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Case report

Mitochondrial cardiomyopathy with involvement of skeletal muscles

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Summary. In this report we describe an idiopathic hypertrophic cardiomyopathy in a 21 month old infant girl, who died shortly after a small surgical intervention for cardiovascular failure. Fine structural investigation disclosed an extreme increase of often abnormally structured and enlarged mitochondria and a great loss of myofibrils in the heart muscle cells. Furthermore, mitochondrial hyperplasia was observed focally in all skeletal muscles investigated. The pathogenesis of this mitochondriopathy in heart and skeletal muscle is unknown. It might be a consequence of a functional mitochondrial defect with compensatory hyperplasia of mitochondria. Differential diagnosis of this very rare infantile cardiomyopathy from the myopathies of storage diseases, typical hypertrophic cardiomyopathy and carnitine defiency is discussed, as is the distinction from the oncocytic or so-called histiocytic transformation of heart muscle cells. Methological hints for diagnostic procedures are given.

Key words: Mitochondrial cardiomyopathy – Mitochondrial myopathy – Idiopathic hypertrophic cardiomyopathy in children – Abnormal mitochondria – Hyperplasia of mitochondria in heart and skeletal muscle

Introduction

Mitochondrial myopathy, a congenital muscle disease with an increased number of abnormal mitochondria in the muscle fibers, has been observed quite frequently in recent years (for literature see Schröder 1982). However, mitochondrial cardiomyopathies with augmentation of abnormal mitochondria in the heart muscle cells are quite rare. Nearly all reports on these

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mitochondrial myopathies are concerned with focal transformation of heart muscle cells into oncocytic or so-called histiocytic cells, which are filled with mostly abnormal mitochondria (for literature see Silver et al. 1980). A generalised diffuse mitochondrial cardiomyopathy seems to be extremely rare and to our knowledge there are only a few reports on a probably diffuse mitochondrial cardiomyopathy diagnosed in a endomyocardial biopsy (Hug and Schubert 1970; Mackay et al. 1976; Neustein et al. 1979). For this reason we present a case of generalised mitochondrial cardiomyopathy diagnosed from the autopsy of an infant girl. There was also an additional mitochondrial myopathy in all skeletal muscles investigated.

Case report

An infant girl, second child of healthy parents, was born with a congenital cleft of the soft palate. No infantile muscle or heart disease is known, in the family of the child no cases of sudden death in infancy are reported. The child learned to sit up and to walk later than usual. When, at the age of 6 months, the cleft in the palate was to be corrected, difficulties at the beginning of anaesthesia with halothane and N_2O required the interruption of narcosis. Consecutive cardiac catheter investigation revealed a non-obstructive hypertrophic cardiomyopathy of unknown etiology. At the age of 21 months surgical intervention was undertaken. The preoperative ECG revealed a sinus rhythm of 145/min, signs of a hypertrophy of the left ventricle, a pathological Q profile and a S-T-abnormality. A few hours later, the child developed tachycardia, followed by bradycardia and a short cardiac arrest. The first analysis of blood gases was done 30 min after the beginning of surgery. It revealed a metabolic acidosis (pH 7,25 PCO_2 41,1, base excess $-8,2$ rising to $-13,9$ on the first post-operative night. These values returned to normal within a short time. No measurements of lactate were done. Following recovery from anaesthesia 2 days later the girl showed wide fixed pupils and died 7 days after surgery with the symptoms of brain death and cardiac failure.

Clinical diagnosis. Cardiomyopathy of unknown etiology, possibly glycogen storage disease (Pompe type)

Autopsy

At autopsy the heart was rounded and enlarged, weighing 200 g, more than three times the usual weight at this age. The left ventricle was intensely hypertrophied, especially in the septal region. Its lumen was very small, the left outflow region was moderately narrowed. The right ventricle was also hypertrophied. The heart valves were intact, no defects in the atrial or ventricular septum were observed. The coronary arteries were without abnormalities. Skeletal muscle tissue of different muscles showed no pecularities macroscopically. Congestion of the liver, spleen and kidneys and edema of the lungs indicated circulatory and cardiac failure. The brain was necrotic.

Material and methods

Tissue for light microscopic investigation was fixed in formalin and embedded in paraffin. Sections were stained with H. and E., after van Gieson, and with the PAS reaction. For electron microscopy specimen from both atria and ventricles, the cardiac septum, the liver, both kidneys and the M. quadriceps femoris were fixed in glutaraldehyde (6,25%), postfixed in 2% buffered osmic solution for 2 h, washed in 0,2 M sucrose solution and embedded in Epon. Semithin sections were stained with Azur-II-Methylenblue (Richardson et al. 1960) and PAS respectively. Ultrathin sections were contrasted in uranyl acetate and lead citrate and inspected in a PHILLIPS 300 electron microscope. For further electron microscopic investigation the following paraffin embedded specimens were reembedded in Epon (Hübner 1970):

1. Heart muscle from the left and right ventricle. 2. Muscle tissue of the tongue, intercostal muscles, diaphragm, M. biceps, M. gluteus max., M. quadriceps fem., and of M. tib. ant. 3. One specimen from the wall of the stomach.

Results

Light microscopy

The slightly pale heart muscle showed no interstitial fibrosis or fatty infiltration. After the PAS reaction the glycogen content seemed slightly increased. The muscle cells displayed an indistinct cross striation and a diffuse pale eosinophilic granulation of the whole cytoplasm. This granularity was more obvious in the Epon-embedded semithin sections. The skeletal muscle tissue contained little glycogen and no increase of fat droplets. Focal granularity of some muscle fibers was also seen here, especially in Epon-embedded thin sections (Fig. 1). The other organs displayed non-specific changes only.

Electron microscopy

Fine structural analysis of the heart reveals the same appearance in all specimens: The cells are filled with many rounded, often enlarged mitochondria (Fig. 2), about 40% of which are abnormally structured, containing piles of lamellar or tubular cristae (Fig. 3 and 4). Intramitochondrial glycogen granules are often seen in monoparticular or rosette form (Fig. 3–5).

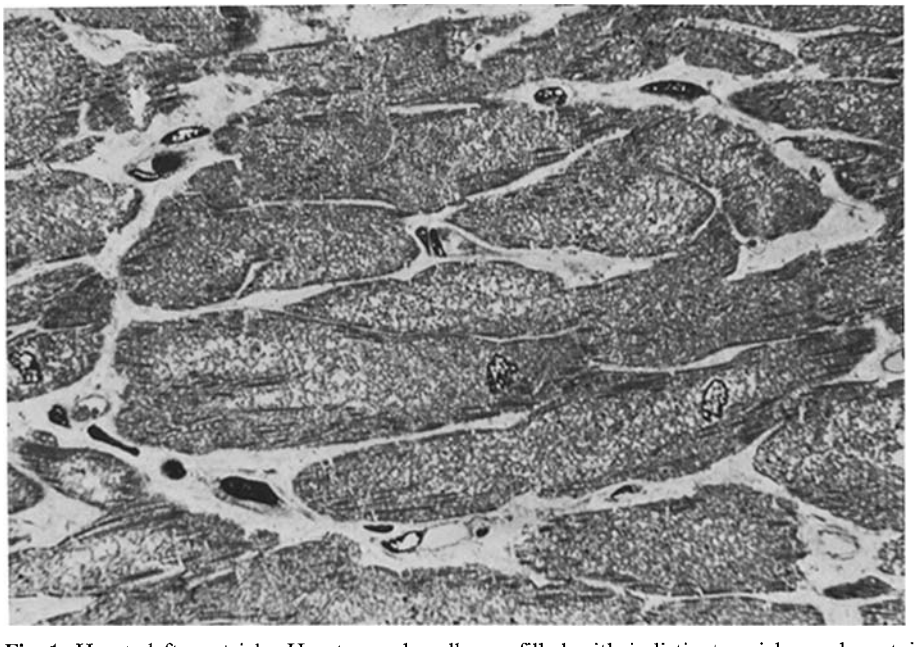

Fig. 1. Heart, left ventricle. Heart muscle cells are filled with indistinct vesicles and contain only few scattered myofibrils. Azur methylene blue stained semithin section. × 540

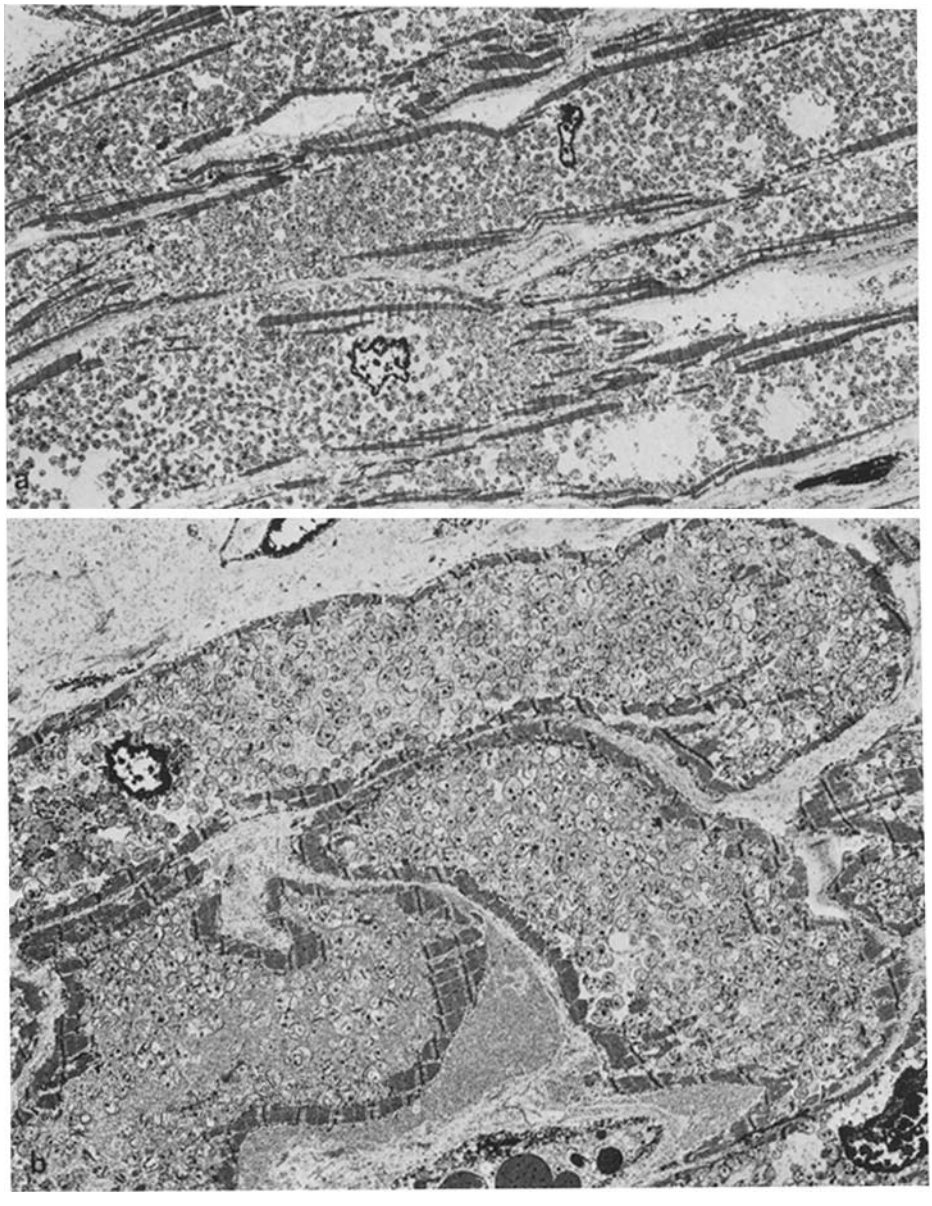

Fig. 2a, b. Heart, left ventricle. Heart muscle cells are filled with rounded mitochondria. Loss of myofibrils. **a** Formalin fixed paraffin embedded specimen reembedded in Epon for electron microscopy. × 1,800. **b** Glutaraldehyde fixed specimen. × 2,200

Some mitochondria contain round electron dense inclusions without a limiting membrane, which measure up to $^1/_2$ µm in diameter (Fig. 5a). The mean diameter of the normally structured mitochondria measures about 1,3 µm, that of the abnormally structured mitochondria from 1,6–2,0 µm. The number of myofibrils in the heart muscle cells is greatly reduced. Many

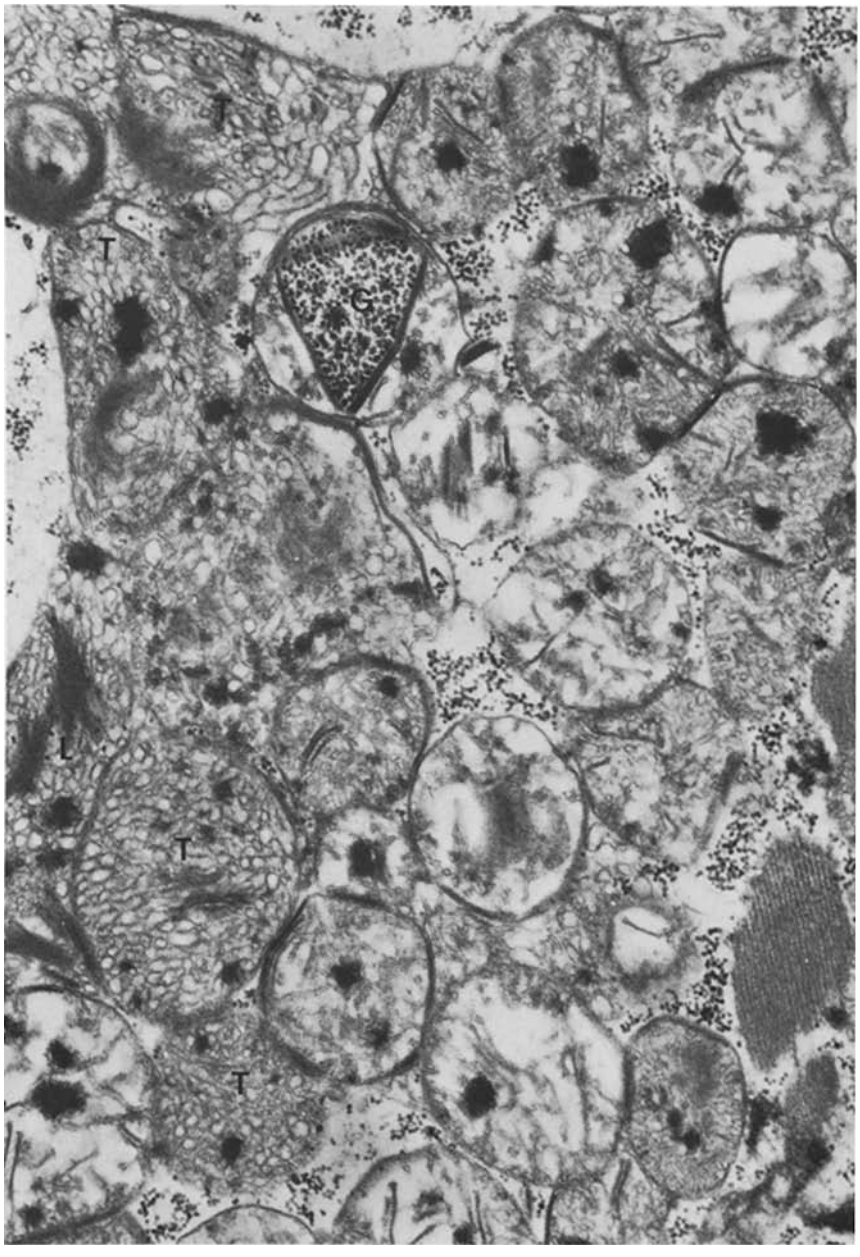

Fig. 3. Heart muscle cell, left ventricle. Many mitochondria contain tubular (*T*) or lamelar (*L*) cristae. *G*, Glycogen within a mitochondrium. × 26,000

cells exhibit only one myofibril directly under the cell surface (Fig. 1 and 2). Small intercalated discs can be observed in the region where the few myofibrils of two heart muscle cells are in mutual contact.

Skeletal muscle tissue of all muscles contains subsarcolemmaly foci of aggregated rounded and enlarged mitochondria with a diameter up to

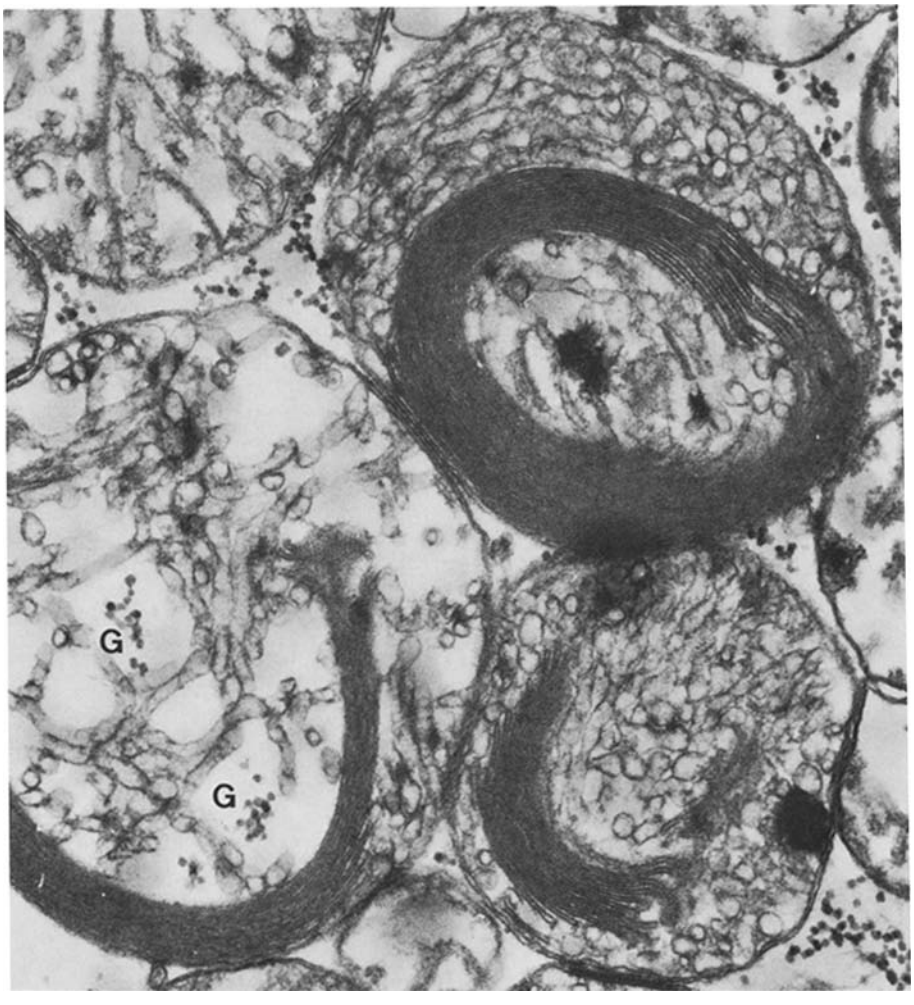

Fig. 4. Heart muscle cell, left ventricle. Rounded enlarged mitochondria are filled with lamellar and tubular cristae. *G*, intramitochondrial monoparticular glycogen granules. × 51,000

1,8 μm. Other muscle fibers are almost totally filled with mitochondria and contain no myofibrils (Fig. 6a). The subsarcolemmal aggregation of mitochondria sometimes consists of disk-like mitochondria which are stacked one on another (Fig. 6b). The mitochondria of the capillary endothelia in skeletal muscle show no abnormalities. All the above-mentioned peculiarities in the heart and skeletal muscle can also be observed in reembedded paraffin material (Fig. 2a and 6a).

The liver cells show no lipid droplets and a normal glycogen content. Some hypoxic vacuoles can be seen. The mitochondria of liver, kidneys and of the smooth muscle cells of the gastric wall were without abnormalities.

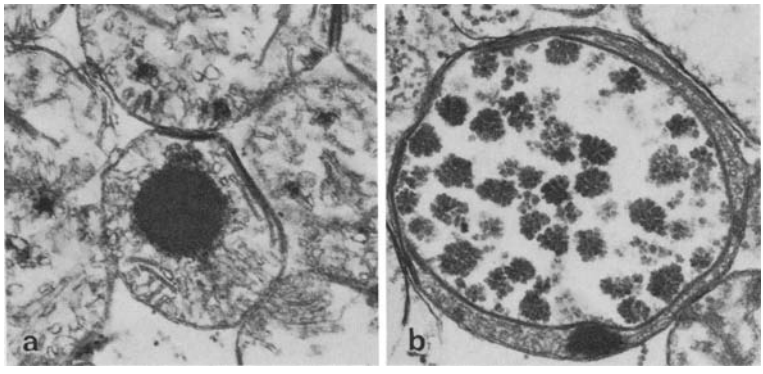

Fig. 5a, b. Heart muscle cell. Mitochondrial inclusions. **a** Round electron dense inclusion. × 32,000. **b** Intramitochondrial glycogen rosettes. × 64,000

Biochemistry

The level of carnitine in the heart muscle and three different skeletal muscles was normal.

Discussion

This case presents as a mitochondrial cardiomyopathy with involvement of skeletal muscle. This cardiomyopathy must be separated from other cases of hypertrophy of the heart in children, especially storage diseases, hypertrophic cardiomyopathy with or without obstruction, and carnitine deficiency. – A storage disease is practically excluded by the histological and fine structural investigation. We found some narrowing of the outflow region in left ventricle but in typical hypertrophic cardiomyopathy the hyperplasia of partially abnormal mitochondria with extreme reduction of myofibrils seen here has never been observed (Roberts and Ferrans 1975; Ferrans 1980). Other morphological pecularities of hypertrophic cardiomyopathy, such as interstitial fibrosis, irregularities in the arrangement of heart muscle cells and of myofibrils within the cells are absent from our material.

The report of Sengers et al. (1975) on seven children from three families with hypertrophic cardiomyopathy and mitochondrial myopathy obviously deals with another type of cardiomyopathy. All these patients suffered from cataracts, furthermore heart and skeletal muscle, in contrast to our case, showed extensive deposition of fat droplets. Carnitine levels in muscle tissue or blood were not reported.

In carnitine deficiency there is only a moderate increase in mitochondria (Hart et al. 1978). The skeletal muscle, in contrast to our material, always shows an intensive fat storage (Karpati et al. 1975; Hart et al. 1978; Pongratz et al. 1979; Müller-Höcker et al. 1982). Finally, the presented case must be separated from the many reports of oncocytic or so-called histiocytic transformation of heart muscle cells (Silver et al. 1980).

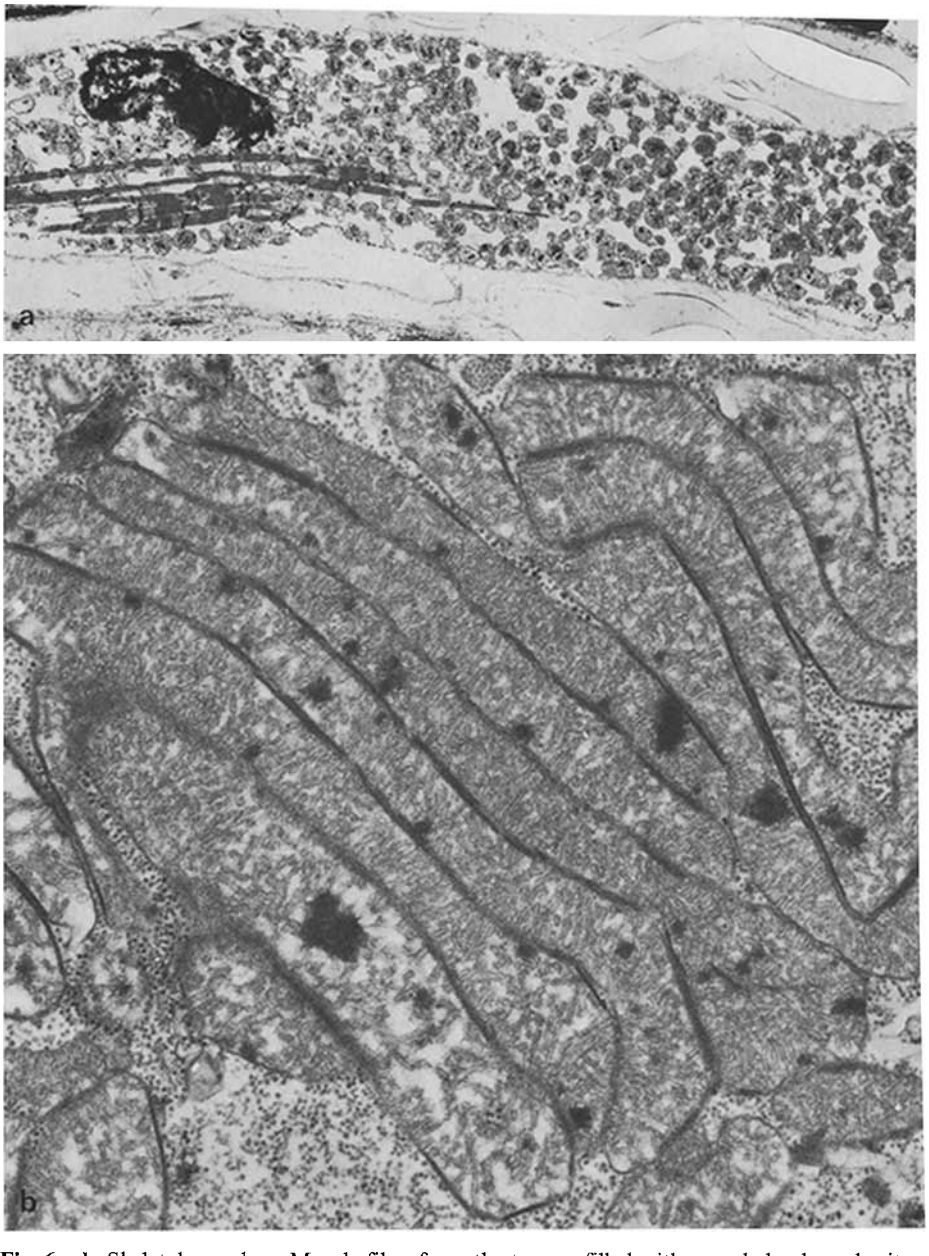

Fig. 6a, b. Skeletal muscle. **a** Muscle fiber from the tongue filled with rounded enlarged mitochondria. Formalin fixed paraffin embedded specimen after reembedding in Epon for electron microscopy. × 2,600. **b** M. quadriceps femoris. Disk-like mitochondria stacked one on another. Glutaraldehyde fixed specimen. × 26,000

In these cases focal transformation of heart muscle cells filled with abnormal mitochondria occurs. The cells are rounded and lack the junctional complexes of intercalated disks. We saw no such rounded oncocytic cells in our case. The diffuse hyperplasia of mitochondria in our material was demonstrable with the same intensity in all regions of the heart, and all heart muscle cells showed the usual elongated form.

In the literature we found only few cases which might be compared with ours:

Hug and Schubert 1970 reported on a 6 month old infant with idiopathic cardiomyopathy. They observed a marked cardiomegaly and a moderate hepatomegaly. An endomyocardial biopsy showed heart muscle cells with the same increase and abnormalities of mitochondria as we have seen. In contrast to our case they also found giant mitochondria in the liver. Skeletal muscle was not investigated.

Mackay et al. (1976) reported on a cardiac biopsy in a 11 year old boy with a proximal myopathy of unusual type. The heart of the child was hypertrophic with a globular contour. The heart muscle cells were filled with enlarged, often ring-shaped mitochondria, the size of which ranged from 1.14–1.50 μm, rarely up to 2.7 μm in diameter. In contrast to our case light- and electron microscopy and histochemistry of a skeletal muscle biopsy showed no abnormalities.

Neustein et al. (1979) demonstrated a family with obviously x-linked recessive cardiomyopathy, in which 5 boys developed dilative cardiomyopathy with endocardial fibroelastosis. In 4 of the children in the heart muscle the occurance of focally arranged rounded (up to 3.0 μm in diameter) or ring-shaped mitochondria was observed. Similar mitochondrial anomalies were seen in one of the boys in skeletal muscles, liver and kidney. In contrast to these cases no congenital heart disease is known in the family of our case. We have not been able to obtain muscle biopsies from the obviously healthy parents of our case. Furthermore, the cardiomyopathy reported by Neustein et al. 1979 was a dilative-one, and the mitochondriopathia showed only a focal occurance; we observed hypertrophic cardiomyopathy with diffuse alterations of mitochondria.

Our knowledge on the pathogenesis of the reported mitochondriopathies in heart and muscle is poor: After an initial report of Luft et al. (1962) on a similar hyperplasia of abnormal mitochondria in skeletal muscle many observations have been published (for literature see Schröder 1982). The mitochondria in these cases often resemble those of oncocytes as in our case (Hübner et al. 1967). Luft and coworkers 1962 found a loosely coupled state of oxidative phosphorylation in their mitochondrial myopathy, the same finding which we later observed in oncocytic mitochondria in adenolymphomas of the salivary glands (Schiefer et al. 1968). The increase in mitochondria might be interpreted as a frustrated attempt to compensate for a functional mitochondrial defect. However, whether a mitochondrial defect in muscle fibers and especially in the heart muscle cells is present in our material we do not know.

Biopsy of liver and skeletal muscle are useful for diagnosis. From these biopsies a storage disease can be readily excluded. Carnitine defiency is confirmed by a low carnitine level in muscle and often in the blood (Karpati et al. 1975; Hart et al. 1978; Pongratz et al. 1979; Müller-Höcker et al. 1982). An endomyocardial biopsy would be helpful, but in infants will mostly be avoided for clinical reasons.

Autopsy material of the heart should be preserved for electron microscopy. However in paraffin sections the peculiar granularity of heart muscle cells with extreme rarefication of myofibrils and loss of cross striation will be valuable in diagnosis. Reembedding of paraffin material in Epon with subsequent electron microscopic investigation allows recognition of increased abnormal mitochondria in heart muscle cells and in skeletal muscle. These procedures will allow more frequently the diagnosis of these unusual conditions.

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