Pathohistological and Ultrastructural Study of Systemic Vasculopathy (Fabry Disease)

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Pathomorphological analysis of skin biopsy specimens from patients with Fabry disease showed edema and mucoid swelling of vascular walls in the skin, pronounced telangiectasias, endotheliocyte degeneration and death, compensatory proliferation of pericytes, and mast cell hyperplasia. Ultrastructural study revealed transformation of vascular cells (endotheliocytes and pericytes) into depocytes accumulating large specific polymorphic granules of varying electron density with fine regular striation, which is pathognomonic for Fabry disease. The complex of these structural changes is interpreted as manifestation of systemic vasculopathy.

Key Words: *diffuse angiokeratoma; skin biopsy; endothelium; optic and electron microscopy*

Different pathways of cell death (necrosis, apoptosis, terminal differentiation) and their role in the pathomorphogenesis of many diseases are now intensively studied. Cell death induced by metabolic disorders during storage diseases, *e.g.* hemochromatosis and Wilson—Konovalov diseases, attracts less attention. A form of glycolipidoses, Fabry disease, is poorly studied [2,4]. Fabry disease (diffuse angiokeratoma) [1,3, 5,6] is a hereditary glycolipidosis from a group of enzymopathies. The genetic defect is linked with X chromosome and is inherited by the recessive type [2]. The key element of pathogenesis is a deficiency of α-galactosyl hydrolase or ceramide trihexoside-α-galactosidase (lysosomal enzyme), as a result of which glycolipids accumulate in various cells, primarily in vascular cells, which leads to systemic involvement of organs and tissues.

The aim of this work was pathohistological and electron-microscopic study of diffuse angiokeratoma.

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MATERIALS AND METHODS

Skin biopsy specimens from patients with Fabry disease were examined. Paraffin sections for light microscopy were stained with hematoxylin and eosin in combination with Perls reaction, by Van Gieson method with post-staining of elastic fibers with Weigert resorcin-fucsine and after Giemza, and periodic acid-Schiff (PAS) test with azur-eosin was carried out. Semithin sections made from blocks embedded in epon-araldite mixture were stained with Schiff reagent and azur II. Ultrathin sections for electron microscopy were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope.

RESULTS

Clinical manifestation of Fabry disease [4] are skin changes (violet crimson-colored angiomas near the navel, on the abdomen, hips; dry skin), causalgic pains in toes, high erythrocyte sedimentation rate, fever, arterial hypertension, decreased tone of the small arteries, deceleration of bloodflow in small arteries and venules, decreased blood supply to the lower extremities and left arm. Biomicroscopy of the eye conjunctiva showed pronounced microcirculatory disorders (twis-

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ted veins, microaneurysms, petechial hemorrhages), aggregation and bloodflow deceleration in limbic and conjunctival capillaries. A drop of leukocytic α-galactosidase activity, pathognomonic for Fabry disease, was diagnosed.

In skin biopsy specimens the epidermis was characterized by notable polymorphism because of alternating foci of acanthosis and atrophy, with predominance of the latter. Degenerative changes in the epidermis were diffuse: karyopyknosis, perinuclear devastation of the cytoplasm, a drop of glycogen content. Fibrous carcass of the derma was formed by fine bundles of collagen fibers and elastic structures with minimally changed tinctorial characteristics. Pronounced degenerative changes in the epithelium of sweat glands, foamy transformation of cells in sebaceous glands, and marked degeneration of all components of the hair bulbs were seen.

Capillaries of the papillary layer and vascular elements of the surface network were extremely heterogeneous. Their walls were thickened, edematous, sharply PAS-positive, somewhere with mucoid swelling. The lumens of some capillaries were visually undetectable because of hyperplasia of the vascular cell; other capillaries, particularly subepidermal, formed large plethoric telangiectasias. The endothelial compartment in general was characterized by sharply pronounced degradation (degeneration and desquamation) and regeneration processes (proliferation, hyperplasia). Endotheliocytes were often hypertrophic, abnormally shaped, with irregular contour of the luminal surface; some cells were sharply thinned, with unclear structures. Analysis of semithin sections showed large cytoplasmatic azurophilic heterogeneous round incorporations in almost all endotheliocytes; these incorporations formed groups, sometimes chains, more often filling the entire cytoplasm (Fig 1).

Perivascular cells were represented by pericytes and numerous mast cells, some cells were degranulated; fibroblasts were also seen. Pericytes were photooptically heterogeneous, but most of them contained azurophilic granules. Vascular cells sometimes formed branching cords, which reflected angiocapillarogenesis processes. The changes were less expressed in arterioles of the deep vascular network; moderate degeneration of vascular cells, edema, and mucoid swelling of the vascular wall and mononuclear perivascular infiltration predominated.

Electron microscopy showed that the majority of endotheliocytes in the surface capillary network were hypertrophic, with euchromic twisted nuclei, thickened peripheral processes, macro- and microclasmatosis signs, which made the relief of the luminal surface peculiar. Large polymorphic granules heterogeneous by electron density (Fig. 2, *a*, *b*), not always surrounded by the membrane, were located perinuclearly. A more than ×40,000 magnification showed lamellar granules with regular striation (straight parallel membrane-like structures at a distance of 50-60 E) against the background of clarifications and osmiophilic zones (Fig. 2, *c*). Some granules looked like large residual bodies with thick concentrical osmiophilic membranes. The granules accumulated and migrated into the supranuclear zone and peripheral processes of endotheliocytes, sometimes potentiating the formation of luminal processes of the cytoplasm with

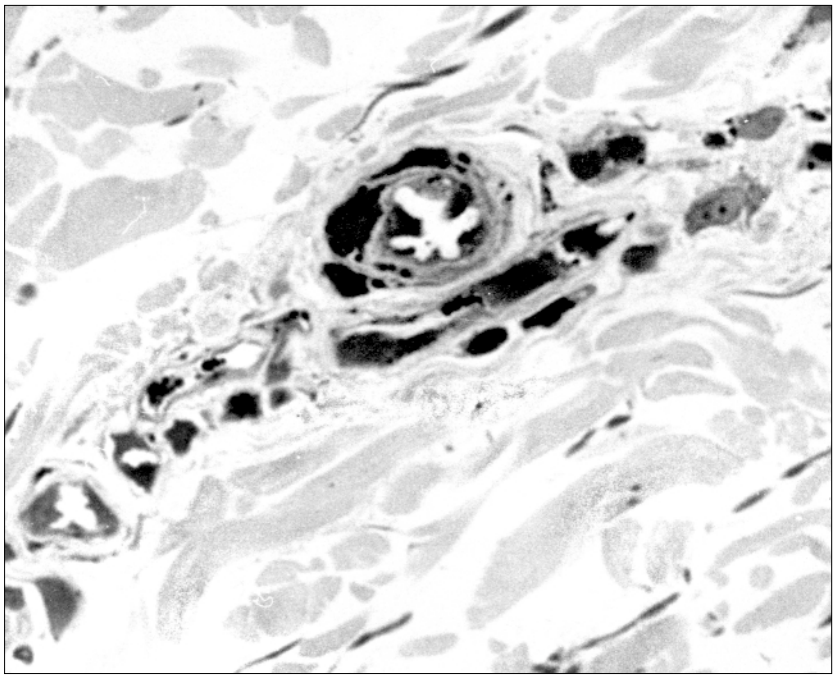

Fig. 1. Pathohistological characteristics of dermal microvessels in skin biopsy specimen from a patient with diffuse angiokeratoma. Endotheliocyte and perivascular cell cytoplasm contains large granules; hypertrophy, hyperplasia, and degradation of vascular cells. Semithin section, azur II staining, ×250.

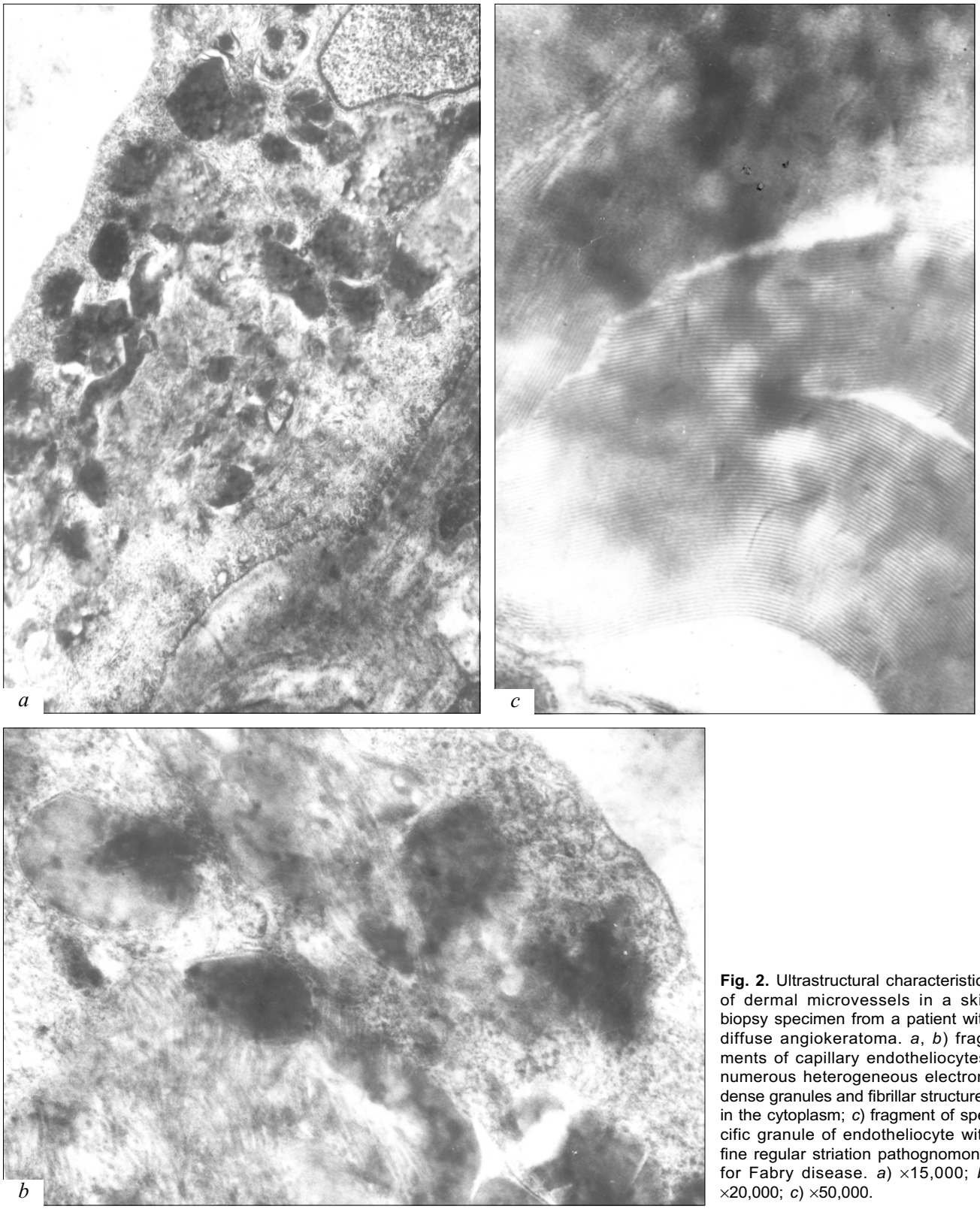

Fig. 2. Ultrastructural characteristics of dermal microvessels in a skin biopsy specimen from a patient with diffuse angiokeratoma. *a*, *b*) fragments of capillary endotheliocytes; numerous heterogeneous electron-dense granules and fibrillar structures in the cytoplasm; *c*) fragment of specific granule of endotheliocyte with fine regular striation pathognomonic for Fabry disease. *a*) ×15,000; *b*) ×20,000; *c*) ×50,000.

numerous pinocytosis vesicles detected also on the basal membrane.

Hyperplasia of the protein-synthesizing organelles and accumulation of mitochondria, elements of Golgi complex, and solitary osmiophilic lysosomes were seen in the endotheliocyte cytoplasm in the zone of granule formation. The entire cytoplasmatic matrix of endotheliocytes contained finely grained substance

with an essential portion of glycogen and numerous bundles of micro- and macrofibrillar components. The endothelial basal membrane was edematous, loosened, stratified, with pericyte processes and collagen fibrils, which indicated repeated desquamation of endotheliocytes.

Most pericytes was hypertrophic, polymorphic, with large euchromic nuclei and other signs of high functional activity; the cytoplasm contained many ribosomes, polysomes, pinocytous vesicles with contents of different electron density, fibrillar structures; heterogeneous polymorphic granules formed perinuclearly. Other part of pericytes looked as depocytes with very large agglomerations of granules presenting as giant osmiophilic residual bodies. Accumulations of poorly differentiated pericytes were seen perivascularly, which sometimes tended to form concentrical structures (capillarogenesis), located near degenerative vessels, in which all cells were transformed into depocytes.

The population of perivascular cells contained numerous round mastocytes with finger-like processes of the plasmalemma and great volume of granular material intra- and pericellularly. Mast cell granules were round and homogenous and were characterized by moderate electron density, sometimes with fine granular or lamellar structure.

Hence, the most significant event in the pathomorphogenesis of hereditary enzymopathy is vascular cell reaction: accumulation in of large specific polymorphic granules of varying electron density with fine regular striation, pathognomonic for Fabry disease the endotheliocyte and pericyte cytoplasm, which leads to endotheliocyte and pericyte degradation. Systemic alteration of capillaries is the basis of diffuse vasculopathy.

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