of the recent concept that L cells belong to the monocyte–histiocyte–phagocyte group of cells (7, 12) rather than to melanocytes (4, 19), their finding that mitochondrial dense granules were seen in both cell types is interesting.

In the present study, however, intramitochondrial crystalline lattice structures were found only in the epidermal L cells—not in any other cell type, including macrophages.

The similarities in structure between the crystalline lattice and those of the internal structures of the L cell granules (7) are of interest. The nature and function of these mitochondrial inclusions are unknown.

Intramitochondrial paracrystalloid inclusions have been described in several human organs in various liver diseases (1, 5), disturbances of lipid metabolism (1, 11) and myopathy (3, 16). Of these inclusions, the most frequent were those showing fibrillar of filamentous subunits. As regards lattice-form inclusion of the skin, intranuclear (14) and intralysosomal (20) inclusions appear somewhat similar to our structure.

In the paracrystalloid mitochondrial inclusions of hepatocytes, which some authors describe as proteinaceous or phospholipidic in nature, enzymes such as succinodehydrogenase and cytochrome oxidase have been found (1). Some inclusions were derived from the membrane of cristae and were related to disordered phospholipid metabolism in mitochondria (15). None of these inclusions resembles the one reported in this study.

Cytoplasmic tubular structure. In the cytoplasm of the epidermal L cell, there were bundles of unusual tubular structures cut transversely and lon-

gitudinally. The aggregation of these tubular structures has not been described previously in the cytoplasm of L cells. Tubular structures described in the skin lesions of discoid (8) and systemic lupus erythematosus (9, 13) and other autoimmune diseases such as dermatomyositis (10) have a larger diameter (200 Å), and are curved and branched. These have been described only in abnormal conditions and are not found in the normal epidermis.

Although there is some difference between the size of the cytoplasmic tubular structures and microtubules, the structure do resemble microtubules, which have a diameter of 200 to 270 Å and a wall 50 to 70 Å thick (2). Microtubules are very important components of the cytoplasm, being essential for cell division, involved in maintenance of cell shape, and of importance in the movements of organelles and inclusions (2).

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REFERENCES

1. Bhagwat, A. G. & Ross, R. C.: Hepatic intramitochondrial crystalloids. Arch Pathol *91:* 70–77, 1971.
2. Bloom, W. & Fawcett, D. W.: A Textbook of Histology. 10th ed. Philadelphia, W. B. Saunders Company, 1975.
3. Bradley, W. G., Hudgson, P., Gardner-Medwin, D. & Walton, J. N.: Myopathy associated with abnormal lipid metabolism in skeletal muscle. Lancet *i:* 495–498, 1969.
4. Breathnach, A. S.: A new concept of the relation between the Langerhans cell and the melanocyte. J Invest Dermatol *40:* 279–281, 1963.
5. Gomez-Lázaro, G., Caro-Paton, A., Prieto, J. & Coca, M. C.: Intramitochondrial paracrystalline inclusions in hepatocytes of patients with biliary lithiasis. Lancet *ii:* 1417–1418, 1972.
6. Gomori, G.: An improved histochemical technique for acid phosphatase. Stain Technol *25:* 81–85, 1950.
7. Hashimoto, K. & Tarnowski, W. M.: Some new aspects of the Langerhans cell. Arch Dermatol *97:* 450–464, 1968.
8. Hashimoto, K. & Thompson, D. E.: Discoid lupus erythematosus. Electron microscopic studies of paramyxovirus-like structures. Arch Dermatol *101:* 565–577, 1970.
9. Hashimoto, K. & Chandler, R. W.: Paramyxovirus-like inclusions in systemic lupus erythematosus. Acta Dermatovener (Stockholm) *52:* 263–277, 1972.
10. Hashimoto, K., Robinson, L., Velayos, E. & Niizuma, K.: Dermatomyositis. Electron microscopic, immunologic and tissue culture studies of paramyxovirus-like inclusions. Arch Dermatol *103:* 120–135, 1971.
11. Kovacs, K., Lee, R. & Little, J. A.: Ultrastructural changes of hepatocytes in hyperlipoproteinemia. Lancet *i:* 752–753, 1972.
12. Morales, A. R., Fine, G., Horn, R. C. Jr. & Watson, J. H. L.: Langerhans cells in a localized lesion of the eosinophilic granuloma type. Lab Invest *20:* 412–423, 1969.
13. Norton, W. L.: Endothelial inclusions in active lesions of systemic lupus erythematosus. J Lab Clin Med *74:* 369–379, 1969.
14. Straile, W., Tipnis, U. R., Mann, S. J. & Clark, W. H.: Lattice and rodlet nuclear inclusions in Merkel cells in rabbit epidermis. J Invest Dermatol *64:* 178–183, 1975.
15. Svoboda, D. J. & Manning, R. T.: Chronic alcoholism with fatty metamorphosis of the liver. Mitochondrial alterations in hepatic cells. Am J Pathol *44:* 645–662, 1964.
16. Sulaiman, W. R., Doyle, D., Johnson, R. H. & Jennet, S.: Myopathy with mitochondrial inclusion bodies. Histological and metabolic studies. J Neuro Neurosurg Psychol *37:* 1236–1246, 1974.
17. Reynolds, E. S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol *17:* 208–212, 1963.
18. Thorne, E. G., Mottaz, J. H. & Zelickson, A. S.: The occurrence of distinctive intramitochondrial granules in epidermal Langerhans cells and dermal mononuclear cells. Acta Dermatovener (Stockholm) *51:* 331–334, 1971.
19. Zelickson, A. S.: The Langerhans cell. J Invest Dermatol *44:* 201–212, 1965.
20. Zelickson, A. S.: Melanocyte, melanine granule and Langerhans' cell. *In* Ultrastructure of Normal and Abnormal Skin (ed. A. S. Zelickson), pp. 163–182. Lea & Febiger, Philadelphia, 1967.

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M. Morohashi, M.D.
Department of Dermatology
Toyama Medical and Pharmaceutical University
School of Medicine
2630 Sugitani
Toyama 930-01, Japan