# The Ultrastructural Study in a Case of Fabry Disease

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**Abstract**

An investigation of the ultrastructural study was conducted on specimens from a typical patient with Fabry disease. Numerous characteristic cytoplasmic inclusions were observed in the vascular endothelial cell, pericyte, smooth muscle cell, nerve and eccrine sweat glands, the lamellar pattern of which were considerably variable in various types of gland cells. Large vacuolar inclusions predominated in clear cells of the secretory coil; lesser vacuoles were also seen in the coiled duct, and the basal cells of the straight duct toward the coiled duct displayed mulberry-like figures. There were some clear cells showing cell damage and necrosis in the secretory coil. Lamellated bodies were noted in the axons and schwann cells around the eccrine sweat glands. The small blood vessels around the eccrine glands were narrowed by swollen endothelial cells with heavy inclusions. These intracytoplasmic deposits may be responsible for the decreased sweating ability in Fabry disease. The factors related to hypohidrosis are also discussed.

**Key word** : Fabry Disease, Ultrastructural Study

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Fabry disease, an inborn error of glycosphingolipid metabolism, results from the defective activity of the lysosomal enzyme, α-galactosidase A. The enzymatic defect, transmitted by an X-linked recessive gene, leads to the progressive deposition of neutral glycosphingolipids (predominantly globotriaosylceramide) with terminal α-galactosyl moieties in most visceral tissues and fluids of the body[1,2]. Although the target cells of the storage process seem to be endothelial cells throughout the body[3], the involvement of organs varies greatly among patients. The birefringent glycosphingolipids are primarily in

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the lysosomes of the vascular endothelium, in perithelial and smooth muscle cells of the cardiovascular-renal system, and to a lesser extent, in reticuloendothelial, myocardial and connective-tissue cells of the cornea, kidney, and other tissues, and in ganglion and perineural cells of the autonomic nervous system. Mild to severe hypohidrosis, is one of the typical cutaneous manifestations of Fabry disease[4]. Inclusions in secretory cells of the eccrine sweat glands have been reported in patients with Fabry disease showing severe hypohidrosis or generalized anhidrosis[5], but the ultrastructural characteristics of the eccrine sweat glands in Fabry disease have not been fully identified, and the mechanism underlying the inability of Fabry disease patients to sweat remains unclear[6]. The ultrastructural characteristics study of this patient provides detailed documentation which confirms earlier findings, adds some previously unreported electron microscopic studies on eccrine sweat glands, and helps to clarify the possible pathological association of lysosomal inclusions with the diminished sweating ability of patients with Fabry disease.

## MATERIAL AND METHOD

### Case report

A 34-year-old man was referred to the dermatology skin department with a 9-month history of multiple red, painless nodules on his right buttock. His skin had been dry and rough for many years. There was no family history of individuals with similar dermatologic findings.

On physical examination, scattered, dark-red, punctuated, telangiectatic eruptions were seen on the lower abdominal region, waist, and buttocks. Slight cardiomegaly was detected, and other laboratory test results including serum hepatic and renal function studies were normal; therefore, the patient's diagnosis was confirmed as Fabry disease.

### Electron microscopy

The cutaneous lesion was excised when the nodule on the patient's right buttock area was operated on, and examined by light and electron microscopy. The tissue for light microscopy was fixed in formalin and embedded in paraffin, and 4 μm sections were stained with hematoxylin and eosin. For routine electron microscopic observation, tissues were fixed in 2.5 per cent glutaraldehyde buffered with phosphate (pH 7.4) at 4°C and postfixed in 1 per cent osmium tetroxide in the same buffer for 1 h. After dehydration in a series of graded concentrations of alcohol, the tissues were embedded in Epon. The 1 μm semithin sections were stained with toluidine blue to select appropriate areas for study. Ultrathin sections were cut by an ultratome with a diamond knife, double stained with uranyl acetate and lead citrate, and examined and photographed under an electron microscope (H-600, Hitachi) at an accelerating voltage of 75 kV.

## RESULTS

The light microscopic examination of the skin lesions revealed dilated subepidermal capillaries which may be enclosed by the hyperplastic epidermis. There was moderate dilatation of the more deeply situated vessels of the skin. There were fibrinous thrombi showing partial organization within some of the dilated capillaries. Large cytoplasmic vacuoles were seen in the endothelial cell, pericytes and eccrine sweat glands in subcutaneous tissue with toluidine blue stains (Fig. 1). No striking findings were shown upon examination of the nodule on the patient's right buttock area.

On electron microscopic examination of the skin, large electron-dense lipid deposits were seen to be present in endothelial cells, pericytes and fibroblasts. They may be seen also in arrector pilorum muscles and in the secretory, ductal, and myoepithelial cells of eccrine glands. On high magnification, these deposits showed a lamellar structure primarily of the zebra body type with a periodicity of 5 nm that is diagnostic for the intracytoplasmic inclusions of Fabry's disease. Many of the inclusions are rounded by a membrane, but others are not. The inclusions with whorls, parallel and fingerprint-like pattern were also seen. At high resolution, the typical pattern of concentric lamellar inclusions with alternating light and dark-staining bands was observed. Large dense bodies substantially decreased the size of the lumen of many small blood vessels in the lower dermis (Fig. 2), including the capillaries and small veins around the eccrine sweat glands. In the secretory portion of eccrine sweat gland, numerous polymorphic dense inclusions were present in the cytoplasm of clear cells, which consisted of parallel arrays, concentric lamellae, a fingerprint-like pattern, and fine granular or amorphous materials, but the most striking feature was that these clear cells containing the dense inclusions were extensively vacuolated. The vacuoles varied in size and shape, and were often delimited by membranes. Some vauoles were partially filled with

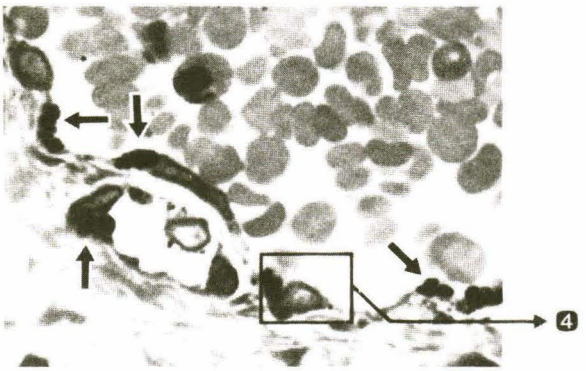

Fig. 1. Photograph of 1 μm Epon section of vascular endothelial cell in subcutaneous tissue from the present patient with Fabry disease, demonstrating the dense granular in vascular endothelial cell (Toluidine blue, x 400).

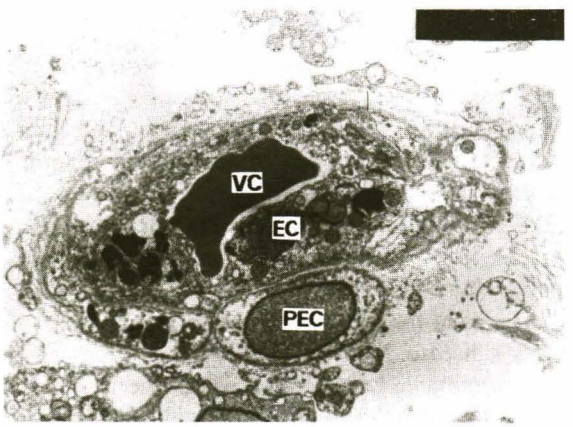


Fig. 2. Electron micrograph showing the swollen endothelial cells with heavy inclusions. the inclusion exhibited whorls, parallel and fingerprint-like patterns. VC = Vascular channel, EC = Endothelial Cell, PEC = Pericyte (x 27,000).

laminated bodies, suggesting that the empty vacuoles were once occupied by laminated bodies and might have been dissolved during fixation; some vacuoles were so large that they occupied almost the entire cytoplasm of clear cells, and the nuclei were often displaced to the cell periphery (Fig. 3 and Fig. 4). Fusion between vacuoles and between vacuoles and

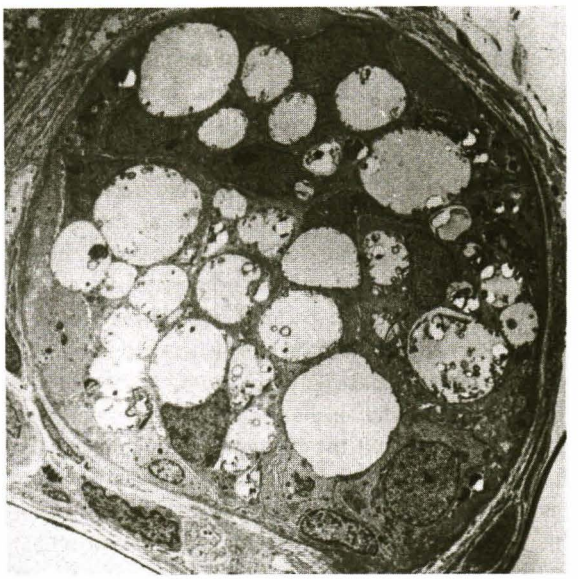

Fig. 3. The secretory segments of the eccrine sweat gland were filled with many vacuoles, some of which were so large that they occupied almost the entire cytoplasm, and displacing the nuclei to the cell periphery (x 2,000).

lamellar inclusions was observed. A relative scarcity of Golgi complex was found in the clear cells, they seemed to be normal in appearance, basal infoldings were undamaged, a few primary lysosomes were occasionally found in the cytoplasm of the clear cells, while some mitochondria exhibited pronounced swelling and even vacuolization. The degeneration of the clear cells was obvious in some secretory coils; the integrity of cytoplasmic membrane and the cytoplasmic structure were lost and the nuclei appeared pycnotic, but the lamellated inclusions were preserved intact (Fig. 5). A few small vacuoles were seen in the dark cells in the same segment. The vacuoles and lamellar lipid granules were observed in the ductal and secretory cells particularly in the basal cells (Fig. 6). However, these vacuoles differed in appearance from those found in the secretory coil; they were obviously smaller in size and almost empty. The changes seen in the basal cells of the straight ducts toward the coiled duct consisted of mulberry-like inclusions in the cytoplasm (Fig. 7). Although the residue of inclusions was frequently observed in the intercellular canaliculi of the secretory portion (Fig. 8), the structure of intercellular canaliculi seemed to be intact. Around the eccrine sweat glands the

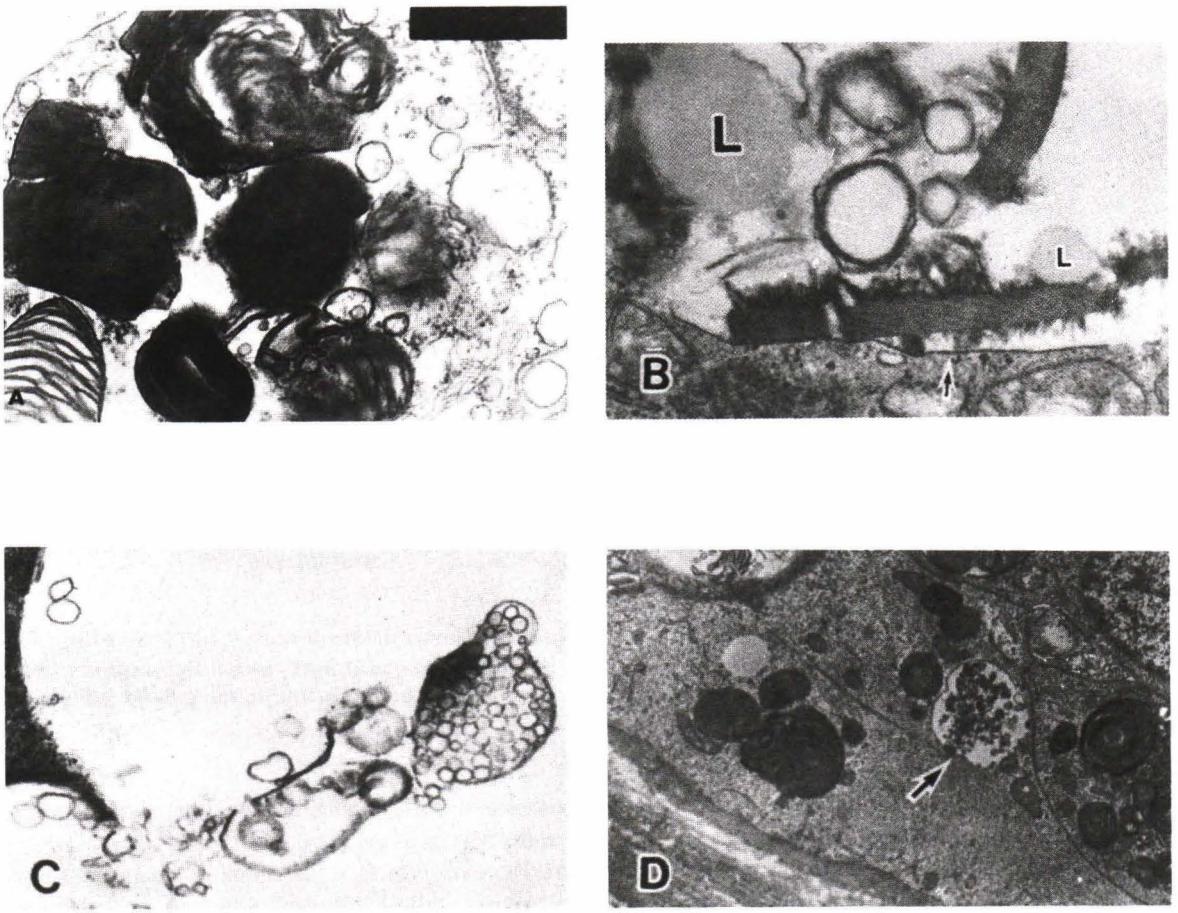


**Fig. 4. Secretory segment showing the polymorphic inclusions. A. Clear cells with a vacuole containing concentric lamellae material (x 140,000). B. Vacuole in a clear cell containing parallel array inclusions and lipid droplet (L). (x 150,000). C. Fish egg-like inclusion in vacuole (x 20,000). D. Myoepitheliel cell with concentric bodies and vacuole containing the granular material (arrow) (x 8,000).**

lamellated and myelin figure- like inclusions could be found in the cytoplasm of schwann cells (Fig. 9), but these schwann cells and axon appeared morphologically normal.

## DISCUSSION

Fabry disease is characterized by a deficiency of lysosomal enzyme α–galactosidase A[3]. The glycosphingolipid is deposited in all areas of the body, occurring predominantly in the lysosomes of endothelial, perithelial, and smooth muscle cells of blood vessels and, to a lesser degree in histiocytic and reticular cells of connective tissue. Lipid deposits are also prominent in epithelial cells of the cornea and glomeruli and tubules of the kidney, in muscle fibers of the heart, and in ganglion cells of the autonomic system[7]. The present investigation confirmed the previous observation that electron-dense, laminated inclusions occurred within the eccrine sweat glands in Fabry disease patients with hypohidrosis or anhidrosis[5]. Interestingly, compared with that in the cytoplasm of endothelial cells, the lamellar pattern of the osmiophilic inclusions was more variable in the cytoplasm of the eccrine sweat gland cells in the present patient. In addition, along the length of the eccrine sweat glands from the secretory segment to the duct segment, the inclusions not only displayed pleomorphism in fine structure, but also showed a continued reduction in size. The former finding is probably due to differences in the chemi-

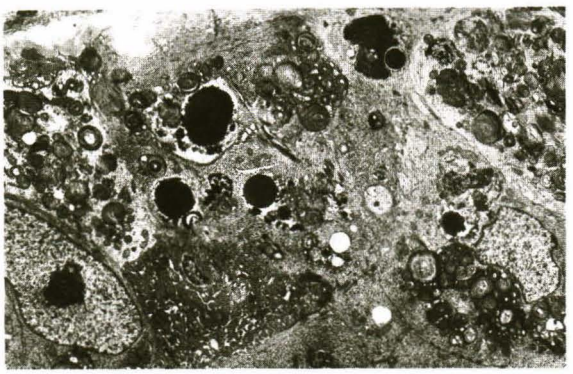

**Fig. 5. Note the degeneration of clear cells in the secretory portion, with loss of cytoplasmic structure and nuclear pycnosis (x 5,000).**

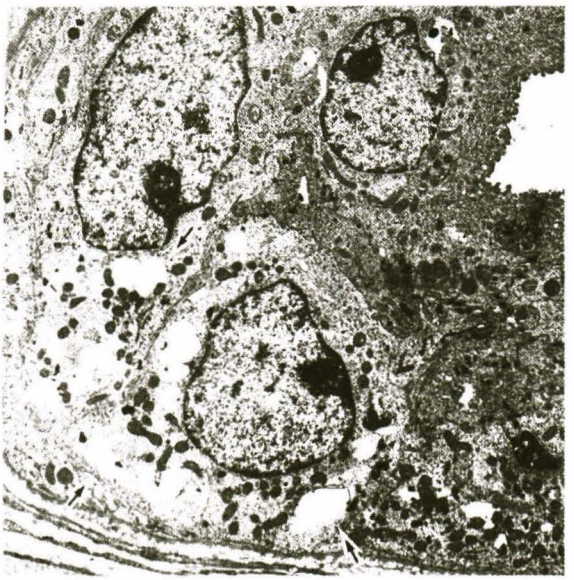

**Fig. 6. Numerous small vacuole (arrows) in the cells of the coiled duct toward the secretory coil, particularly in the basal cells (x 8,000).**

cal composites making up the inclusions in the various types of cells, while the latter finding may be ascribed to the difference of pH in the cytoplasm of different segments of eccrine sweat glands. Glycosphingolipids are degraded in a step-wise fashion by a family of specific exoglycosidases including α-galactosidase A[8].

Some authors have speculated that the decreased sweating in Fabry disease is due mainly to an abnormality at any level in the nerve pathway or to an inability of the secretory glands to form sweat [6]. Various factors have been considered as a cause of hypohidrosis in Fabry disease, but it remains unclear as to whether the inclusion itself directly destroys the sweat glands, thereby causing hypohidrosis, of whether peripheral and autonomic dysfunction is primarily involved. Evidence of impaired autonomic function was found in Fabry cases[9], and lipid-stained inclusion have been noted in the peripheral autonomic ganglia[10]. Unmyelinated and small myelinated fibers were selectively decreased [11,12], some fibers appeared to be undergoing wallerian degeneration. Though the authors could not perform any further study to confirm the damage of cholinergic or adrenergic nerves controlling eccrine sweating, observation confirmed that the inclusions existed in the schwann cells around the eccrine sweat glands. The accumulation of these inclusions may have a toxic effect on the neurons and ultimately destroy the cells[13] some authors have, therefore, suggested that the pathogenic condition might be located in the descending nerve fibers, causing peripheral or/and central autonomic dysfunction, resulting in the decreased sweating ability.

Regarding eccrine sweat glands, the present findings are rather similar to those demonstrated by Lao et al[5], i.e., that most of the eccrine sweat gland cells contain numerous lipid inclusions, and the large vacuole-like inclusions occupy almost all cytoplasms of the clear cells and the main secretory cells. These morphological changes influence the sweating function to some degree. Another interesting finding of the present study is that some of the intracytoplasmic inclusions were excreted into the intercellular canaliculi, suggesting that inclusions were released through a ruptured cell membrane. Large ruptures of the cell membrane may kill the gland cells, resulting in cell death[14], as shown in Fig. 5.

In addition to the eccrine sweat gland itself and its descending nerves, it is probable that an alteration of the blood supply is also related to hypohidrosis. To the authors' knowledge, the present des-

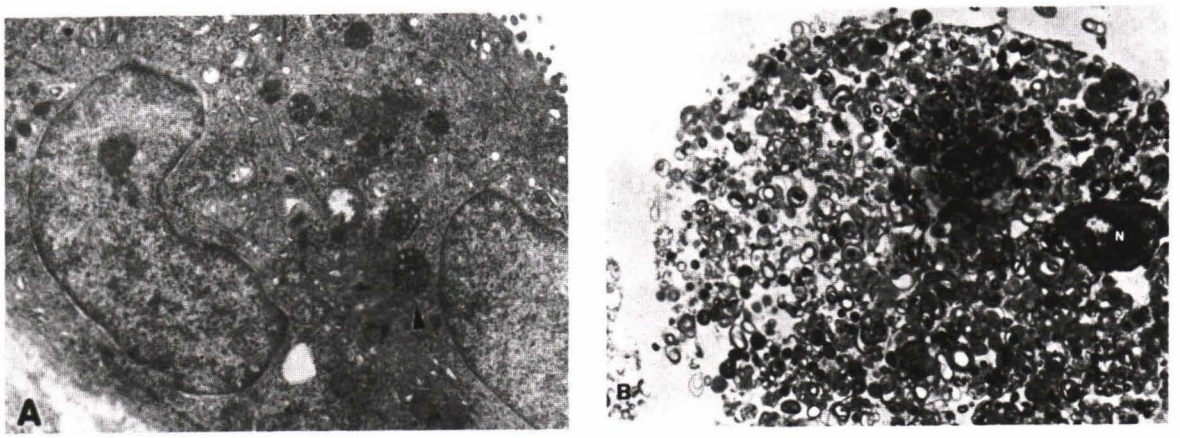


Fig. 7. A. Dense bodies (arrowhead) in a basal cell of the straight duct toward the coiled duct (x 8,000). B. High magnification of the body showing the mulberry-like appearance (x 80,000).

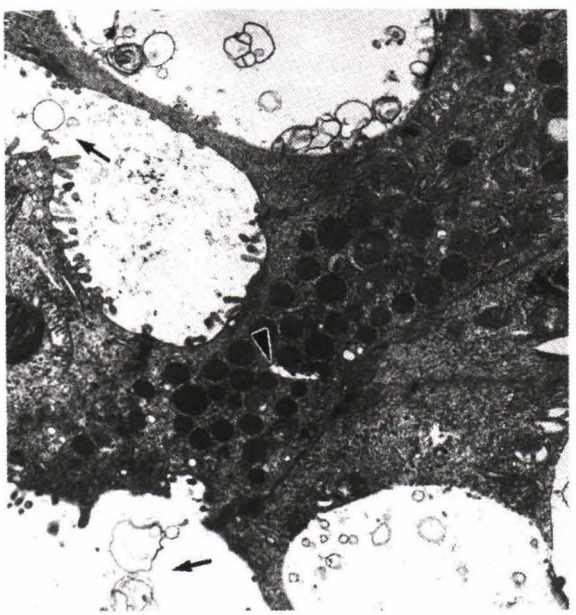

Fig. 8. Excretion of the loosely bound lamellae (arrows) into the intercellular canaliculi (C), and small vacuole (arrowhead) in the dark cell (x 10,000).

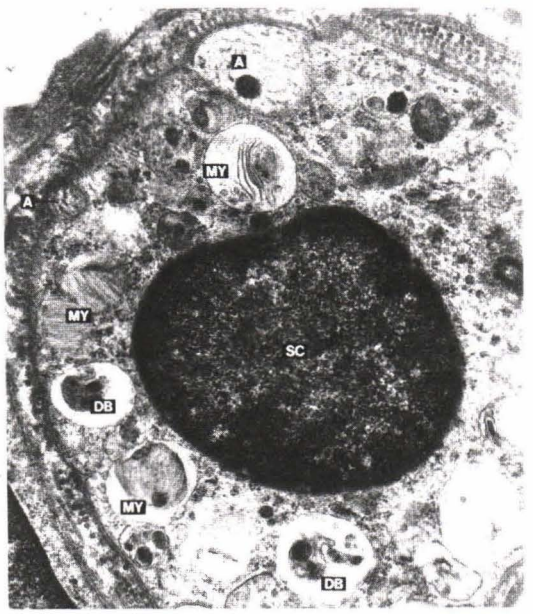


Fig. 9. Lamellated bodies (DB) and myelin figure (My) in the Schwann cell close to the vacuolated eccrine sweat gland, A = Axon (x 4,000).

cription of this relationship is the first such report. A capillary network surrounds each sweat gland, transports water, electrolytes, oxygen and other nutrients to the secretory glands. The present electron microscopy revealed widespread accumulation of concentric lamellar inclusions in the lysosomes of the vascular endothelium. The progressive lysosomal deposition of the glycosphingolipid substrate leads to the narrowing and eventual occlusion of the vascular lumen. An excessive accumulation of lysosomal deposits may injure the endothelial cells and lead to the occlusion of the vascular lumen(15), subsequently reducing the blood flow around the eccrine glands. These changes would eventually interfere with the

formation of primary sweat and the nutrition of the eccrine gland cell itself, causing the dysfunction of sweating.

It remains to be studied further, however, if it is reasonable to postulate that the occurrence of impaired sweating ability in Fabry disease patients results concurrently from the sweat glands themselves, is rendered inactive by the lipid deposition and peripheral and/or central autonomic dysfunction, as well as a poor supply of fluid and nutrients from the involved vessels, which are all secondary to the storage of glycosphingolipids in all areas of the body.

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# จุลทรรศน์อิเล็คตรอนในผู้ป่วยโรค Fabry 1 ราย

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จากการศึกษาจุลทรรศน์อิเล็คตรอนในผู้ป่วยโรค Fabry 1 ราย พบลักษณะของ lamellar inclusion จำนวนมากภายในผนังหลอดเลือดเอ็นโดทีเลียล เพอริไซต์ กล้ามเนื้อเรียบ ต่อมเหงื่อ axon และ Schwann cell บางแห่งพบลักษณะ vacuolar inclusion บริเวณ clear cell ของต่อมเหงื่อ และท่อต่อมเหงื่อ พร้อมลักษณะของการทำลายเซลล์ร่วมด้วย หลอดเลือดเล็ก ๆ ของต่อมเหงื่อพบว่าตีบแคบลง ซึ่งเข้าใจว่าเป็นผลจากการสะสมของ inclusion เหล่านี้ภายในไซโตพลาสซึม จากการศึกษาในครั้งนี้ทำให้สามารถอธิบายถึงสาเหตุของการพบปริมาณเหงื่อซึ่งลดน้อยลงในโรค Fabry

**คำสำคัญ** : โรคแฟบรีย์, จุลทรรศน์อิเล็คตรอน

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