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*Case report*

# Ultrastructural abnormalities of mitochondria and deficiency of myocardial cytochrome *c* oxidase in a patient with ventricular tachycardia *

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**Summary.** A 30-year-old woman presented with life-threatening ventricular tachycardia without overt heart disease. Ultrastructural investigation of endomyocardial biopsy disclosed abnormally structured and often enlarged mitochondria. Morphometry revealed the ratio of volume density of mitochondria to myofibrils to be markedly increased to 0.667 as compared with five controls (mean: 0.46; range: 0.445–0.479). Investigation of mitochondrial respiratory chain enzymes revealed a 90% reduction in activity of cytochrome *c* oxidase. Our data suggest that mitochondrial cardiomyopathy may induce malignant ventricular arrhythmias.

**Key words:** Mitochondrial cardiomyopathy – Ultrastructure – Cytochrome *c* oxidase deficiency – Ventricular tachycardia

## Introduction

Ventricular tachycardia and fibrillation are life-threatening arrhythmias, usually secondary to coronary artery disease. Other causes include hypertrophic and dilated cardiomyopathy, aortic valve disease, long QT syndrome, mitral valve prolapse, Wolff-Parkinson-White syndrome, arrhythmogenic right ventricular disease, coronary artery spasm and acute myocarditis (Segal et al. 1985; Dunnigan et al. 1987). In a few patients, malignant arrhythmias have occurred without obvious heart disease and metabolic abnormalities have been suggested as the underlying cause (Lemery et al. 1989).

We report here the case of a young woman with life-threatening arrhythmias in whom morphological and biochemical investigations of right septal endomyocardial biopsy revealed a mitochondrial cardiomyopathy.

## Case report

A 30-year-old woman suffered a cardiac arrest, was resuscitated by her husband and subsequently defibrillated by an emergency physician. After resuscitation there was no evidence of acute myocardial infarction, intoxication or metabolic disorder.

Skeletal muscle atrophy had been documented since childhood. Her mother and an aunt have also had muscular weakness since childhood. There was no family history of cardiac disease. In 1982, she was diagnosed as a hereditary motor and neuropathy type I according to the criteria of Dyck (1984). In 1983, she complained of palpitations and an electrocardiogram showed negative T-waves in V3–6. Echocardiography disclosed thickening of both the septum and the free wall of the left ventricle to 12 mm. Left ventricular diameter and contractility were normal. The patient was without medication and felt well until her cardiac arrest.

On the intensive care unit numerous attacks of ventricular tachycardia at a rate of 200/min were observed. Laboratory investigations revealed repeatedly normal values for electrolytes, creatinine, cardiac and liver enzymes, folic acid, phytanic acid, cobalamine, carnitine, thyroid hormones and immunological variables including antinuclear antibodies. Red and white blood cell counts, blood smear and bone marrow histology were normal. There was no evidence of acute virus infection in multiple serological tests. Ophthalmological examination gave normal findings.

The chest radiograph was normal. The electrocardiogram showed sinus rhythm, normal electrical axis, prominent P-waves in lead I and II and pre-terminal negative T-waves in I, II, aVF and V3–V6. The echocardiogram revealed slightly reduced contractions, but the other dimensions were as in 1983. Cardiac catheterization excluded valvular and coronary heart disease as well as intraventricular obstruction. Left ventricular end-diastolic pressure was elevated (27 mmHg); ejection fraction was slightly reduced (60%). Electrophysiological investigation showed normal atrioventricular and intraventricular conduction. Using programmed right atrial and ventricular stimulation, no tachycardias could be induced. However, programmed left ventricular stimulation at 300 beats/min induced a monomorphic ventricular tachycardia reproducibly. This had to be terminated by external defibrillation. An automatic implantable cardioverter defibrillator (AICD) was implanted. During half a year on therapy with sotalol (160 mg t.i.d.), she received five shocks by the AICD. On one of these

* Dedicated to Professor Waldemar Hort on the occasion of his professor emeritus

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occasions a monomorphic ventricular tachycardia at a rate of 280 beats/min was documented. Therapy with coenzyme Q (100 mg/day) was added and in the following 1.5 years only one shock was recorded.

## Methods

A right septal endomyocardial biopsy was done under fluoroscopic control in our patient and in five controls (3 men, 2 women; mean age: 52, range 40–66 years) who underwent cardiac catheterization for the exclusion of coronary and myogenic heart disease. Left ventricular end-diastolic pressures ranged from 6 to 8 mmHg, mean 7 mmHg; ejection fractions from 71 to 89%, mean 75% in controls. Morphological (3–5 pieces per patient) and biochemical studies (2 pieces per patient) were done on biopsies. An open skeletal muscle biopsy specimen was also taken from the right gastrocnemius muscle of the young woman for light and electron microscopy and biochemical investigations. All patients gave their written consent to the investigations.

One part of the right septal endomyocardial biopsy was fixed in 4% formaldehyde and embedded in paraffin for light microscopy. Sections were stained with haematoxylin and eosin and elastic van Gieson. For electron microscopy pieces were fixed in 2.5% cacodylate-buffered glutaraldehyde, dehydrated in graded ethanol series and embedded in Epon. Semi-thin sections were stained with methylene blue; ultra-thin sections were contrasted with uranyl acetate and lead citrate. Specimens were examined with a Zeiss electron microscope EM 109 at 50 kV.

The diameter of the cardiomyocytes was determined across the nucleus from 3 biopsy pieces per patient, measuring 75 cardiomyocytes in each biopsy sample at a magnification of $\times 1250$. The proportion of fibrous tissue was determined from the elastic van Gieson stained sections of 3 biopsy pieces per patient by point counting [8 superimposed grids (100 points each) on random sections per biopsy piece at $\times 500$, according to a previously described protocol (Schwartzkopff et al. 1987)]. Volume densities of myofibrils, mitochondria, and sarcoplasm were determined between the nucleus and the intercalated disc of cardiomyocytes from 10 electron microscopic pictures per patient at a magnification of $\times 10100$

**Table 1.** Morphometric findings

| | Cardiomyocyte diameter (μm) | Fibrosis (Vv%) | Myofibrils (Vv%) | Mitochondria (Vv%) |
|---|---|---|---|---|
| Controls | | | | |
| Mean ± SD | 12.9 ± 0.8 | 1.8 ± 0.7 | 57.8 ± 1.8 | 26.7 ± 1.3 |
| Range ($n=5$) | (11.8 – 13.8) | (0.6 – 2.5) | (55.5 – 60.1) | (25.3 – 28.2) |
| Patient | | | | |
| Mean ± SD | 16.4 ± 2.8 | 6.3 ± 2.4 | 43.3 ± 8.9 | 28.9 ± 9.5 |

SD Controls, standard deviation derived from the mean of patients; SD Patient, standard deviation of repeated measurements

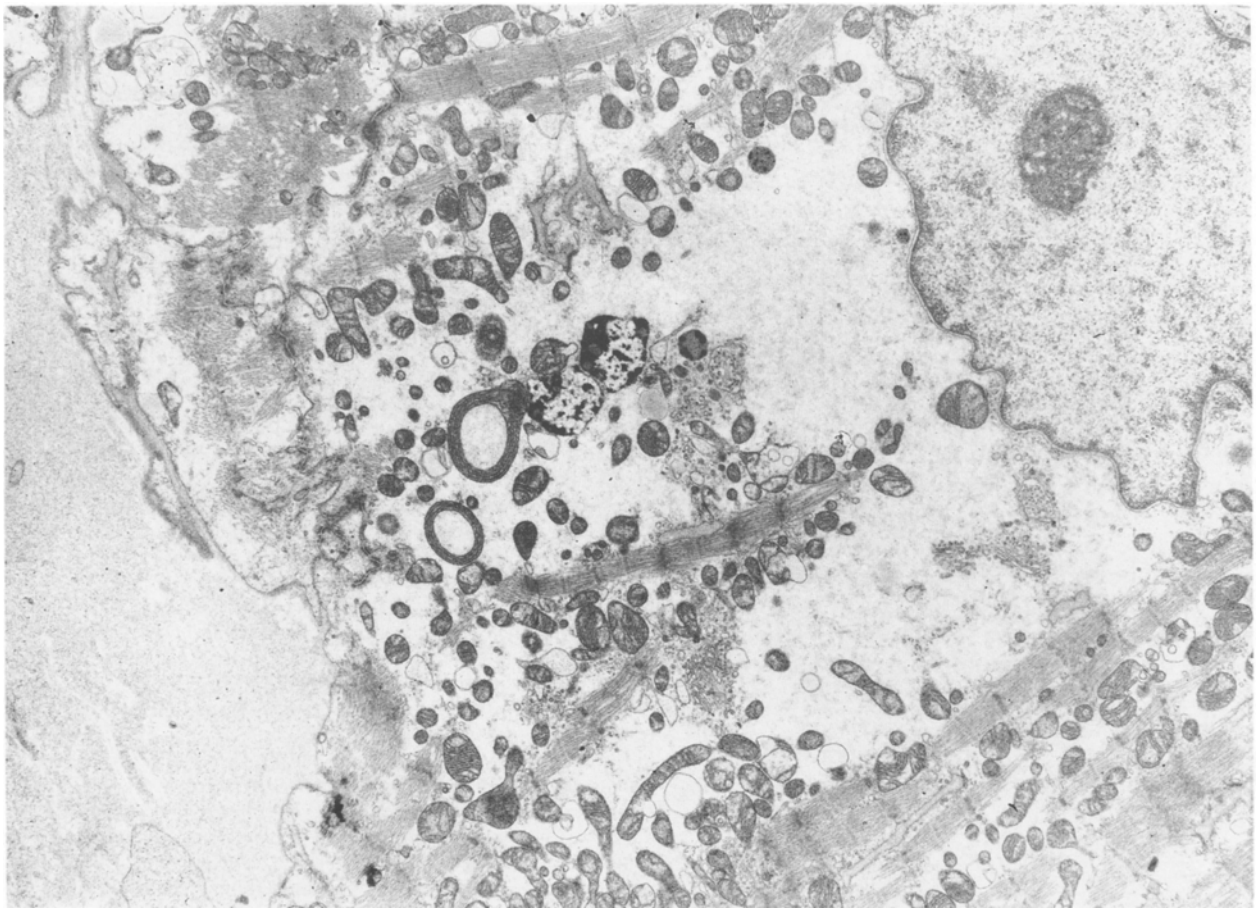

**Fig. 1.** Cardiomyocyte with pleomorphic mitochondria; cristae are arranged in parallel or concentric. In the increased content of sarcoplasm the myofibrils are reduced. Electron microscopy, $\times 12000$ (Reduced to 70%)

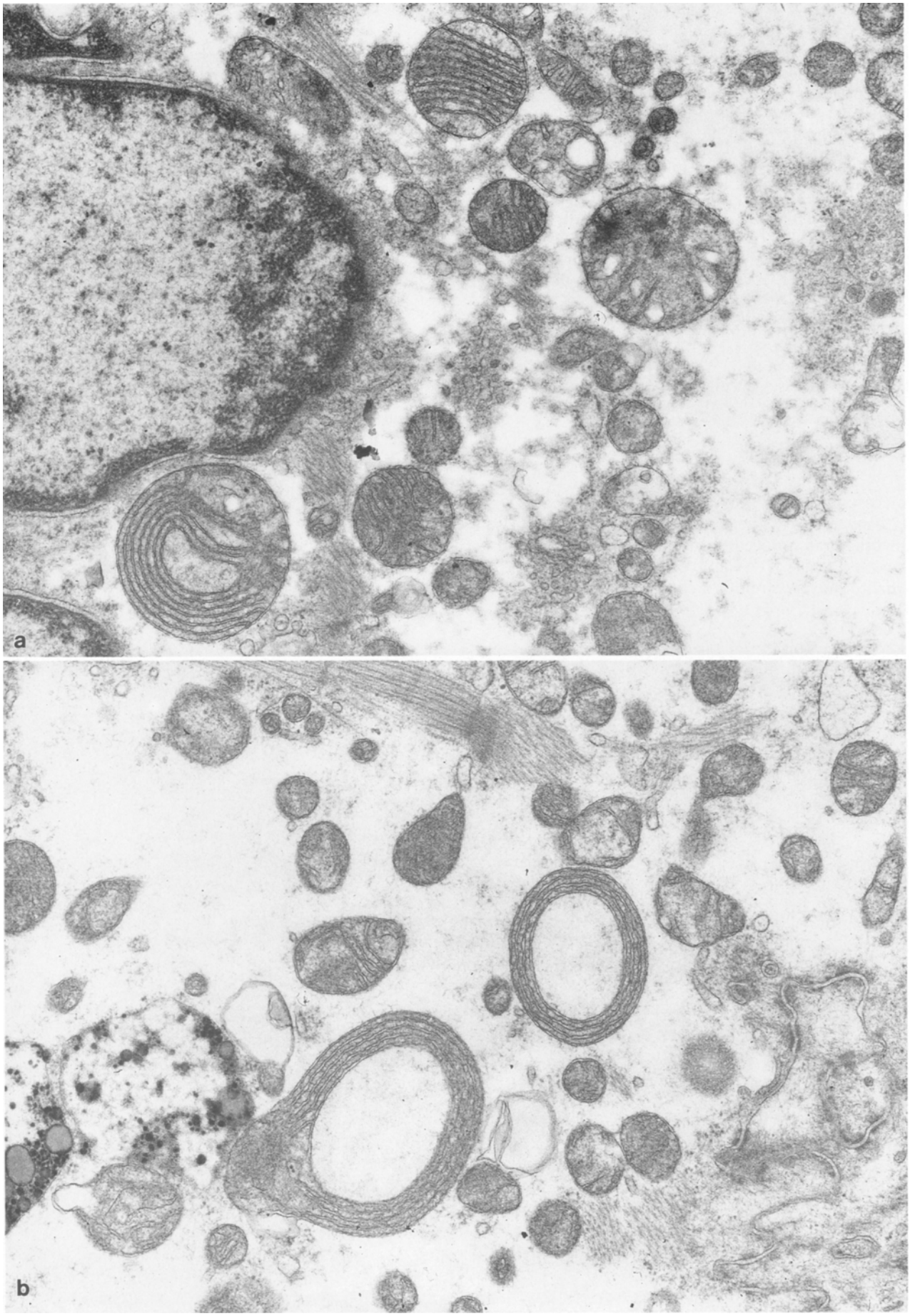


**Fig. 2. a** Cardiomyocyte with numerous bizarre mitochondria of very small and very large size near to the indented nucleus. Some mitochondrial cristae are tubular-like swollen, often fragmentary. A few mitochondria have a parallel or concentric arrangement of their cristae. Electron microscopy, ×40800 (Reduced to 70%). **b** Two large mitochondria with concentric cristae surrounded by small mitochondria with fragmented cristae. Electron microscopy, ×43200

by point counting according to well-established morphometrical rules using a grid with 360 points (Weibel 1969; Mall et al. 1982).

For biochemical analyses heart and skeletal biopsies were frozen immediately at −150° C and then stored under liquid nitrogen. Specimens were homogenized with 29 vol. ice-cold Chappel-Perry medium containing 50 mM Tris-HCl (pH 7.4), 100 mM potassium chloride, 5 mM magnesium chloride, 1 mM EDTA using a glass/glass homogenizer. Activities of respiratory chain enzymes were measured spectrophotometrically in heart muscle homogenates. Total activity of NADH-cytochrome *c* reductase was measured as described by Hatefi and Rieske (1967). The activity of complex I+III was determined as the antimycin A and rotenone sensitive fraction of total NADH-cytochrome *c* reductase in the presence of antimycin A (5 μg/ml) and rotenone (5 μg/ml). Succinate cytochrome *c* reductase (complex II+III) was measured as described by Tisdale (1967), succinate dehydrogenase (part of complex II) as described by Hatefi and Stiggal (1978), and cytochrome *c* oxidase (complex IV) as described by Wharton and Tzagaloff (1967). Non-collagenous protein was determined according to Lowry et al. (1951).

## Results

Light microscopy of the myocardium showed cardiomyocyte hypertrophy with some atrophic fibres. Abnormal branching was noted and mean cardiomyocyte diameter was increased (Table 1). Perinuclear vacuolation was frequent. Nuclei were often enlarged. There was interstitial and focal fibrosis. There were few intramural arteries but they appeared to be normal. No evidence of acute or chronic myocarditis was seen.

Electron microscopy revealed abnormal mitochondria in numerous cardiomyocytes (Figs. 1, 2). Mitochondria were pleomorphic and focally increased in number. Mitochondrial cristae were often oriented in parallel or were partially fragmented (Fig. 2a, b). About 40% of all cardiomyocytes showed a few mitochondria with concentric cristae. Electron-dense globular bodies could be found in some mitochondria but there were no paracrystalline inclusions. The volume fraction of myofibrils was reduced (Table 1) and the mitochondria to myofibrils ratio was increased (0.667 vs controls: $0.460 \pm 0.016$; range 0.445–0.479). Glycogen as well as lipid droplets seemed increased in some myofibres. No alterations were seen in the biopsies of controls (Fig. 3). Quantitative data of controls were comparable with those reported as normal by Schaper et al. (1985).

Light microscopy of the skeletal muscle showed chronic neurogenic muscular atrophy with secondary myopathic changes. Modified Gomori trichrome stain as well as electron microscopy gave no evidence of mitochondrial myopathy.

Biochemically the patient's cardiac muscle cytochrome *c* oxidase activity was markedly decreased when compared with controls (Table 2). The activities of the other respiratory chain enzymes were within the control

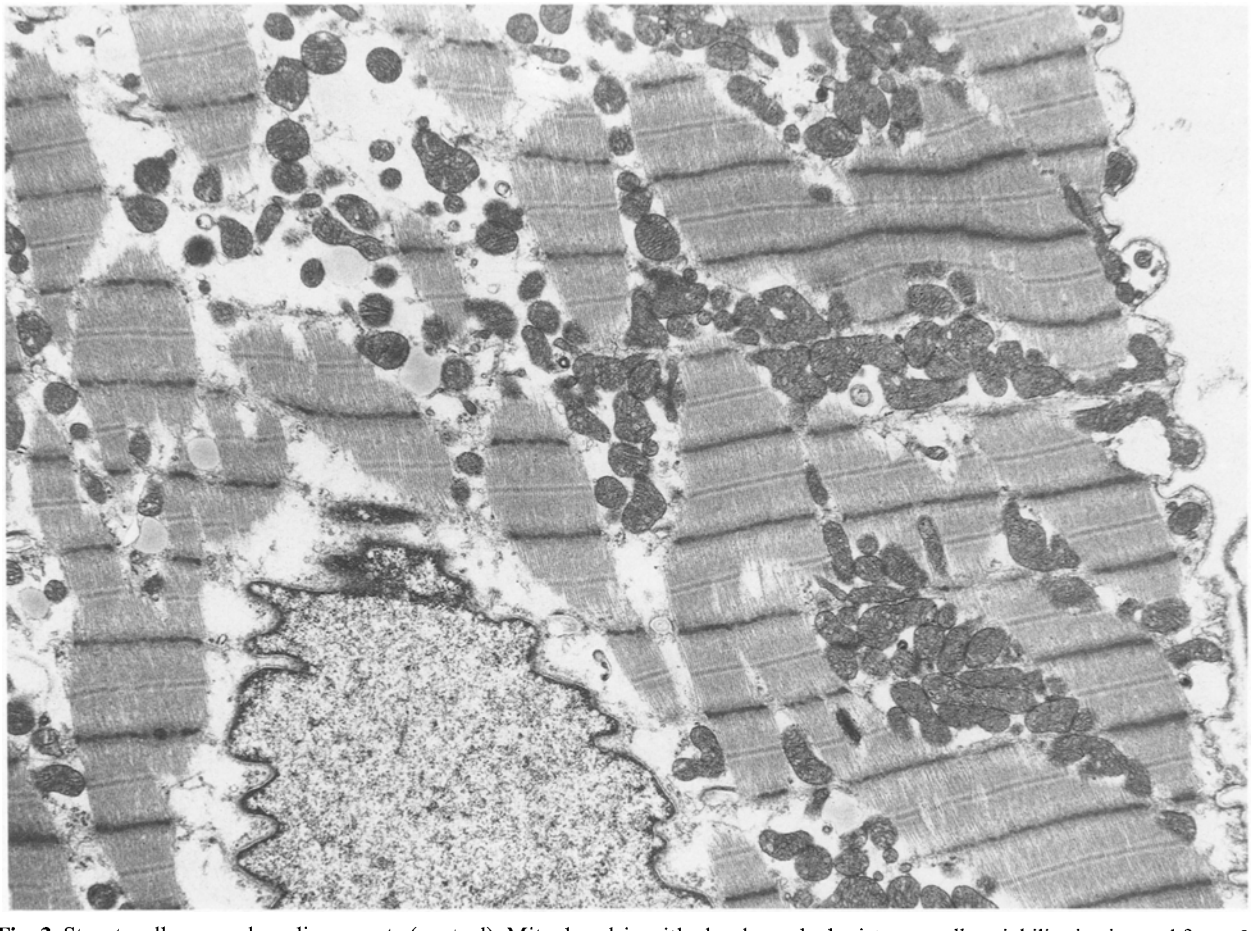

**Fig. 3.** Structurally normal cardiomyocyte (control). Mitochondria with closely packed cristae, small variability in size and form. Myofibrils are arranged in parallel. Electron microscopy, ×12000 (Reduced to 70%)

**Table 2.** Biochemical findings: activities of mitochondrial respiratory chain enzymes in homogenates of heart and skeletal muscle

| | Total NADH-cytochrome *c* reductase | I+III | SDH | II+III | IV |
|---|---|---|---|---|---|
| Heart controls | | | | | |
| Mean±SD | 28.8±14.5 | 7.0±4.7 | 86.1, 49.5 | 42.9±21.6 | 185.5±100.9 |
| Range | 13.0–49.8 | 2.0–14.2 | | 22.4–66.8 | 99.8–325.0 |
| *n* | 5 | 5 | 2 | 4 | 5 |
| Patient | 33.7 | 5.9 | 53.3 | 18.9 | 18.3 |
| Skeletal muscle Controls ($n=24$)[a] | | | | | |
| Mean±SD | 23.0±7.8 | 9.9±3.7 | 28.1±10.3 | 14.9±6.5 | 136.0±39.7 |
| Range | 10.1–40.5 | 4.2–16.2 | 12.5–56.2 | 6.1–31.6 | 81.5–216.4 |
| *n* | 24 | 24 | 24 | 24 | 24 |
| Patient | 23.5 | 4.2 | 16 | 8.4 | 141.3 |

Enzyme activities are expressed as $\mu mol \times min^{-1} \times g$ non-collagen protein$^{-1}$. I+III, Antimycin A-sensitive activity of total NADH-cytochrome *c* reductase, i.e. complexes I+III, SDH, succinate dehydrogenase; II+III, complexes II+III; IV, complex IV, i.e. cytochrome *c* oxidase

[a] According to Zierz et al. (1989)

range. In the patient's skeletal muscle the activities of respiratory chain enzymes were all normal (Table 2) (Zierz et al. 1989).

## Discussion

Structural heart disease is the main cause of ventricular tachycardia and fibrillation. In our patient, mild left ventricular hypertrophy, elevated end-diastolic pressure and slightly reduced contractions are not sufficient to explain the occurrence of malignant ventricular arrhythmias. The long history of minor cardiac abnormalities suggests a latent myocardial disease. It is noteworthy that there was an associated neuromuscular disease. Myocardial hypertrophy, heart failure, as well as malignant arrhythmias and sudden death have been described in various hereditary neuromuscular diseases (Welsh et al. 1963; Lascelles et al. 1970; Perloff 1972; Grigg et al. 1985). In Friedreich's ataxia, a cardiomyopathy of metabolic aetiology was suspected because of a deficiency in mitochondrial malic enzyme in fibroblasts (Stumpf 1982). In our patient the cardiac process seems to be unrelated to the neurogenic muscle disease, because only the cardiac mitochondria were pathological.

Light microscopical findings of right septal endomyocardial biopsy suggest a cardiomyopathy because of the fibrosis and the hypertrophy of cardiomyocytes. Electron microscopy shows mitochondrial changes which are typical but non-specific for mitochondrial disease (DiMauro et al. 1985). The quantitative data reveal a marked reduction of myofibrils and an increase in the mitochondria to myofibrils ratio, suggesting a reduced protein synthesis per mitochondrial volume (Askanas et al. 1978). It is possible that in our case mitochondriosis and cardiac hypertrophy are compensatory mechanisms for a pathological mitochondrial metabolism.

There are a few reports of mitochondrial cardiomyopathies with mitochondria of abnormal size and concentric cristae (Hug and Schubert 1970), tubular or lamellar cristae (Hübner and Grantzow 1983), tubular and myelinic transformation of cristae (Langes et al. 1985), vacuolized or packed with concentric or parallel cristae (Schwartzkopff et al. 1988) or mitochondria showing concentric cristae, dense bodies and sometimes paracrystalline arrays (Neustein et al. 1979) and mitochondria consisting of ring forms or fingerprint pattern (Urie and Billingham 1988). The slight differences in the structural mitochondrial abnormalities, the severity of heart failure, the differences in inheritance, the involvement of other organ systems and the variety of clinical symptoms may perhaps be explained by different biochemical defects in the reported cases.

In our young patient structurally abnormal mitochondria are associated with a markedly reduced activity of cytochrome *c* oxidase, the complex IV of the respiratory chain enzymes. These enzymes are located in the inner mitochondrial membranes. Physiological ageing of the heart is combined with a decrease of cytochrome *c* oxidase activity without grossly abnormal mitochondria (Müller-Höcker 1989). In myopathy, associated with a cytochrome *c* oxidase deficiency, there are usually structural abnormalities of mitochondria (Müller-Höcker 1988).

The most common clinical symptoms of cytochrome *c* oxidase deficiency are congenital myopathy, renal dysfunction or encephalomyopathy. However, a variety of other clinical symptoms may occur and the heart is not regularly involved (DiMauro et al. 1987). The association of myopathy and cardiomyopathy was described by Zeviani et al. (1986) in an 8.5-month-old girl who died from cardiac failure. Activity of cytochrome *c* oxidase in this case was reduced in cardiac mitochondria to 12.2% of normal and in skeletal muscle to 7.3%. Deficiency of cytochrome *c* oxidase was also demonstrated by immunohistochemical studies in the myocardium of a 26-year-old man with Kearns-Sayre syndrome who suffered from malignant arrhythmias and died in intractable congestive heart failure (Müller-Höcker et al. 1986). Cardiomyocytes with normal and abnormal numbers of mitochondria showed enzyme defects, suggesting that the biochemical abnormalities precede the structural changes.

In our patient the coincidence of normal skeletal but abnormal cardiac mitochondria provides evidence for tissue specificity of the enzyme. Cytochrome *c* oxidase is composed of at least 13 subunits (Kadenbach et al. 1983). Three catalytic subunits (I–III) are encoded by mitochondrial DNA, but ten small subunits by nuclear DNA (Kadenbach et al. 1987; DiMauro et al. 1988). The latter are tissue specific. Therefore, our observation might be explained by abnormalities of the nuclear-encoded and tissue-specific subunits of cytochrome *c* oxidase. Whether the mitochondrial pathology is secondary or primary, caused by abnormal enzyme subunits with secondary loss of protein components or by reduced protein synthesis cannot be answered by our investigations. Nevertheless, the case presented here indicates that malignant arrhythmias in patients without obvious heart disease may be induced by mitochondrial cardiomyopathy.

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