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Hereditary mitochondrial hypertrophic cardiomyopathy with mitochondrial myopathy of skeletal muscle, congenital cataract and lactic acidosis

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Summary. A six day old boy died from an hereditary hypertrophic cardiomyopathy which was associated with mitochondrial myopathy of skeletal muscle, congenital cataract and lactic acidosis. In heart and skeletal muscle identical mitochondrial abnormalities were found: paucity and abnormal arrangement of cristae, formation and extrusion of vesicle-like structures and crystalline inclusions in the matrix compartment. Electron-cytochemistry revealed that only part of the mitochondria reacted positively for cytochrome oxidase activity. Morphometric analysis indicated that the cardiomegaly was due to cellular hypertrophy, which might be caused by an increase in the mitochondrial mass. The cardiac hypertrophy in this syndrome can be classified histopathologically as mitochondrial hypertrophic cardiomyopathy.

Key words: Mitochondrial cardiomyopathy – Mitochondrial myopathy – Morphometry – Congenital cataract – Lactic acidosis

Introduction

The association of hypertrophic cardiomyopathy with mitochondrial myopathy of skeletal muscle, congenital cataract and lactic acidosis after exercise is known as a syndrome which follows an autosomal recessive pattern of inheritance (Sengers et al. 1975). The syndrome is registered number 21235 on the McKusick register. The histopathological abnormalities in skeletal muscle were described previously (Sengers et al. 1975, 1985). They include abnormal arrangement and loss of mitochondrial cristae, crystalline inclusions in the mitochondria and deposition of lipid and glycogen.

This syndrome is either fatal in infancy or follows a more benign course with death occurring in adolescence or young adulthood (unpublished data). Recently we had the opportunity to study the histopathological changes in the heart and skeletal muscle of an infant who suffered from the fatal infantile form of the syndrome. To our knowledge the histopathology of heart and skeletal muscle in this infantile form of this syndrome has not been described.

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Case report

The patient, a boy, was born after an uneventful pregnancy and delivery as the second child of healthy, non-consanguinous parents. His birth weight was 3250 g and the Apgar scores were 7 and 10 at 1 and 10 min, respectively. Tachypnoea and perioral cyanosis were noted within a few hours after birth. On the first day of life radiological and electrocardiographic examination indicated the presence of right ventricle hypertrophy. Echocardiography revealed septal hypertrophy (septum thickness 6 mm; left ventricular posterior wall thickness 4 mm; right ventricular anterior wall thickness 3 mm; normally below 4.5, 4.2, and 3.4 mm respectively). Repeated echocardiography at the fourth day showed an increase in septum thickness up to 8 mm. Cataract of the anterior capsules of the lenses was noted at that time. The lactic acid concentration in blood was 12 mmol/l (normal < 1.8), in urine 1.57 mmol/mmol creatine (normal $<$ 0.2) and in cerebro spinal fluid 12 mmol/l (normal < 1.8). The usual therapeutic procedures were unsuccessful and at the age of six days the patient died due to cardiorespiratory insufficiency. With informed consent of the parents specimens were taken from the apex of the left ventricle and from the right femoris muscle within ten minutes after death. Autopsy took place four hours later. Results of the examination of heart and skeletal muscle are described in detail below. Apart from the bilateral cataracts no relevant abnormalities were noted in other organs and tissues. The first child of this couple, also a boy, died eight days after birth; at autopsy hypertrophic cardiomyo-

pathy was found. Eyes and skeletal muscle were not examined. Examination of both parents by an ophthalmologist (including split lamp examination) and by a cardiologist (including electrocardiography and echocardiography) excluded ophthalmological and cardiological disorders. Since the parents had no complaints of muscle disease a biopsy was not performed. Urine analysis revealed normal lactic acid levels. The parents of our patient were not aware of abnormalities of eyes, heart or skeletal muscular system in other members of their family.

Material and methods

Autopsy material was fixed in formalin and embedded in paraffin. Sections were stained with haematoxylin-eosin and also for elastin and reticulin. The specimens of skeletal muscle and myocardium taken 10 min after death were immediately fixed in 2% buffered glutaraldehyde, postfixed in 2% buffered osmium tetroxide and embedded in Epon 812. Semithin Epon sections were stained with toluidine blue, with PAS or were treated with para-phenylene diamine to demonstrate fat deposition. Ultrathin sections were contrasted with uranyl acetate and lead citrate. For the electronmicroscopic histochemical demonstration of cytochrome oxidase activity vibratome sections were treated according to Seligman et al. (1968) with modifications according to Angermüller and Fahimi (1981).

Profile diameters of cardiac muscle cells were estimated on projected images of the 10 µm reticulin stained sections. In cases of ellipsoid profiles the lesser diameter was considered to be the profile diameter. A MOP-Videoplan measuring instrument (Kontron, Federal Republic of Germany) was used for morphometric measurement. Electron micrographs for the estimation of volume densities of myofibrils, mitochondria, nuclei and lipid droplets were obtained by systematic random sampling. The point counting method described by Weibel (1979) was used for the morphometric-stereological procedure. A total area of 0.01 mm^2 heart tissue was analysed at a final magnification of 21600 ×.

Results

Examination of the heart

The whole heart was severely hypertrophied and it weighed 47.5 g (normal weight 23 g; Kissane

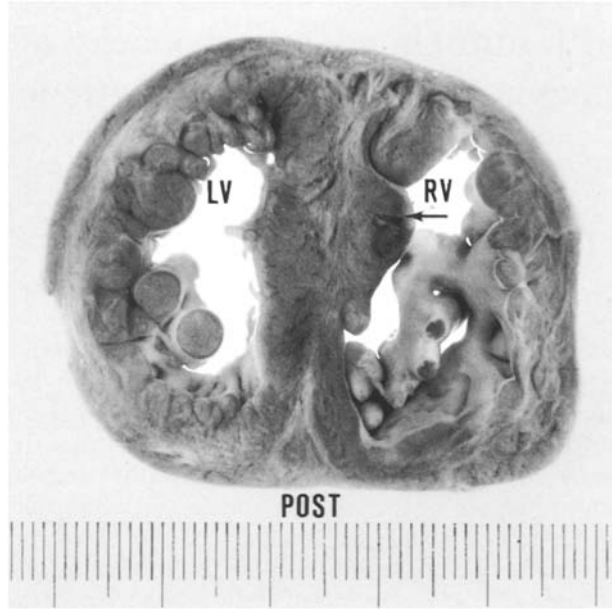

Fig. 1. Transversal section through the ventricles. Extreme hypertrophy and mild dilatation of both cardiac ventricles. Unusually thick trabeculae in the left ventricle. Asymmetric thickening of the ventral portion of the septum, evidently due to incorporation of an atypical trabecula and/or papillary muscle (*arrow*). *LV*: left ventricle; *RV*: right ventricle; *POST*: posterior. Metric scale. ×1.4

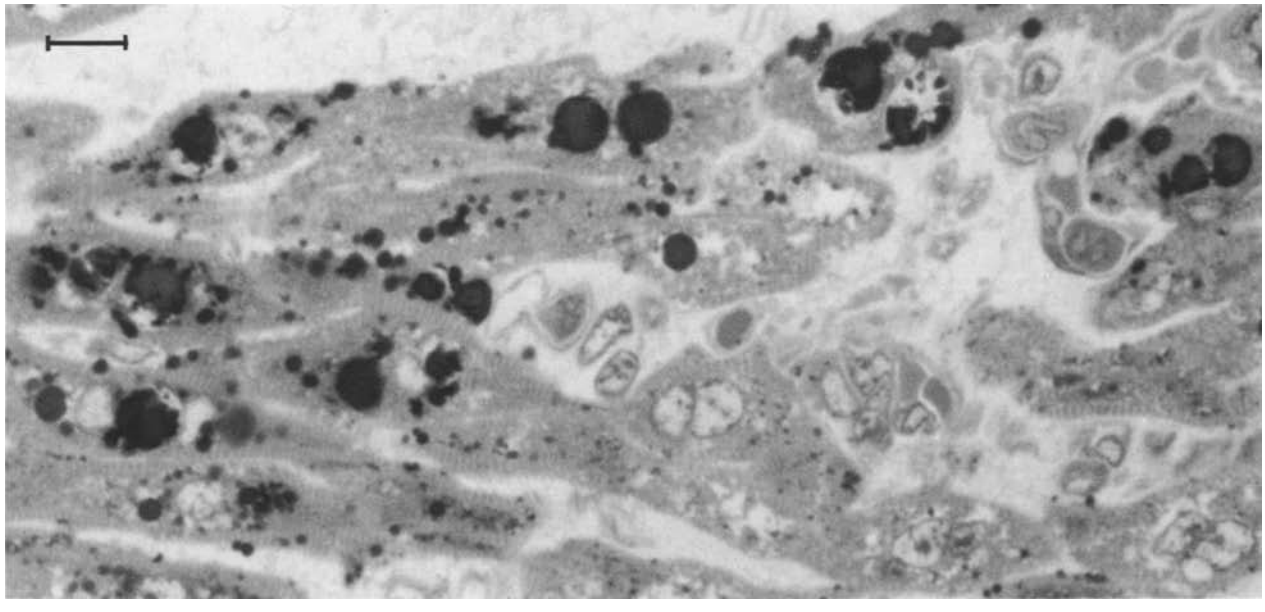

Fig. 2. Heart muscle cells of left ventricle, some of which contain large lipid depositions. Indistinct vacuolisation. Space bar 10 µm. Para-phenylene diamine stained semithin section. ×1100

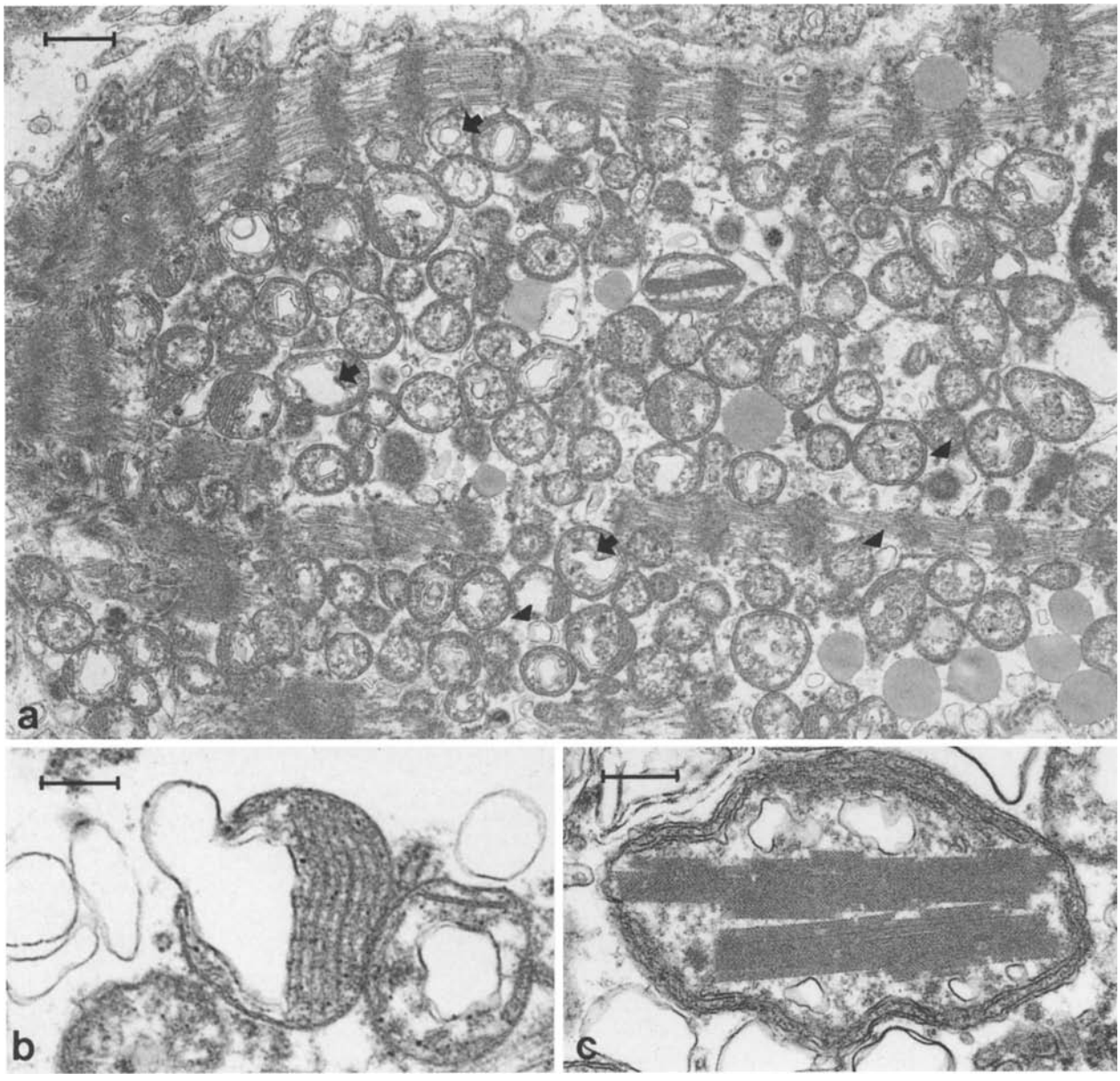

Fig. 3. a Heart muscle cell of left ventricle almost completely filled with abnormal mitochondria. Vesicles in mitochondria (*arrows*) and in cytoplasm (*arrowheads*). Lipid deposition and few myofibrils. Space bar 1 μm. Uranyl acetate led citrate staining. ×11000; **b** Vesicle emerging from mitochondrion. Space bar 0.25 μm. Uranyl acetate lead citrate staining. ×48000; **c** Crystalline inclusion in matrix space. Space bar 0.25 μm. Uranyl acetate lead citrate staining. ×46000

1975). This hypertrophy was most pronounced in the ventricular septum (Fig. 1). The ventricular and atrial lumina were slightly dilated.

A slight subendocardial interstitial fibrosis, mainly in the septum, is present. Fibre disarray is not noted. Neither inflammatory cells nor necrotic fibres are seen. Many cardiac muscle cells show vacuolisation. Paraphenylenediamine staining reveals large areas of lipid droplet deposition (Fig. 2). PAS-positive material is present in localized areas. Subepicardial and perivascular regions are less affected by lipid and glycogen accumulation.

Electronmicroscopically the sarcolemma plus lamina basalis as well as the intercalated discs of the cardiac muscle cells appear normal. One or two myofibrils, consisting of partly dispersed myofilaments, are present beneath the sarcolemma. Lipofuscin is seen. The nuclei contain normally dispersed chromatin; karyolysis or karyorrhexis is not noted. In part of the cardiac muscle cells the nuclei are surrounded by "empty" sarcoplasmic zones,

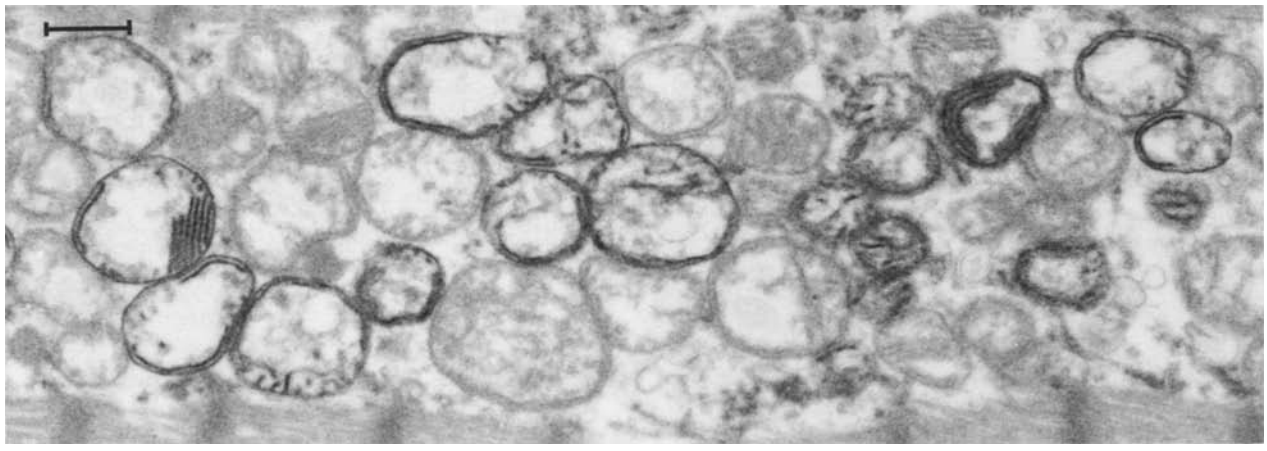

Fig. 4. Electron micrograph of abnormal mitochondria of heart muscle cell of left ventricle, stained for cytochrome oxidase activity. Darkly stained membranes are cytochrome oxidase positive, while the other membranes do not show activity. Space bar 0.5 μm. ×23000

but mostly the perinuclear areas are filled with closely packed, abnormal mitochondria and 1 to 6 μm large lipid droplets (Fig. 3a). The mitochondria are rounded and uniformly large. The mitochondria characteristically contain one or two cristae, lying immediately beneath the outer membrane. Some of the mitochondria contain stacks of transversely oriented cristae. Only about half of the mitochondria show cytochrome oxidase activity in the inner membrane or, if present, in the remnants of cristae (Fig. 4).

Another characteristic finding is the presence of matrix vesicles which are usually limited by two membranes. Many mitochondrial profiles suggest a process of extrusion of these vesicles into the cytoplasmic space (Fig. 3b). The same type of vesicle is seen in the sarcoplasm. In the matrix compartment dense granules are absent. However, rectangular crystalline inclusions are regularly seen (Fig. 3c).

The cardiac muscle cells had a mean diameter of 9.9 ± 2.8 μm (mean ± standard deviation; $n = 150$; normal 6 μm, David et al. 1978). In Table 1 the results of stereologic analysis are summarized. Since such data are not available from age matched controls, data from children aged 5–15 years are included in the table. With exception of the volume density of nuclei, which decreases during postnatal life, no major changes in the relative contents of the cardiac muscle cells occur during postnatal life (Page et al. 1974; David et al. 1978).

Examination of skeletal muscle

In the skeletal muscle tissue no necrotic fibres occur. The modified Gomori trichrome staining does not reveal ragged-red fibres. Accumulations of PAS-positive material are seen in the enlarged intermyofibrillar and subsarcolemmal spaces, incidently forming subsarcolemmal blebs.

In electron microscopy the enlarged intermyofibrillar and subsarcolemmal spaces are filled with abnormal mitochondria and accumulations of glycogen particles. The skeletal muscle mitochondria are irregularly outlined and partly have the same abnormalities as described above regarding the heart muscle mitochondria (Fig. 5). Cytochrome oxidase activity is lacking in about half of the mitochondria. In particular many mitochondria possess vesicles. Matrix crystals are also seen occasionally.

The myofibrils appear normal with only incidentally some derangement of the sarcomere pattern. Lipofuscin is occasionally seen.

Table 1. Results of stereological analysis of samples of the left ventricle and literature data

	Patient Mean [%] ± SER	Literature data
Volume fraction per total tissue volume		
Volume density of myocardiocytes	68.8 ± 2.1	76.1
Volume fractions per myocardial tissue volume		
Volume density of nuclei	6.9 ± 1.5	6.5
Volume density of myofibrils	29.0 ± 1.6	47.1
Volume density of mitochondria	30.1 ± 1.2	34.5
Volume density of lipid	3.4 ± 0.5	not registered

SER: Standard Error of the Ratio. Literature data from Fleischer et al. (1978)

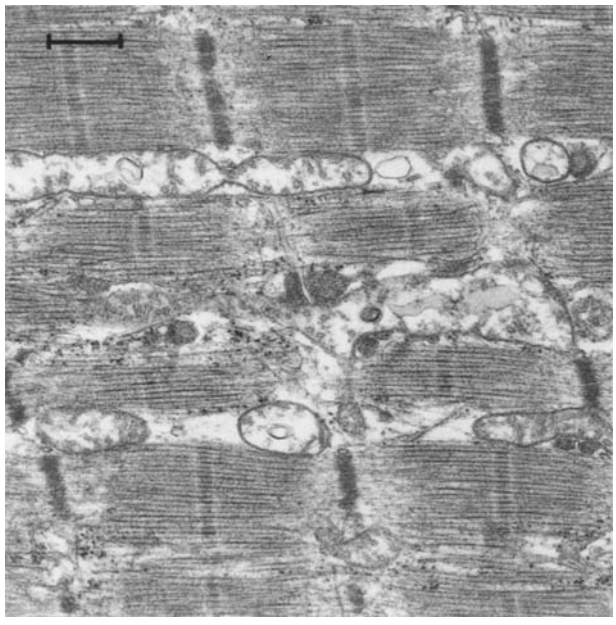

Fig. 5. Skeletal muscle fibre of right femoris muscle. Abnormal mitochondria with vesicles between normal myofibrils. Uranyl acetate lead citrate staining. Space bar 0.5 μm. × 21 000

Discussion

This patient suffered from hypertrophic cardiomyopathy, mitochondrial myopathy of skeletal muscle, congenital cataract and lactic acidosis. We have knowledge of 17 patients with this syndrome, eight of which are described in literature (Sengers et al. 1975, 1985). Four patients died within the first months of life and six died between the age of 14 and 23 years. The cause of death in all these patients was cardiorespiratory insufficiency. The age of the living patients varies between 15 and 33 years. They all suffer from a moderate to severe exercise intolerance. In all cases surgical extraction of the cataracts had to be performed at young age.

In our patient there was increasing hypertrophy of the heart in the six days of life. The distinctly increased mean cardiac muscle cell diameter indicates that the heart hypertrophy must mainly be due to hypertrophy of the cardiac muscle cells. The morphometric studies indicate that the mitochondria in particular contribute to this cellular hypertrophy. Assuming that the tissue samples studied are representative for all cardiac muscle cells and assuming an equal specific gravity for all tissue components, it can be calculated that the total mitochondrial mass in the cardiac muscle cells of our patient is 9.7 g (mass of heart × cardiac muscle cell volume density/100 × mitochondria volume density/100). The total mitochondrial mass of the cardiac muscle cells of a healthy newborn (heart mass 23 g; Kissane et al. 1975) can be calculated to be 6.1 g, using the stereological data of Fleischer et al. (1978). This would indicate an increase of the mitochondrial mass in the heart of our patient of about 60%. Myofibrils do not contribute much to the hypertrophy since the increase in myofibrillar mass is only about 14% (calculations as described above). The stereological data of Fleischer et al. (1978) used above are obtained from 5 to 15 year old children. Use of these data in our calculations on the heart of a newborn seems justified since there are no major changes in the mitochondrial and myofibrillar volume densities during postnatal life (Page et al. 1974; David et al. 1978). Lipid accumulation may also have contributed to the cellular hypertrophy, but to a far lesser extent since lipid volume density is only about 1/10 th of mitochondrial volume density.

The increase in mitochondrial mass may be an attempt of the cardiac muscle cell to compensate for a defect in mitochondrial function. Deficient mitochondrial function is indicated by the marked lactic acidosis in our patient and by the observed lipid and glycogen accumulation. Furthermore the electron-cytochemical findings of cytochrome oxidase activity in our patient show that about half of the mitochondria in both heart and skeletal muscle fibre react negatively. It is important to note, however, that we do not have comparative information about the cytochrome oxidase activity in the heart and skeletal muscle of healthy newborns.

Negatively reacting mitochondria have also been demonstrated in the skeletal muscle fibres of patients with progressive external ophthalmoplegia (Müller-Höcker et al. 1983). In the fibres of these patients, where there is also an increase in mitochondrial mass, all mitochondria in one fibre are either positive or negative.

Other authors have used the term mitochondrial cardiomyopathy (Hübner and Grantzow 1983; Langes et al. 1985; Hübner et al. 1986) for myocardial disease in which the most prominent histopathological findings are structural and/or quantitative changes in the chondrioma of the cardiac muscle cell. The analogy with description in skeletal muscle pathology, in which mitochondrial myopathy is a generally accepted term (Sengers et al. 1984; DiMauro et al. 1985) seems to justify the use of the term mitochondrial cardiomyopathy for these diseases. This is supported by the fact that the same mitochondrial changes are present in both heart and skeletal muscle in our patient.

Besides the pathogenetic characterization "mitochondrial cardiomyopathy" we also use the de-

scriptive term "hypertrophic". The latter term seems to be justified, not only by the severe hypertrophy of the whole heart, but also by the striking, preponderant thickening of the ventricular septum. The hypertrophic mitochondrial variant of cardiomyopathy must not be mistaken for the idiopathic form, which is characterized by cardiac muscle cell disorganization (Maron et al. 1987) without any metabolic and usually also without dystrophic alterations of the myocardium.

Mitochondrial cardiomyopathy is also known to occur in the Leigh syndrome (Langes et al. 1985) and in the Kearns Sayre syndrome (Hübner et al. 1986). Comparing the findings in these reports with our findings, the increase of the mitochondrial mass of the cardiac muscle cell seems to be the most consistent finding in mitochondrial cardiomyopathy. The extensive fat deposition, the occurrence of intramitochondrial vesicles and the presence of crystalline inclusions in the matrix compartment (and not in the intracrystal space as usual, cf Stadhouders and Sengers 1987), are findings which have not been described by the above mentioned authors.

Electronlucent vesicles which seem to emerge from the mitochondrion have been seen in rabbit hearts which were rendered ischaemic and consequently reperfused (Post et al. 1985). The occurrence of these vesicles was dedicated to a pH drop and a rise in Ca^{2+} concentration. Our patient suffered from severe lactic acidosis, which may also have caused a strong decrement in the intracellular pH.

In summary it can be stated that the histopathological alterations of the heart muscle in the infantile form of the syndrome of hypertrophic cardiomyopathy, mitochondrial myopathy of skeletal muscle, cataract and lactic acidosis can best be described as a mitochondrial hypertrophic cardiomyopathy. In our case cellular hypertrophy plays an important role in the enlargement of the heart. Of all structural cellular components the mitochondria appear to contribute most to the cellular hypertrophy.

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