Virchows Archiv A Pathol Anat (1991) 419:355–362

Virchows Archiv A
Pathological Anatomy and Histopathology

Case report

Fatal infantile mitochondrial cardiomyopathy and myopathy with heterogeneous tissue expression of combined respiratory chain deficiencies

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Received May 6, 1991 / Received after revision July 15, 1991 / Accepted July 16, 1991

Summary. A 5-month-old boy died of progressive heart failure that started at the age of 3 months. Autopsy revealed a mitochondrial cardiomyopathy and a mitochondrial myopathy of the limb muscle and diaphragm. Cytochemically random defects of cytochrome *c* oxidase were visualized by light and electron microscopy in the diaphragm and especially the heart muscle, the limb muscle showing a diffuse attenuation whereas the liver and kidneys reacted normally. The activities of NADH-dehydrogenase (complex I) and cytochrome *c* oxidase (complex IV) were severely diminished (20% residual activity of controls) in the skeletal and heart muscle. In the heart, succinate cytochrome *c* reductase (complex II/III) was additionally decreased to the same degree. Loss of cytochrome *c* oxidase activity was based on a reduction of both mitochondrial and nuclear derived subunits in the heart and diaphragm as revealed by immunohistochemical analysis, whereas the limb muscle showed a normal immunoreactive protein content. The results illustrate heterogeneous tissue expression of respiratory chain enzyme defects and demonstrate that a cardiomyopathy may be the leading presentation of a mitochondrial disorder in early infancy.

Key words: Mitochondrial myopathy – Cardiomyopathy – Respiratory chain enzyme deficiency

Introduction

Disorders of the respiratory chain typically occur either as an isolated myopathy or as a multisystemic disease, especially involving the skeletal muscle, the brain (encephalomyopathy), either in early infancy or later in adolescence (Di Mauro et al. 1987a; Morgan-Hughes 1986; Morgan-Hughes et al. 1988). Besides the skeletal muscle and the brain, other organs, especially the liver, kidneys and the heart, may be affected. The associated defects of the respiratory chain (complexes I–V) may occur either singly or in combination. However, there is no strict correlation between the biochemical side of the defect and the clinical-morphological presentation (Petty et al. 1986). Deficiency of cytochrome *c* oxidase (complex IV) and of NADH-dehydrogenase (complex I) of the respiratory chain are the most commonly observed defects, running either a benign or fatal course (Di Mauro et al. 1987b; Morgan-Hughes et al. 1988). In this report we describe a 5-month-old boy who died of a mitochondrial cardiomyopathy associated with combined deficiencies of respiratory chain enzymes and heterogeneous tissue expression.

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Case report

The boy was the second child of healthy, non-consanguineous parents. Pregnancy and delivery were normal. Three days after birth, however, an episode of respiratory arrest and seizures were observed. Three months later an infection of the upper respiratory tract led to progressive dyspnoea. The heart was severely enlarged and ventricular contractility was greatly reduced. Continuous respiratory ventilation was required until the age of 5 months when irreversible cardiac arrest occurred. There was no clinical evidence of a cataract, but muscular hypotonia was conspicuous.

Laboratory investigations. Total carnitine was reduced to 13.9 μmol/l in the serum (control: 44.2–79.3 μmol/l) just as free carnitine: 8.45 μmol/l (control: 30.8–69.5 μmol/l), short-chain acyl carnitine being normal (5.25 μmol/l). In the skeletal muscle total carnitine was slightly reduced to 12.4 μmol/g non-collagen protein (control: 16.1–39.0 μmol/g NCP), free carnitine being in a low normal range 9.8 μmol/g NCP (control: 12.1–25.6 μmol/g NCP). In the heart muscle total carnitine (6.20 μmol/g NCP) and free carnitine (5.90 μmol/g NCP) were both in the normal range.

Lactic acidosis (4.5 μmol/l, pH 7.20), hyperammonaemia (370 μmol/l) and hypoglycaemia (0.22–1.38 μmol/l) developed repeatedly. No ketonuria was observed. In the urine there was excretion of 3-methylglutaconic and 3-methyl glutaric acid (Ibel et al., to be published). Glycogen storage diseases, hereditary fructose intolerance, fructose-1,6-diphosphatase deficiency as well as urea cycle disorders were excluded.

Biochemical determination of the respiratory chain enzyme activities in the limb muscle revealed an approximately 80% reduction of NADH cytochrome *c* oxidoreductase (2.44 units/g NCP, controls 11.3–28 units/g NCP) and of cytochrome *c* oxidase (19.4 units/g NCP, controls 90–281 units/g NCP) while succinate cytochrome *c* reductase was normal (6.4 units/g NCP, controls 6.0–25 units/g NCP), indicating severe combined deficiency of complex I (NADH-dehydrogenase) and complex IV (cytochrome *c* oxidase) of the respiratory chain. In the heart a similar severe deficiency of complex I (0.6 units/g NCP, controls 4.1–31.6 units/g NCP) and of complex IV (20.0 units/g NCP, controls 102–288 units/g NCP) existed. Succinate cytochrome *c* oxidoreductase was normal in the limb muscle, but was severely diminished in the heart (1.3 units/g NCP, controls 10.5–29.7 units/g NCP), indicating generalized impairment of the respiratory chain function. In the liver and the kidney no defects of the respiratory chain could be detected.

Materials and methods

Autopsy was performed 10 h after death. Tissue samples of the limb, heart muscle and diaphragm, liver and kidney were deep frozen in isopentane cooled liquid nitrogen and stored at −70° C for biochemical/cytochemical studies. Tissue samples were fixed in glutaraldehyde (6.25% in phosphate buffer pH 7.4) for 2 h, followed by washing in 0.2 M buffered sucrose and then processed further for routine electron microscopy. Cytochrome *c* oxidase activity has been determined histochemically as previously described (Müller-Höcker et al. 1983), but without the addition of hydrogen peroxide in the incubation medium. Succinate dehydrogenase (complex II) has been visualized according to Lojda et al. (1976).

Immunohistochemistry of cytochrome *c* oxidase was carried out as previously described (Müller-Höcker et al. 1989), but on unfixed frozen sections. The sections were fixed in 4% formaldehyde for 45 min, dehydrated in ethanol 70%, 95%, 100%, 95% and 70%, for on the whole 20 min, pretreated with hydrogen peroxide (7.5% in distilled water) for 15 min. The sections were then preincubated with normal goat serum [1:10 in phosphate-buffered saline (PBS)] for 20 min and afterwards incubated with the specific primary and secondary antibody (goat and rabbit antibody peroxidase conjugated) in PBS for 20 min using an ABC kit (Dako, Hamburg). Aminoethylcarbazol (0.01%) was used as chromogen in the peroxidase medium (0.0015% hydrogen peroxide). Specific antisera against the subunits I, II/III, IV, Vab, VIa, VIbc, VIIa, VIIbc and VIII of cytochrome *c* oxidase (Kadenbach et al. 1987) were used for the immunohistochemical study. The production and characterization of these antisera was previously described (Johnson et al. 1988; Merle et al. 1981). In this study the antisera against the subunits I, II/III, IV, Vab, VIa, VIbc, VIIbc were applied at ratio 1:50 (I, VIa, VIIa, VIII) and 1:300 (II/III, VIbc, VIIbc), 1:600 (Vab), 1:900 (IV).

Results

The heart showed marked biventricular hypertrophy (Fig. 1A), weighing 93 g (normal 35 g). The thickness of the free ventricle wall was 0.3 cm in the right chamber and 1.3 cm in the left. There was no fibroelastosis of the endocardium. The pericardial space was filled with serous fluid (150 ml), the epicardium being smooth. No valvular defects were apparent. The coronary arteries were normal. No anatomical obstruction of the left outflow tract existed.

Many cardiomyocytes showed a coarse-granular cytoplasm and a reduced content of myofibrils (Fig. 1B). There was no fibre disarray. No inflammatory cells or necrotic fibres were seen. In a lipid stain, massive accumulation of neutral lipids was detected. The liver also showed severe panlobular fatty metamorphosis with predominantly large lipid vacuoles, as did the kidneys, though to a lesser degree. Permission to examine the brain at autopsy was not obtained.

The ultrastructure of the heart revealed accumulations of enlarged mitochondra (up to 4 μm) with abnormal cristae showing tubular formations and a reduced content of myofibrils (Fig. 1C). The lamina basalis and the intercalated discs of the cardiomyocytes were normal. Lipofuscin was not conspicious. In the limb muscle and diaphragm no necrotic fibres were seen. The myofibrils were loosened by accumulation of lipid droplets and of mitochondria forming large subsarcolemmal aggregates, especially in the diaphragm with slight structural abnormalities, including the formation of tubular cristae. In the kidneys and the liver the mitochondria were inconspicuous. No mitochondrial microangiopathy was seen.

Cytochrome *c* oxidase staining revealed aggregates of cardiomyocytes devoid of enzyme activity randomly distributed in the heart muscle (Fig. 2A). Similar defects affecting disseminated fibres were present in the diaphragm (Fig. 2C). In the limb muscle a more diffuse attenuation of the reaction was seen. Ultracytochemistry disclosed that in the heart (Fig. 2D) and the diaphragm all the mitochondria of an involved cell/fibre had deficient activity. In the skeletal muscle a moderately reduced staining of mitochondria was present in the muscle fibres, indicating residual enzyme activity. No defects were present in the liver and the kidney for cytochrome *c* oxidase, and in all organs, including the heart and skeletal muscle, succinate dehydrogenase reacted normally (Fig. 2B).

Immunohistochemistry for the detection of cytochrome *c* oxidase protein revealed a defect pattern similar to the reduced staining for cytochrome *c* oxidase activity in the heart. There were randomly distributed cardiomyocytes with reduced enzyme protein content. Immunoreaction was most severely reduced in the reaction with antibodies recognizing the mitochondrially coded subunit II/III and the nuclear derived subunits Vab, VIIbc and to a lesser degree in the nuclear subunits IV, VIbc (Fig. 3). No defect was seen in subunit VIIa. The subunits I, VIa and VII generally reacted weakly, just as in controls.

In the diaphragm an identical defect pattern existed, affecting single fibres. In the limb muscle the enzyme protein content was normal.

Discussion

The clinical picture of our patient was dominated by a progressive cardiomyopathy leading to fatal cardiorespiratory failure at the age of 5 months. Muscular hypotonia indicated involvement of the skeletal muscle.

Biochemical analysis revealed a defect both of NADH-dehydrogenase and of cytochrome *c* oxidase (complex I and IV of the respiratory chain) in the skeletal and heart muscle, the latter also showing an equally

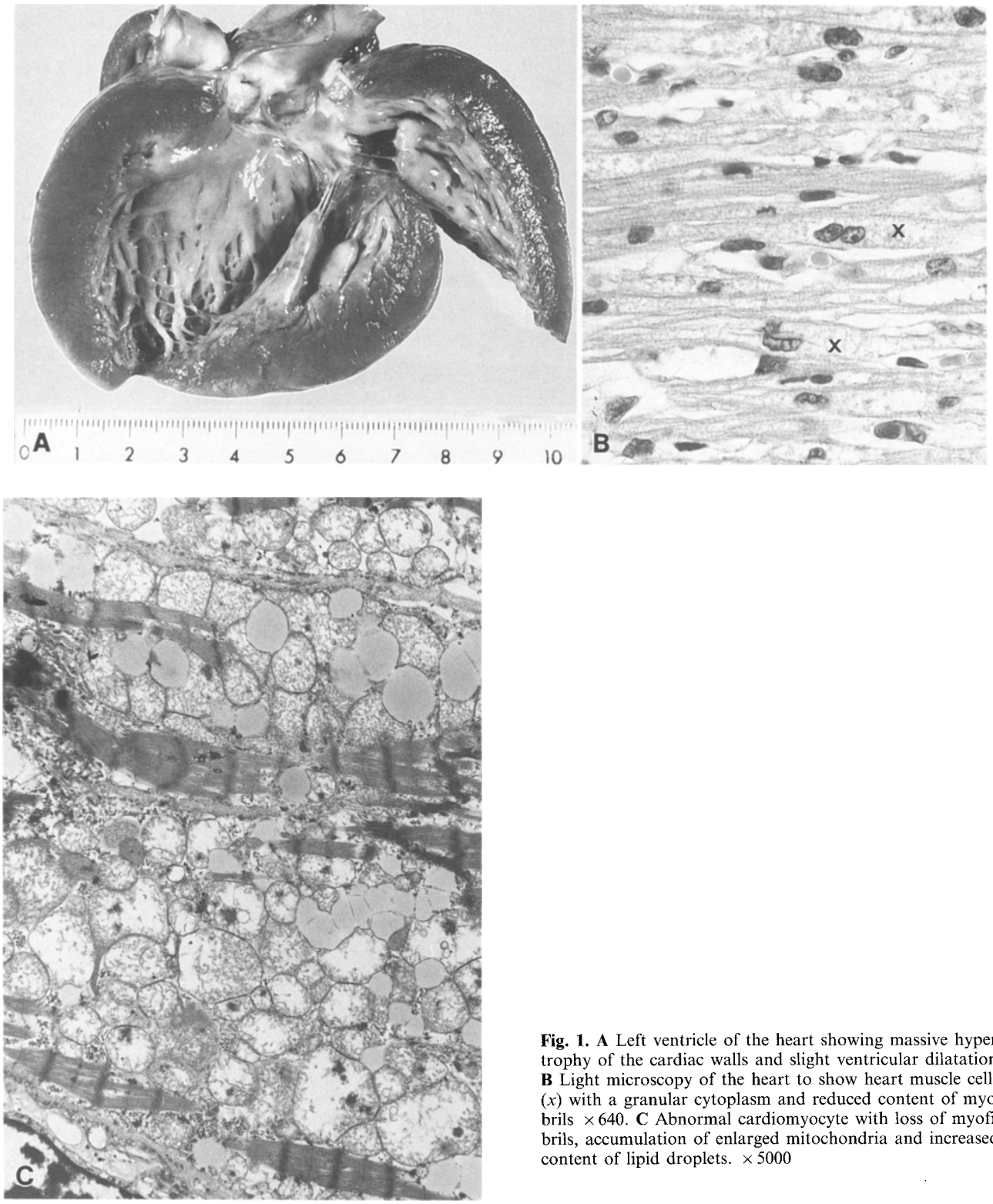

Fig. 1. **A** Left ventricle of the heart showing massive hypertrophy of the cardiac walls and slight ventricular dilatation. **B** Light microscopy of the heart to show heart muscle cells (*x*) with a granular cytoplasm and reduced content of myofibrils ×640. **C** Abnormal cardiomyocyte with loss of myofibrils, accumulation of enlarged mitochondria and increased content of lipid droplets. ×5000

severe loss of succinate cytochrome *c* oxidoreductase activity (complex II/III), which was normal in the skeletal muscle. In addition carnitine deficiency was evident in the serum and skeletal muscle. This is a well-known feature of mitochondrial disorders often due to loss of acylcarnitine in the urine (Engel and Rebouche 1984), in this case probably as 3-methylglutaconyl-carnitine and 3-methylglutaryl-carnitine (Ibel et al., to be published).

Enzyme histochemical studies of cytochrome *c* oxidase disclosed a random defect pattern in the heart with defective heart muscle cells next to normally reacting cardiomyocytes. In contrast to a recent report (van Ekeren et al. 1987), ultracytochemistry revealed that all the mitochondria of an affected heart muscle cell had lost enzyme activity and there was no evidence of intracellular heterogeneity. Single defective fibres occurred in the

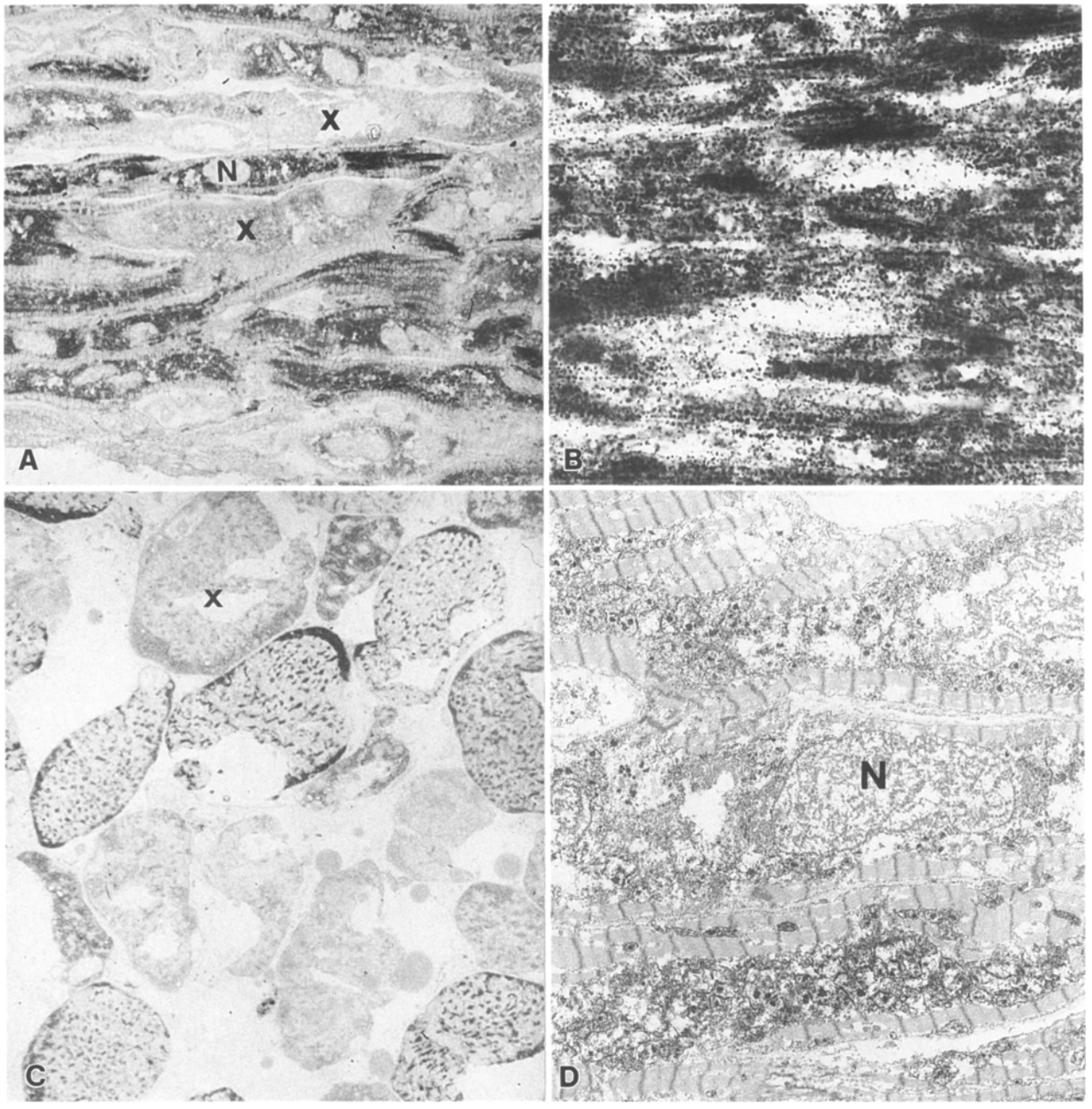

Fig. 2. A Cytochemistry of the heart and skeletal muscle. Randomly distributed cardiomyocytes with loss of cytochrome *c* oxidase activity (*x*). *N*, Nucleus. ×640. **B** Succinate dehydrogenase activity of the heart being intact. ×640. **C** Diaphragm with multiple cytochrome *c* oxidase deficient fibres, some of which show subsarcolemmal aggregates of mitochondria (*x*). ×640. **D** Ultracytochemistry of cytochrome *c* oxidase to show reduced activity in the mitochondria of heart muscle cells. The mitochondria of an adjacent cardiomyocyte react normally. *N*, Nucleus. ×3300

diaphragm, whereas in the limb muscle the reaction intensity was homogeneously diminished.

Various arguments point to the specificity of the heart lesion. First, the pattern of single defective cardiomyocytes is not typical of ischaemic damage and there were no signs of ischaemia. In addition, in ischaemic damage succinate dehydrogenase would also be defective. Second, in contrast to the present case, in myocardial infarction the enzyme protein of cytochrome *c* oxidase would be preserved (unpublished results). Third, the defects are not due to heart hypertrophy by itself, as they were lacking in a typical case of mitochondrial cardiomyopathy, the routine morphology of which has recently been published (Hübner and Grantzow 1983). Fourth, similar defects have been seen in a case of mitochondrial cardiomyopathy occurring as MELAS syndrome (Müller-Höcker et al., to be published) and in chronic progressive external ophthalmoplegia (Müller-Höcker et al. 1986), but also in the normal heart during ageing (Müller-Höcker 1989). Immunohistochemical studies for the detection of the enzyme protein revealed that loss of cytochrome *c* oxidase activity was based on a reduc-

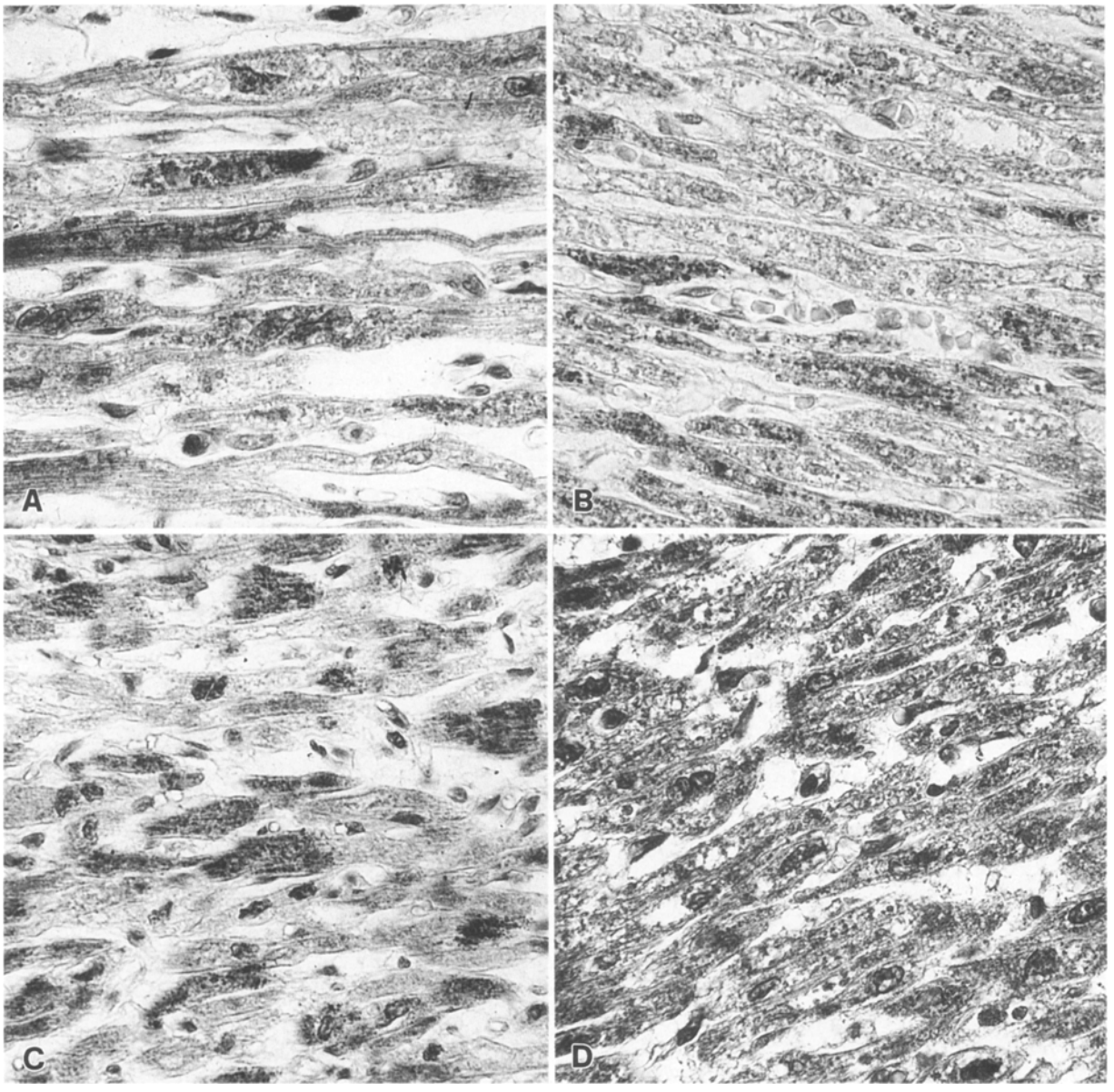

Fig. 3. Immunohistochemical detection of cytochrome *c* oxidase in the heart. There is loss of enzyme protein in the subunits II/III (**A**), IV (**B**), Vab (**C**), but not in subunit VIIa (**D**). **A–D** ×640

tion of enzyme protein in the heart and diaphragm but not in the skeletal muscle, both types of protein profile being well known in cytochrome *c* oxidase deficiency (Di Mauro et al. 1987a, b, 1988).

Involvement of the heart occurs in various muscular disorders (Perloff 1988; Rahlf et al. 1982; Ziter and Tyler 1986). In mitochondrial myopathies involvement of the heart is known in selective defects of complex I, III and IV (Di Mauro et al. 1987a, b, 1988; Katoch et al. 1985; Moreadith et al. 1984; Morgan-Hughes et al. 1988; Papidimitriou et al. 1984; Tanaka et al. 1986; Zeviani et al. 1986) and in combined deficiencies especially of complex I and IV, as seen in the present case (Müller-Höcker et al., to be published; Nishizawa et al. 1987; Tanaka et al. 1986; Zheng et al. 1989). Notably, the combined defect may be restricted to the heart, the skeletal muscle and the liver showing isolated complex I deficiency in a case of MELAS syndrome (Nishizawa et al. 1987).

However, cardiomyopathy may coexist with isolated cytochrome *c* oxidase deficiency in the skeletal muscle, the heart being unaffected (Rimoldi et al. 1982). Even in a case of a morphologically typical mitochondrial myopathy and cardiomyopathy, evidence of mitochondrial dysfunction may be lacking (Smeitink et al. 1989). Furthermore loss of cytochrome *c* oxidase activity may occur in the heart without clinical or morphological evidence of a cardiomyopathy (Arts et al. 1987; Koga et al. 1990), showing that there is no strict correlation between morphology, biochemistry and function.

Complex I and complex IV deficiency are the most common combined respiratory chain defects (Bleistein

and Zierz 1989; Koga et al. 1988a, b; Korenke et al. 1990; Mizusawa et al. 1988; Müller-Höcker et al. 1991; Peiffer et al. 1988; Roodhooft et al. 1986; Schapira et al. 1990a; Sherrat et al. 1984; Tanaka et al. 1987a, b). More seldom combined deficiencies of complex IV with complex III (Di Mauro et al. 1987a; Kennaway et al. 1987; Takamiya et al. 1986) or complex V (Müller-Höcker et al. 1985) are seen besides more complex defects involving more than two sides of the respiratory chain (Barth et al. 1983; Desnuelle et al. 1989; Malczewski and Whitfield 1982; Ruitenbeek et al. 1989; Schapira et al. 1990b; Tanaka et al. 1988).

In cases of predominant complex I deficiency the associated complex IV deficiency has been regarded as a secondary phenomenon (Koga et al. 1988a, b; Morgan-Hughes et al. 1988; Tanaka et al. 1986) developing in the progress of the disease, but the converse situation of pre-existing complex IV deficiency and ensuing complex I deficiency is also known (Koga et al. 1988b).

Generally, as both complex I and complex IV deficiency are the most common defects of the respiratory chain their coincidence might occur by chance. However, mutations of mitochondrial DNA may cause such combined defects as seen in cases with mitochondrial deletions (Gerbitz et al. 1990; Harding et al. 1990; Holt et al. 1989; Morgan-Hughes et al. 1990). Alternatively, a nuclear-coded protein involved in the control of translation and processing of the polycistronic mitochondrial messenger RNA might be defective (Takamiya et al. 1986), leading to extended deficiency of respiratory chain complexes. In yeast, for instance, about 30 nuclear proteins are necessary for the correct assembly of cytochrome *c* oxidase (McEwen et al. 1986). In our patient, however, the short course and the equally severe reduction of the complexes I and IV favour a true primary combined deficiency of the respiratory chain. Moreover, we cannot exclude the possibility that the 3-methylglutaconic and 3-methylglutaric aciduria observed in this infant was secondary to the deficiencies of complex I and/or IV, since the activity of 3-methylglutaconyl-CoA hydratase was found to be normal (Ibel et al. 1991).

The tissue-selective and heterogeneous tissue expression of enzyme defects of the respiratory chain as seen in the present patient is a well-known feature in mitochondrial myopathies (Di Mauro et al. 1987a, 1988; Morgan-Hughes et al. 1988; Müller-Höcker et al. 1989; Sperl et al. 1990), being due either to the presence of tissue-specific isoenzymes (Clay and Ragan 1988; Kuhn-Nentwig and Kadenbach 1985; Schlerf et al. 1988; Walker et al. 1988) or to unbalanced segregation of mutated and wild mitochondrial DNA, leading to the biochemical manifestation of the defect when a certain threshold level of mutated mitochondrial DNA is reached. In fact, recent studies indicate that mutations of mitochondrial DNA occur in cases of infantile and adult cardiomyopathy (Ozawa et al. 1990; Tanaka et al. 1990).

Acknowledgements. The authors thank Mrs. Karin Schneiderbanger for excellent technical assistance and Mrs. Petra Tränkel for skilful work in preparing the manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft (Mu 755/1–2).

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