

NEW LIGHT MICROSCOPIC SKIN FINDINGS IN FABRY'S DISEASE

Study of four patients using plastic-embedded tissue

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Light microscopic examination of 0.5 μ to 1.5 μ sections of tissue fixed for electron microscopy and embedded in plastic has yet to be fully exploited. Using this technique we report a hitherto unrecorded finding in skin biopsies of four patients with Fabry's disease [a variety of glycolipid lipidoses possibly the result of ceramidetrihexosidase deficiency (1)].

Materials and Methods

Punch biopsies of skin were taken from four men with Fabry's disease. Three were brothers; the fourth was unrelated to them. Electron microscopic findings in the skin of two of these patients have been reported previously (7, 17). One specimen was fixed for four hours in 0.1 M phosphate-buffered 3% glutaraldehyde before osmium fixation. All specimens were fixed for two hours in veronal acetate-buffered 1% osmium tetroxide, dehydrated with graded concentrations of ethanol and propylene oxide and embedded in epoxy casting resin (Araldite). Sections 0.5 μ to 1.5 μ thick were cut with glass knives on a Porter-Blume microtome and fixed to slides. Thin

sections (600 to 1000 Å) were cut immediately before or after a thick section so that corresponding areas might be compared. The former were picked up on uncoated copper grids.

Thick sections were stained with either 1% Toluidine blue or 1% Azure B in 1% aqueous sodium borate (pH approximately 12). Thin sections stained with uranyl acetate (16) and lead citrate (13) were studied in an RCA-3G electron microscope. As controls, blood vessels in skin biopsies of several other patients were studied. These specimens were fixed and embedded similarly to those from the patients with Fabry's disease and included several specimens of normal skin, one cavernous hemangioma, one capillary hemangioma, one capillary hemangioma of the "pyogenic granuloma" type, one glomus tumor, and one hemangioendothelioma.

Results

Typical dilated thin-walled superficial vessels filled with erythrocytes were found in the upper dermis. Many were completely surrounded by the epidermis (Fig. 1) giving

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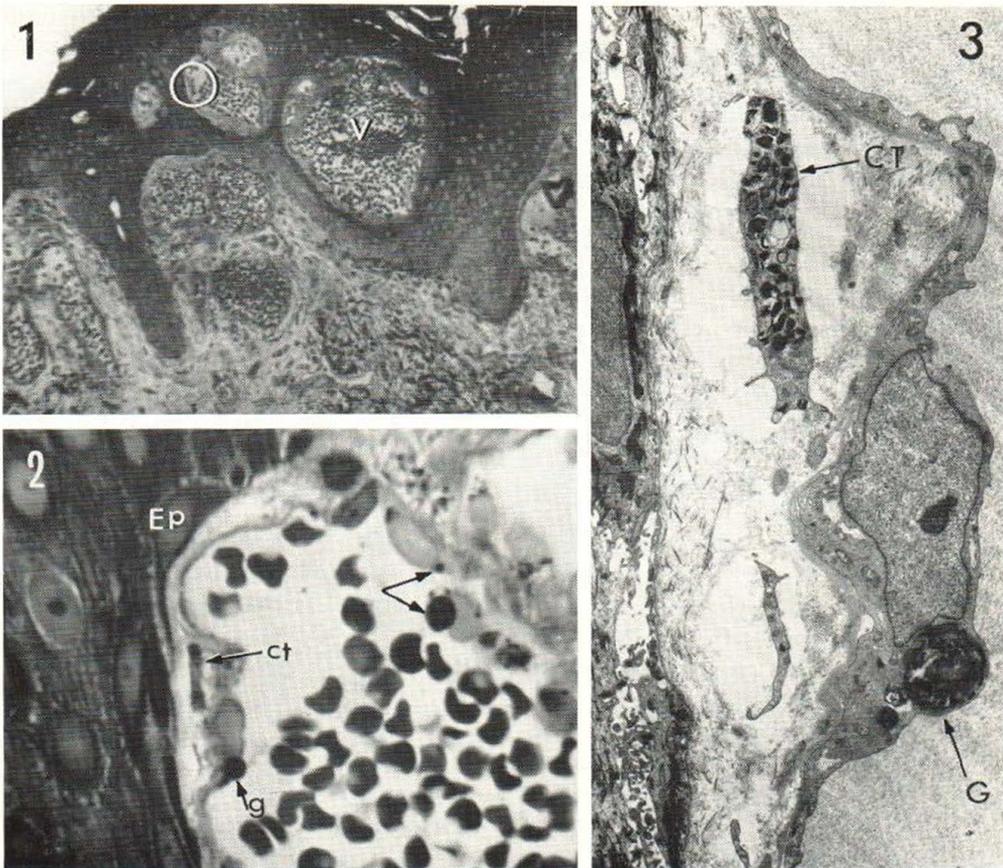


Fig. 1 (upper left). Shows many erythrocyte-filled dilated vessels some of which (v) are completely surrounded by epidermis. Encircled area shown at higher magnification in Figs. 2 and 3. Osmium fixation, toluidine blue stain. $\times 100$.

Fig. 2 (lower left). Light micrograph of a portion of dilated vessel circled in Fig. 1. Unlabeled arrows point to dense granules within endothelial cells illustrating variability in size. In black and white photographs large granules appear similar to red cells, but when viewing the slide, the difference in density and color is immediately apparent. Portion of a connective tissue cell (ct) and a dense granule (g) within an endothelial cell correspond to those illustrated in the electron micrograph (CT and G in Fig. 3). Ep=epidermis. $\times 700$.

Fig. 3 (right). Electron micrograph of a portion of vascular space shown in Fig. 2. The dense-granule-filled process of a connective tissue cell (CT) and a large dense granule within an endothelial cell (G) correspond to those features in Fig. 2 (ct and g). The basement membrane surrounding the vessel and the epidermal basement membrane are evident. Uranyl acetate-lead citrate. $\times 3200$.

the appearance of an intraepidermal vascular space. The endothelial cells of virtually every vessel (Fig. 2) and most dermal connective tissue cells contained blue-black (with either toluidine blue or Azure B), densely stained, round to oval granules varying in size from less than one micron to about five microns in diameter. Dense

granules were not detectable in unstained sections. Mast cell granules stain metachromatically with these stains and can be distinguished from the lipid granules.

Electron microscopic examination of thin sections cut immediately adjacent to thick sections established that the dense granules seen with the electron microscope corre-

spond to the lamellar lipid inclusions first described in the kidney in Fabry's disease by Henry and Rally (8) (compare Figs. 2 and 3).

Dense granules were not observed within vessel walls of any of the other specimens enumerated under Materials and Methods.

Comment

In Fabry's disease, while one can suspect lipid deposition with routine fixation and staining of heart muscle (15), kidney (20), and bone marrow macrophages (19), the amount of lipid in skin lesions is too small to detect in routinely fixed and stained sections (9). One must resort to special fixation and lipid staining techniques (11, 14).

Dense granules have been found by light microscopy of methacrylate-embedded kidney in two laboratories (3, 6, 10) studying Fabry's disease. One group (3, 6) alludes to a "smudgy" Alcian Blue-PAS staining material in methacrylate-embedded cutaneous vessels of their patient but no micrographs illustrating this finding were published by them or by others (4, 5, 12, 18) studying plastic-embedded skin biopsies in this disease.

Our four patients were clinically affected but it is likely that similar granules would be found in the skin of asymptomatic female carriers since special staining reveals lipid deposits in the cutaneous vessels of those subjects (2).

The uniform presence of dense granules in vessel walls in our four patients with Fabry's disease and their absence in normal as well as the pathological vessels which we have examined suggests that study of plastic embedded skin may be an adjunct to diagnosis in Fabry's disease as well as the carrier state.

SUMMARY

Skin biopsies from 4 patients with Fabry's disease were fixed for electron microscopy, embedded in plastic, cut into 0.5 to 1.5 micron sections, stained with an aniline dye and examined with the light microscope. Densely stained granules were found in virtually every endothelial cell and most

dermal connective tissue cells. Electron microscope examination of adjacent ultra-thin sections established that the dense granules observed by light microscopy correspond to the lamellar lipid inclusions previously described in Fabry's disease.

Blood vessels from normal skin and from a variety of vascular lesions prepared and examined in the same way did not contain similar granules.

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