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DERMAL LYMPHATICS IN MYOTONIC DYSTROPHY

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ABSTRACT

Myotonic dystrophy is an hereditary disorder of several organ systems. Skeletal muscle is a principal target organ, but abnormalities also occur in the peripheral microcirculation. Because morphological and functional changes in the dermal blood microcirculation may affect interstitial fluid drainage of the skin, we examined dermal lymphatic morphology in adult patients with myotonic dystrophy. Skin biopsies were taken from the big toe from patients with myotonic dystrophy (age 18-50 years) and subjected to light and electron microscopy; five healthy subjects served as controls. The salient findings in myotonic dystrophy were ultrastructural changes of the lymphatic endothelial cells and the fibrillar elements that surround the lymphatic wall. These abnormal lymphatic findings are interpreted in light of changes in the blood microvasculature and loose connective tissue in this disorder.

Myotonic dystrophy is an hereditary disease transmitted as an autosomal dominant with great variability of penetrance. The disorder may have manifestations in a multitude of organs including skeletal muscle, eyes, gonads, hair and the pituitary gland (1-3). In recent years, morphological changes in arterioles, capillaries and venules have been demonstrated in the skin and in other organs from myotonic patients. Ultrastructurally, the endothelium in dermal blood vessels is characteristically thickened and the blood vascular basement membrane is reduplicated. These changes occur in myotonic subjects without diabetes mellitus and a hypertension (4,5).

There are also descriptions of the morphology and function of dermal lymphatic capillaries in normal and in pathological conditions (6-10). These studies have stressed the key role of dermal lymphatics in immunoresponsiveness and in the regulation of cell hydration and osmosis. In this report, we examined, after digital biopsies were taken from adult patients with myotonic dystrophy, light and electron microscopic alterations of dermal lymph vessels and how these lymphatic changes related to alterations in adjacent arterioles and blood capillaries.

MATERIALS AND METHODS

Seven adult men and one woman (18 to 52 years old) with myotonic dystrophy and 5 normal male volunteers (20 to 54 years old) were studied. The most sensitive clinical tests for identifying myotonic dystrophy were lens cataract using slit-lamp examination and myotonia as confirmed by electromyography. Particular attention was taken to exclude overt or subclinical hypertension and also diabetes mellitus by demonstrating a normal oral glucose tolerance test in each patient.

Digital biopsies were taken from the base of the hallux according to Curri (12). Small pieces of skin were fixed in a mixture

of glutaraldehyde (2.5%) and paraformaldehyde (2%) in a sodium cacodylate buffer (0.1M, pH 7.4, Karnovsky half strength) (13) and then dehydrated and embedded in epoxy resin. Semithin sections were stained with toluidine blue, for light microscopy. Ultrathin sections (40-100nm) were stained with uranyl acetate and lead citrate for electron microscopy (Zeiss EM109). Several ultrathin sections were contrasted with orcein in aqueous solution (2%) to detect elastic fibers.

RESULTS

Light microscopy

In samples taken from healthy subjects, the dermal lymph vessels were characterized by different shapes and sizes. They were easily recognized by a wide lumen and thin wall. The smaller lymphatics (i.e., initial lymphatics 50-60μm) were located in the papillary and subpapillary layers of the dermis. These lymphatics were numerous

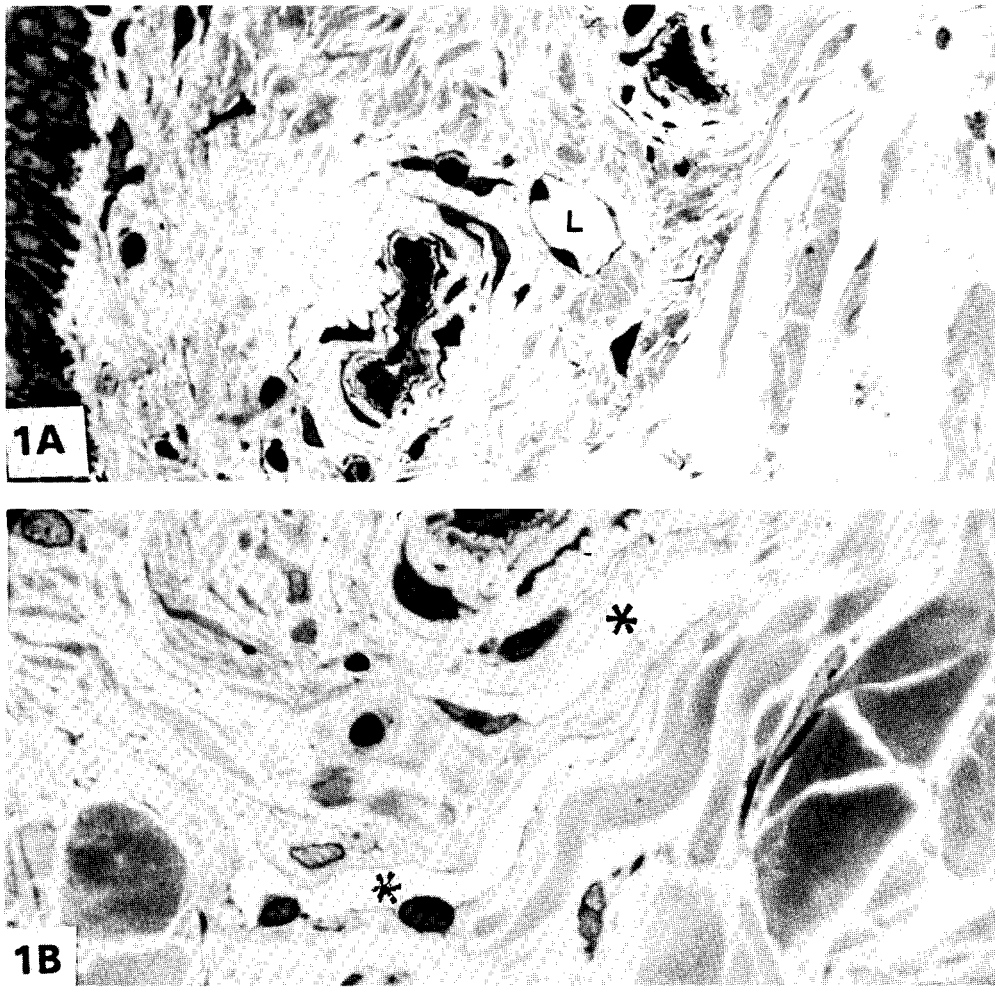


Fig. 1. Skin specimens from normal control subjects. A—a lymphatic vessel associated with blood vessels is visible in the subpapillary layer of the dermis. L=lymphatic vessel (semithin section x800). B—a large lymphatic vessel is seen in the subdermis (asterisk) (semithin section x800).

and often appeared associated with arterioles and venules *(Fig. 1A)*. In the deeper layers of the skin (i.e., subdermis) lymph vessels were less numerous and were larger (200-300μm) than those of the dermis. The larger size and lower density indicate they were precollector or collector lymphatics *(Fig. 1B)*.

In the skin of patients with myotonic dystrophy, the small lymphatics of the dermis were not easily seen because they were partially collapsed *(3 subjects—Fig. 2A)* or completely collapsed *(5 subjects—Fig. 2B)*. In the subdermis, large lymphatics with thin and indented walls were surrounded by several layers of connective tissue and cells *(Fig. 3)*.

Electron microscopy

Dermal lymphatics of healthy subjects showed characteristic ultrastructural features: the vascular wall was thin and flattened; the endothelial cells had a scanty

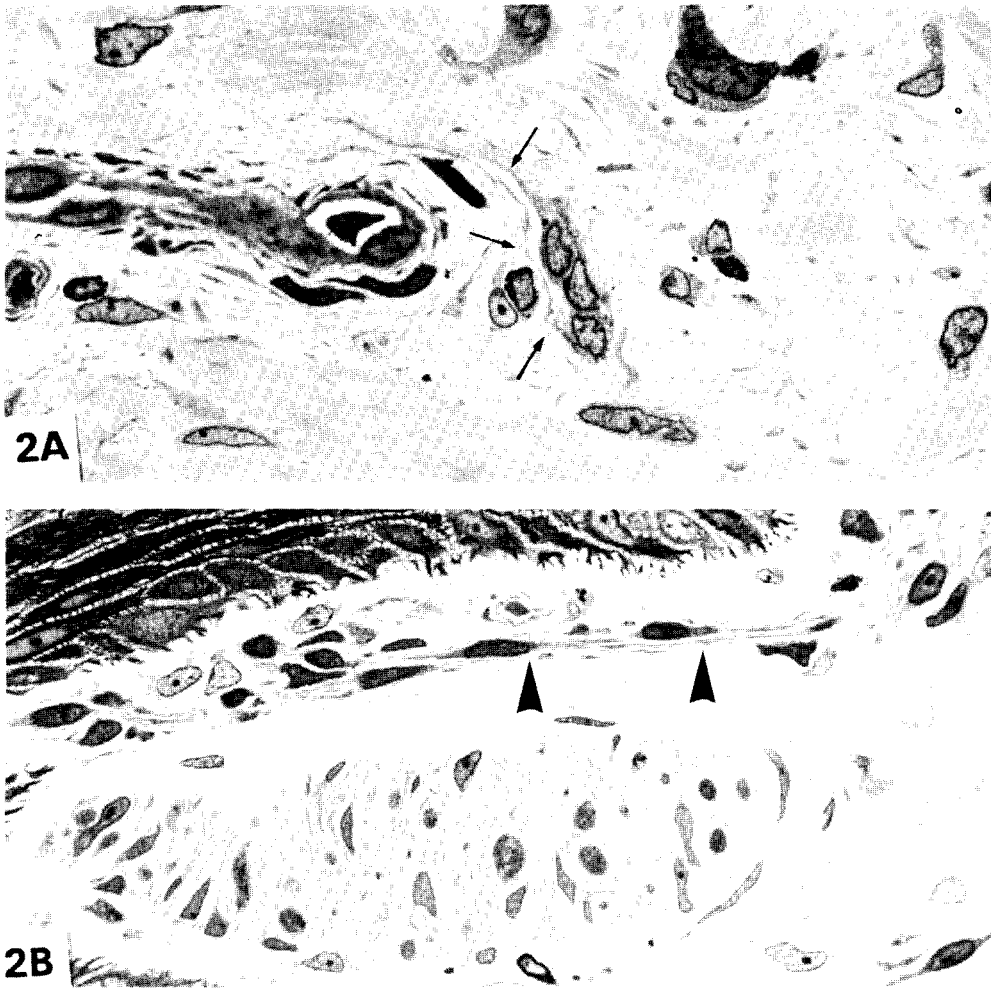


Fig. 2. Skin specimens from patients with myotonic dystrophy. A—a partially collapsed lymphatic vessel is seen in the dermal subpapillary layer (arrows) (semithin sections x800). B—a completely collapsed lymphatic is seen in the dermal papillary layer (arrowheads) (semithin section x800).

cytoplasm and organelles were detected only near the nuclei; micropinocytotic vesicles were regularly distributed on both the luminal and abluminal surface of the lymphatic; the endothelial basal lamina appeared thin and discontinuous and a fine network of filaments anchored the endothelial cells to the surrounding connective tissue *(Fig. 4A)*. Collagen and elastic fibers were also abundant around the lymphatics. The endothelial cells overlapped over a long distance and maintained close contact through specialized junctions. Often the connection between neighboring endothelial cells was end-to-end. Junctions between two contiguous endothelial cells were often open *(Fig. 4B)*.

In most patients with myotonic dystrophy, the ultrastructure of the dermal lymphatic showed differences with that of healthy subjects. Junctions between contiguous endothelial cells were more complex and often two or more edges of neighboring endothelial cells were connected by complex interdigitations and united by desmosomes *(Fig. 5A)*. Intercellular channels also coursed along the endothelial wall and were delimited by the cytoplasmic protrusions of adjacent endothelial cells. The main feature common to most of these lymphatics was a continuous electron-dense basal lamina that surrounded the endothelial wall *(Figs. 5B-5D)*. The lumen of the dermal lymphatics was usually collapsed and the endothelial cells on opposite sides of the lymphatic wall were joined together *(Fig. 5C)*. In many instances, the basal lamina was thick and composed of a homogeneous electron-dense matrix; the fine network of filaments that normally adhere to the endothelial wall was separated from it by this electron-dense material. Collagen and elastic fibers were also seen outside this dense sheet *(Fig. 5C)*. Small blood vessels were characterized by a thick and reduplicated basal membrane *(Fig. 6)*.

DISCUSSION

Morphological changes in the microvasculature in patients with myotonic dystrophy have been described previously including abnormally thick blood vessels in the skeletal muscle of newborn children (14) and of adults (4), in the capillaries of the choroid, retina, and ciliary body of the eye, and also in the brain (5,15,16). Ultrastructure of the peripheral microvasculature

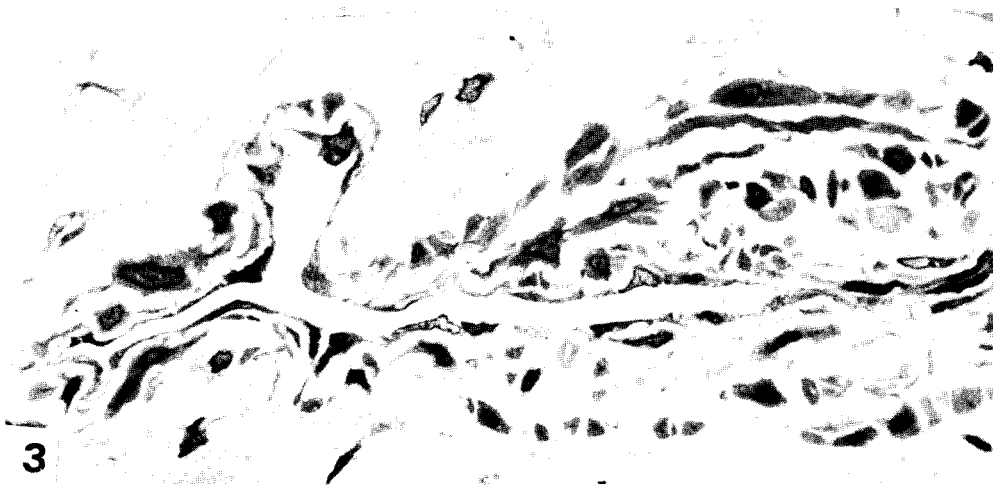


Fig. 3. Skin specimen from a patient with myotonic dystrophy. A lymphatic collector surrounded by several layers of connective tissue and cells is seen in the subdermis (semithin section x800).

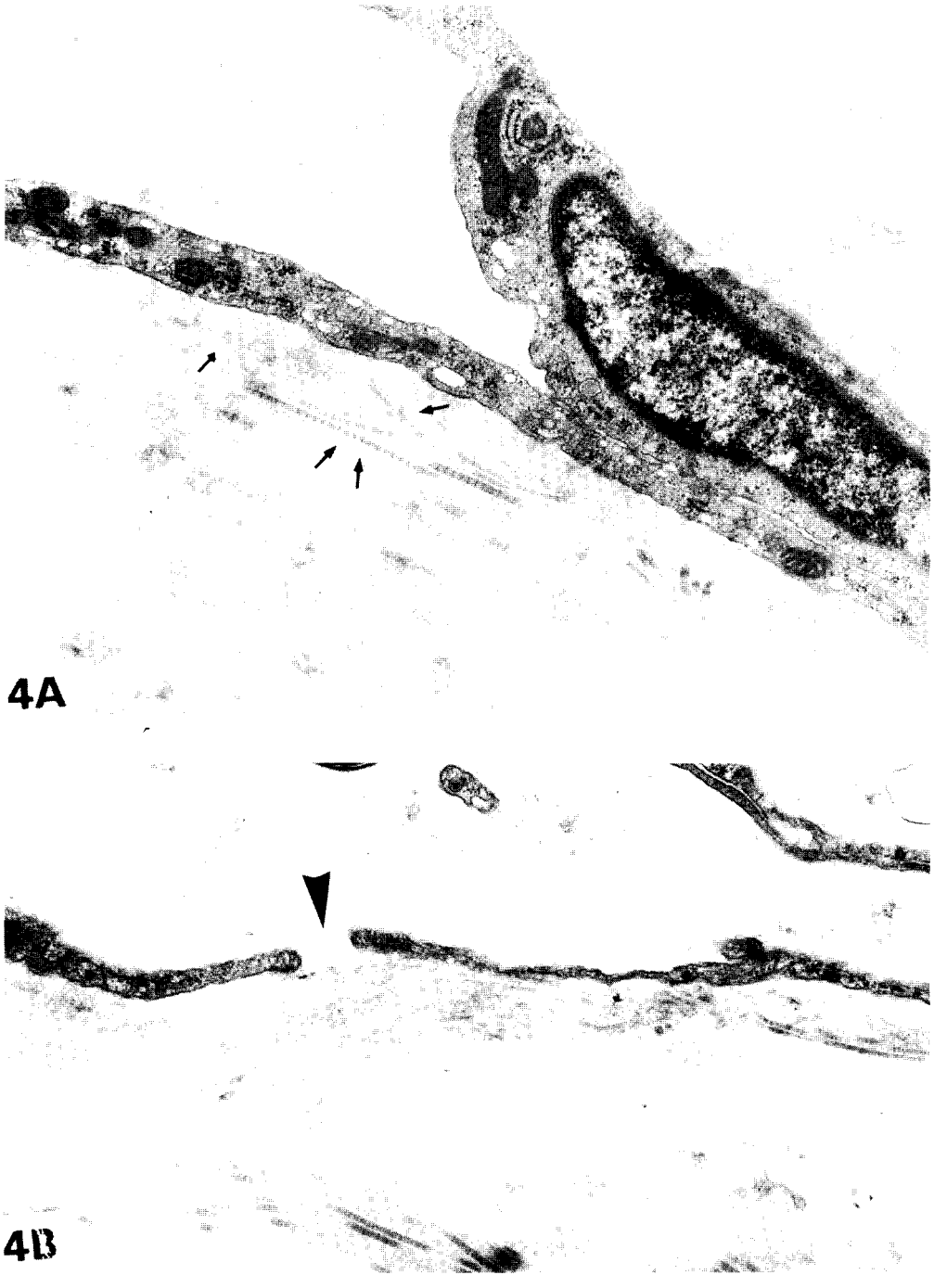
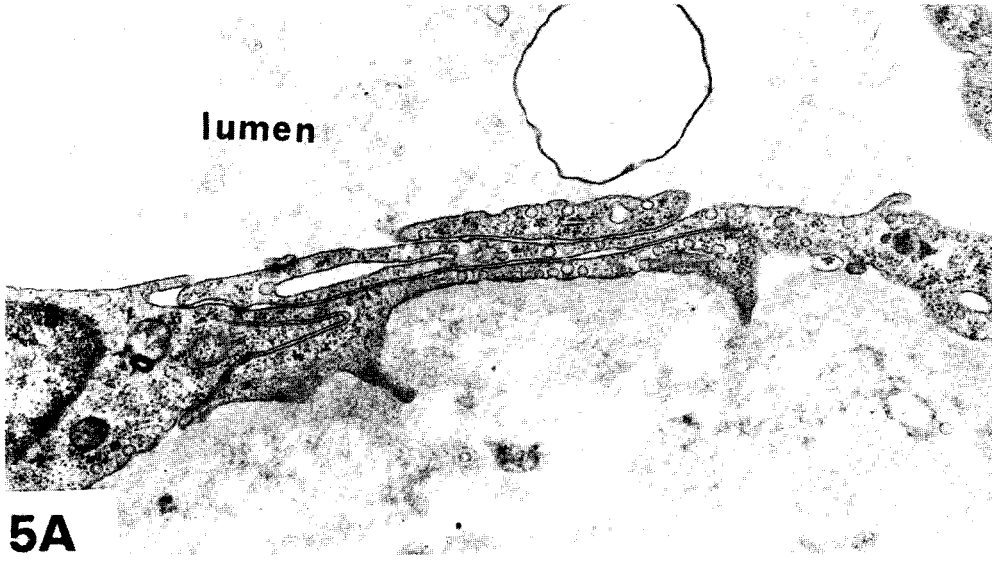


Fig. 4. Ultrastructure of lymphatics from a normal subject. A—thin filaments and collagen fibers are seen adjacent to the endothelial membrane (arrows) (x16200). B—an endothelial wall displaying an "open junction" (arrowhead) (x16200).



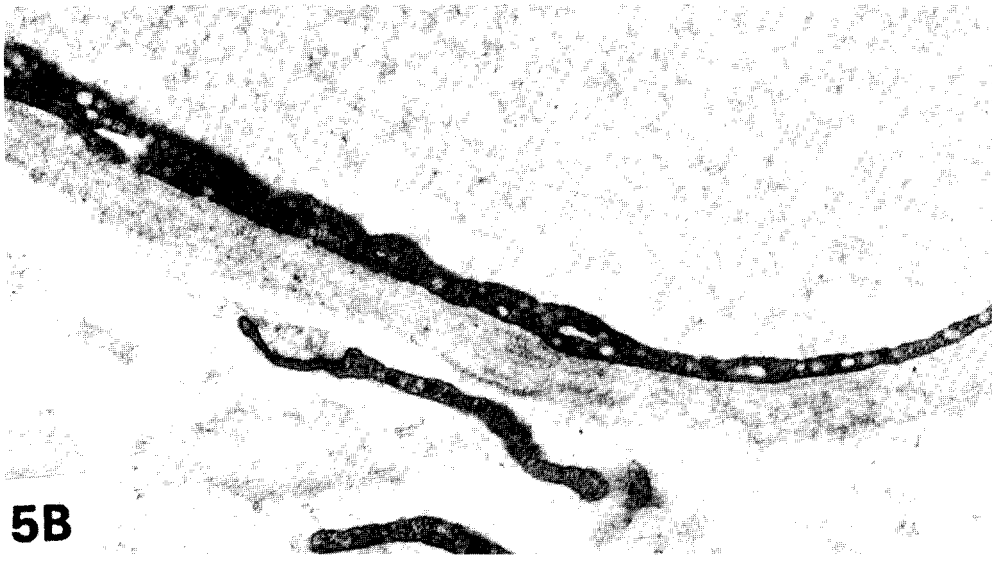
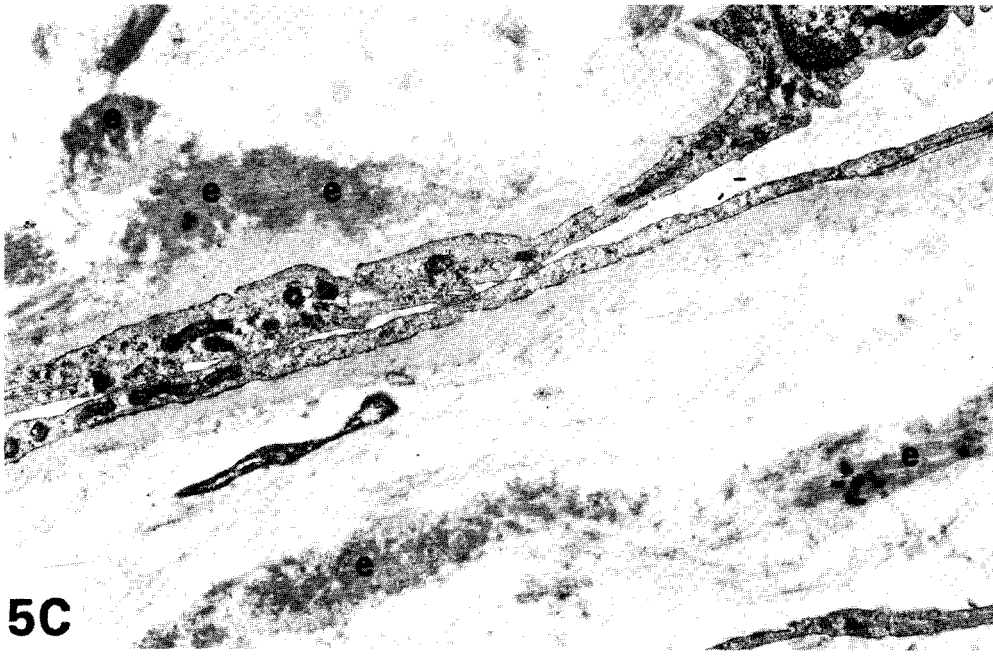


Fig. 5. Ultrastructure of dermal lymphatics from patients with myotonic dystrophy. A—complex interdigitations connecting contiguous endothelial cells (x16200). B—electron-dense material surrounding the endothelial membrane (x16200). C—elastic fibers with orcein stain are abundant around the lymphatic. e=elastic fibers (x16200). D—the endothelial wall is surrounded by a continuous and electron-dense material (x16200).



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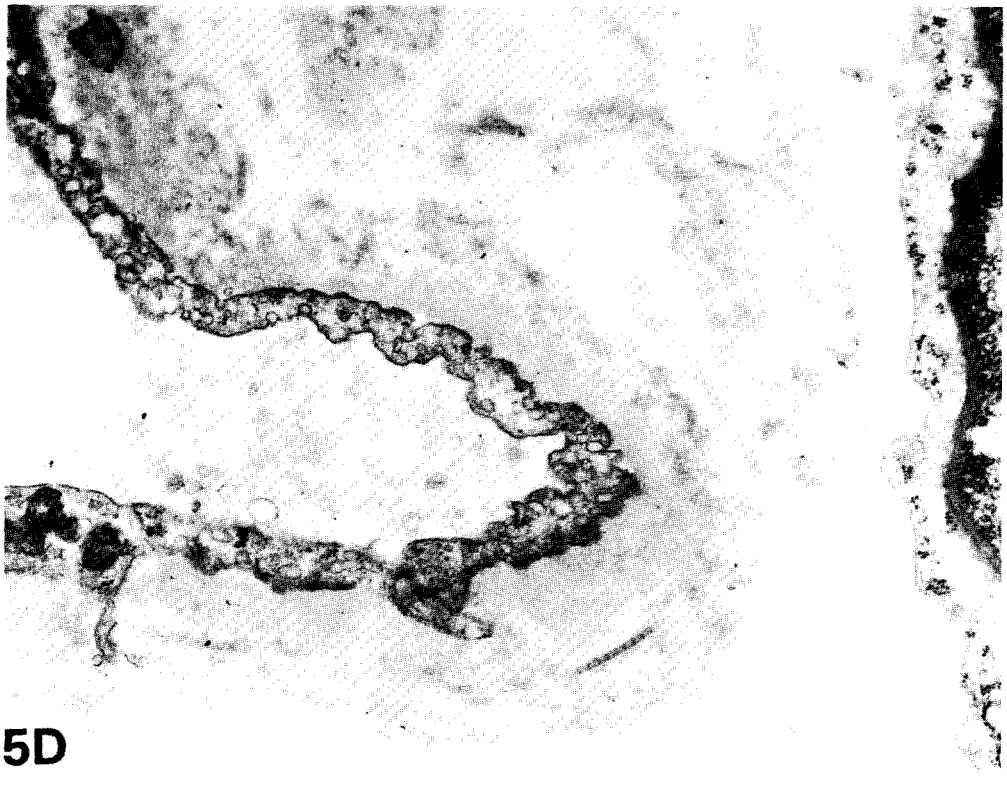

5D

of the skin of patients with myotonia also show a marked thickening of the basement membrane of the capillary wall and "hemodynamic-proliferative" arteriosclerotic changes in the arterioles and in arteriovenular anastomoses (4). These variations are also unrelated to age and in the patients with myotonia are unrelated to diabetes, hypertension and other angiopathic-inducing disorders.

In the biopsies from healthy subjects, the dermal lymphatics showed light microscopic and ultrastructural features similar to those previously described in normal human skin (6-11). Thus, by electron microscopy the lymphatic endothelial wall

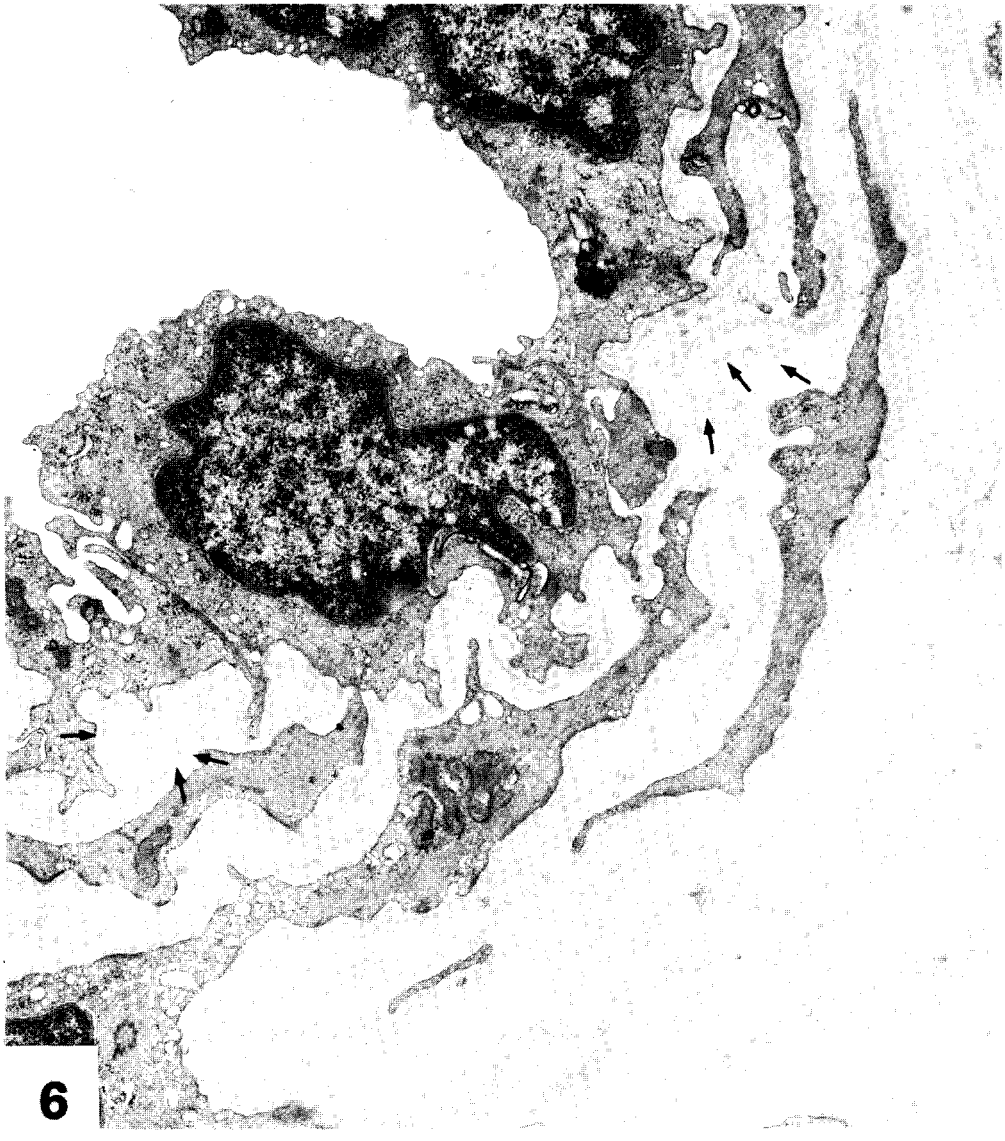

Fig. 6. Ultrastructure of a dermal blood capillary from patient with myotonic dystrophy showing an endothelial wall with reduplication of the basal lamina (arrows) (x9750).

was characterized by an attenuated endothelium, a discontinuous basement membrane, loose gaps between contiguous endothelial cells, and a fibrillar apparatus that surrounded the lymph vessel. Moreover, the lymphatic lumen was moderately wide and filled with proteinaceous material.

In myotonic subjects, the dermal lymphatic network undergoes changes presumably related to the underlying disease, and similar to those previously described in the blood microvasculature. By light microscopy, the dermal lymph vessels are collapsed as is the lumen of most blood capillaries. In the lymphatics with a collapsed lumen, the endothelial cells are united by highly complex junctions. Because circulating macromolecules enter the lumen through these structures, it is reasonable to assume that free access to and from the interstitial space is impeded. This hypothesis is supported by a continuous and thick basal lamina that closely surrounds the endothelial wall. This feature is also common for small blood vessels which, in the skin from dystrophic patients, are characterized by a thick and irregular reduplicated basement membrane (4). In fact, in the dermal blood vessels, these changes may relate to a widespread disturbance of cell membranes that are unable to regenerate because of deficient enzymes (17,18). Moreover, beyond the basement membrane of the dermal lymphatic vessels, the anchoring filaments normally present are not detected. These filaments ordinarily extend from the abluminal surface of the endothelial cells into the perilymphatic connective tissue and connect to elastic and collagen fibers (8,11). This "fibrillar apparatus" seems to be critical for widening the lymphatic lumen during the filling and emptying phase of these vessels. Although collagen and elastic fibers are seen beneath the basement membrane in the dermal lymphatics of patients with myotonia, the lymphatic lumen is occluded. This abnormality may relate to lack of anchoring filaments and defective function of the collagen and elastic fibers, reducing access of circulating elements into and from the vessel lumen.

Thus, in patients with myotonic dystrophy the blood and lymph microvessels in the tissues are similarly altered.

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