

Fabry's disease: specific inclusions found on electron microscopy of fibroblast cultures*

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Summary. Electron microscopy of fibroblasts cultured from skin biopsies of two sibs with Fabry's disease has shown characteristic crystalline cytoplasmic inclusions.

Leucocytes (Kint, 1970) and cultured fibroblasts (Romeo and Migeon, 1970) in Fabry's disease have been shown to lack α -galactosidase activity, and cultured fibroblasts to accumulate mucopolysaccharide and glycolipid, mainly trihexosyl ceramide (Matalon *et al*, 1969).

The cultures examined in the present investigation were obtained from two sibs by courtesy of Miss S. E. Gardiner (MRC Human Biochemical Genetics Unit). Miss Gardiner has shown both leucocytes and fibroblasts from the sibs to be deficient in α -galactosidase activity.

Materials and Methods

The fibroblast cultures were grown in Minimal Essential Eagles Medium with 10% fetal calf serum, and divided by trypsinization. At the sixth subculture the cells were transferred to Leighton tubes and when confluent, were prepared for electron microscopy. Cultures were fixed *in situ* with 2% glutaraldehyde in 0.05 M cacodylate buffer pH 7.2 at 4° C for three hours. Maintaining the temperature at 4° C, the cells were washed for several hours in 0.1 M cacodylate buffer containing 4.5% sucrose and postfixed for one hour in 1% osmium tetroxide in 0.05 M cacodylate buffer containing 5% sucrose. Cells were dehydrated in ethanol and embedded in Araldite.

Sections were cut using a Porter-Blum MT2 ultramicrotome, stained with methanolic uranyl acetate and lead citrate, and viewed in a Siemens 101 electron microscope at 80 kV.

Received 20 September 1973.

* This study is part of an electron microscopic survey of skin fibroblasts cultured from patients with inherited neurological disorders. Dr J. McLean is in receipt of a grant from the National Fund for Research into Crippling Diseases.

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Results

The cells contained crystalline cytoplasmic inclusions in which periodicities of approximately 6 and 10.5 nm were recognized along the two axes (Fig. 1). The inclusions were mostly 0.5 to 1 μ m in diameter, surrounded by a single membrane, and are presumably lysosomal. In addition to these bodies, other non-specific features of lipid accumulation were found in lysosomes of the Fabry cells. These included myelin figures, whorls, and bodies with irregular parallel lamellae (Fig. 2).

Discussion

The crystalline cytoplasmic inclusions are distinctive. Similar bodies have not been seen in cells of a series of 60 cultures from a variety of neurological disorders including Tay Sachs disease and other neuropilidoses, and cells from normal individuals.

Examination of biopsy material from patients with Fabry's disease has shown myelin-figure inclusions in kidney (Malmqvist *et al*, 1971) and nerve (Bischoff *et al*, 1968; Kocen and Thomas, 1970); Rae, Lee, and Hopper (1967) noted inclusions with a lattice-like structure in both kidney and skin. The appearance of zebra bodies in brain material (Grunnet and Spilsbury, 1973) may be due to post-mortem and fixation changes in similar aggregates.

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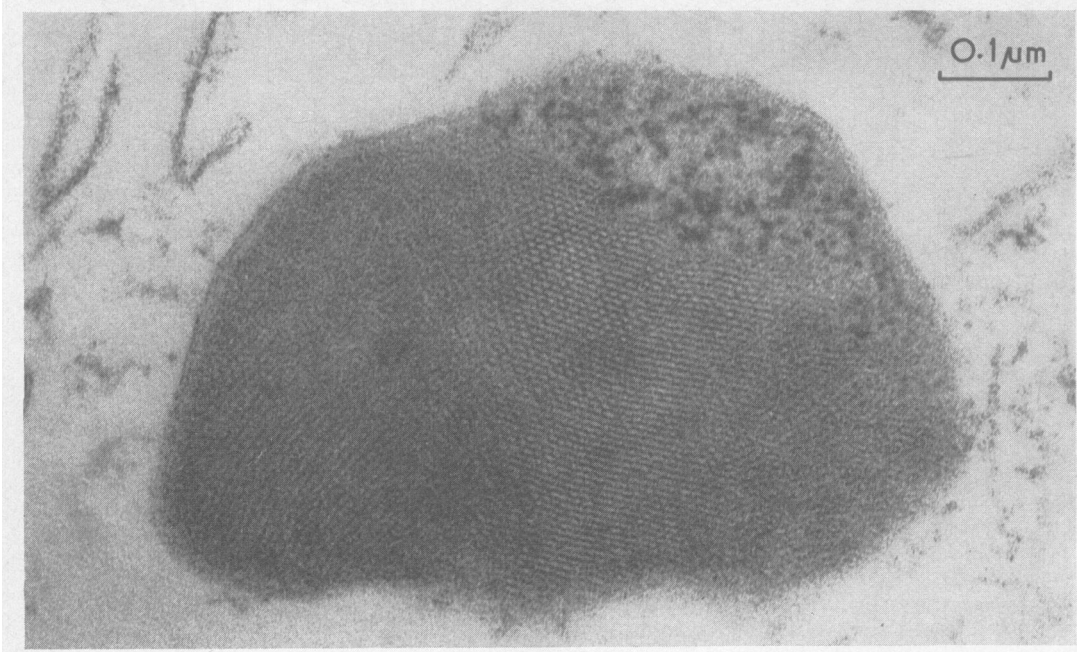


FIG. 1. A crystalline inclusion in the cytoplasm of a fibroblast cultured from the skin of a patient with Fabry's disease.

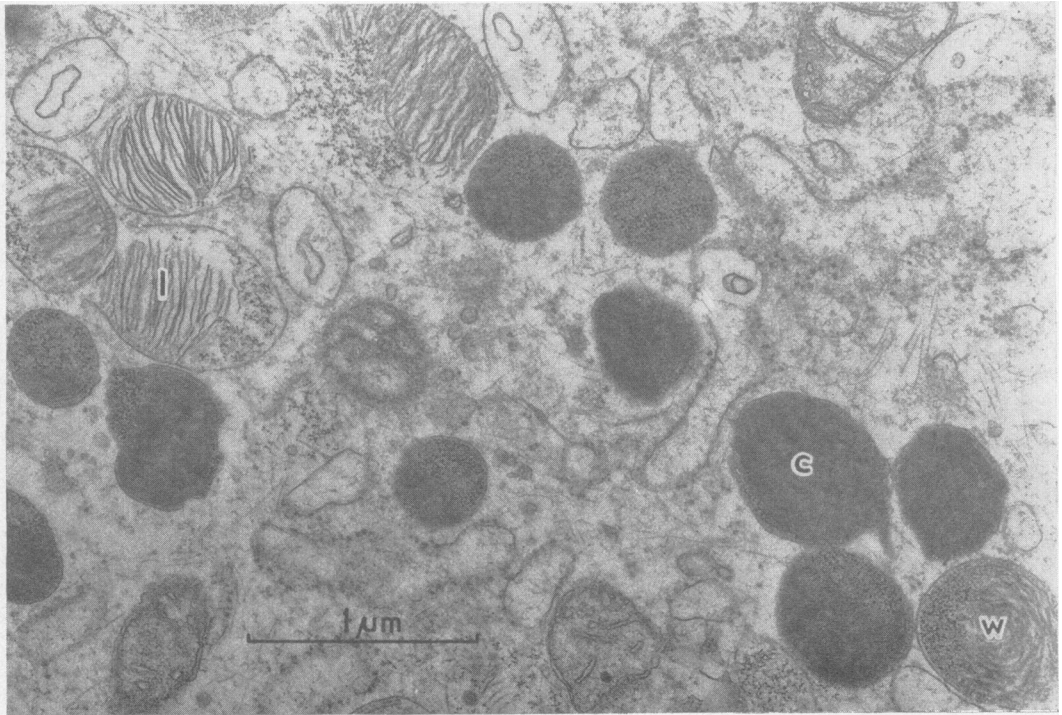


Fig. 2. The cytoplasm of a similiar fibroblast to show crystalline inclusions (c), whorls (w), and inclusions with irregular parallel lamellae (l).

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