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

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

The clinical and biochemical findings in a patient with β-methylcrotonyglycinuria have been de-
scribed 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 metabo-
lites from the urine occurred with the admin-
istration of biotin, a cofactor for β-methyl-
crotonyl 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 tryp-
sinization 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 over-
night in 0-1M cacodylate buffer containing 4-5% suc-
rose. 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 Ultra-
microtome, stained with methanolic uranyl acetate and
lead citrate and viewed in a Siemens 101 electron micro-
scope at 80 kV.

Results

Mitochondria in the culture from the patient con-
tained 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 ex-
amining 60 other cultures from skin biopsies on
normal subjects and on patients with a variety of
neurological diseases. Fig. 2 shows a mitochon-
drion from a normal fibroblast culture.

Discussion

Such mitochondrial abnormalities have not pre-
viously been reported in fibroblast cultures. Mitochon-
drial abnormalities have been noted in a wide
variety of muscle diseases. Many of these are re-
ports of crystalline, lamellar, or rod-shaped inclu-
sion 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 myo-
pathic patient who had hyperglycaemia and hyper-
ketonaemia.

Amorphous mitochondrial inclusions, similar to
those described in this study, were illustrated in the
description of axonal dystrophy produced by vita-
min 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).
The occurrence of mitochondrial deposits in cells cultured from a patient with $\beta$-methylcrotonylglycinuria is interesting since the presumed enzyme defect (of $\beta$-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.

We thank Drs D. Hull and D. Gompertz for their help and cooperation and Miss J. Workman for technical assistance. Dr J. McLean received a grant from the National Fund for Research into Crippling Diseases.

REFERENCES


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doi: 10.1136/jmg.11.3.257

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