Mitochondrial inclusions in fibroblast culture from a patient with β -methylcrotonylglycinuria

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Summary. Electron microscopy of fibroblasts grown from a patient with biotin responsive β -methylcrotonylglycinuria revealed unusual deposits within their mitochondria.

The clinical and biochemical findings in a patient with β -methylcrotonylglycinuria have been described by Gompertz *et al* (1971) and Gompertz and Draffan (1972). Briefly the patient at 5 months of age presented with metabolic acidosis and ketosis. β -Methylcrotonylglycine, β -hydroxyisovaleric acid, and tiglylglycine were found in the child's urine. Clinical improvement and disappearance of the metabolites from the urine occurred with the administration of biotin, a cofactor for β -methylcrotonyl CoA carboxylase.

In this study, skin fibroblasts from the patient and his parents were grown in culture and examined by electron microscopy.

Materials and methods

Skin biopsies were performed on the patient at the age of 9 months—after biotin treatment had been started—and on his parents.

Fibroblasts were grown in 10% newborn calf serum and Eagle's medium; the culture was divided by trypsinization five times before preparing cells for electron microscopy. It is unlikely that any biotin in the original biopsy persisted after these multiplications.

Cells subcultured into Leighton tubes were prepared for electron microscopy when confluent. Cultures were fixed *in situ* with 2% glutaraldehyde in 0.05M cacodylate buffer pH 7.2 at 4°C for three hours and washed overnight in 0.1M cacodylate buffer containing 4.5% sucrose. They were then treated for one hour in 1% osmium tetroxide in 0.05M 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.

Results

Mitochondria in the culture from the patient contained amorphous deposits of an unidentified material (Fig. 1). This was often associated with derangement of the mitochondrial cristae in the neighbourhood of the deposits. Similar but less marked abnormalities were seen in the cultures from both parents.

Deposits of this nature have not appeared in examining 60 other cultures from skin biopsies on normal subjects and on patients with a variety of neurological diseases. Fig. 2 shows a mitochondrion from a normal fibroblast culture.

Discussion

Such mitochondrial abnormalities have not previously been reported in fibroblast cultures. Mitochondrial abnormalities have been noted in a wide variety of muscle diseases. Many of these are reports of crystalline, lamellar, or rod-shaped inclusion bodies in the muscle mitochondria (discussed by Mair and Tomé, 1972). Salmon, Eseri, and Ruderman (1971) described rounded electron-dense mitochondrial inclusions in the muscle of a myopathic patient who had hyperglycaemia and hyperketonaemia.

Amorphous mitochondrial inclusions, similar to those described in this study, were illustrated in the description of axonal dystrophy produced by vitamin E deficiency in the rat by Schochet (1971). Other reports of mitochondrial inclusion bodies seem associated with various types of cell injury or necrosis (Svoboda and Higginson, 1963; Minick *et al*, 1965; Trump, Goldblatt, and Stowell, 1965; Gritzka and Trump, 1968).

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FIG. 1. β -Methylcrotonylglycinuria: amorphous deposits in a mitochondrion of a fibroblast cultured from the patient.

FIG. 2. Control culture: a mitochondrion from a normal fibroblast culture.

The occurrence of mitochondrial deposits in cells cultured from a patient with β -methylcrotonylglycinuria is interesting since the presumed enzyme defect (of β -methylcrotonyl CoA carboxylase) is of a mitochondrial enzyme. The less marked changes found in cultures from the patient's parents may indicate that they are heterozygotes and that the disease is recessively inherited.

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