A study of a family with inherited disease of cardiac and skeletal muscle

Part II. Skeletal muscle morphology and mitochondrial oxidative phosphorylation

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Summary

Skeletal muscle morphology and mitochondrial oxidative phosphorylation capacity were examined in a family whose members showed varying combinations of mental subnormality, cardiomyopathy and muscle weakness. Light and electron microscopic findings suggested a neuropathic process, while tests of mitochondrial function indicated a state of tight coupling of oxidative phosphorylation, a feature in marked contrast to those in biochemical studies so far reported.

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Pathological changes in cardiac muscle in association with a variety of disorders of voluntary muscle are well known,[1-3] the clinical emphasis almost always falling upon the neuromuscular component of the disease. Conversely, patients presenting with a primary cardiomyopathy who show additional clinical or electrophysiological evidence of skeletal muscle involvement now form a documented group, although the pathological process affecting voluntary muscle has proved difficult to define.[4-7]

In part I of this report,[8] we described a family in which a cardiomyopathy was the presenting complaint in 3 of 6 brothers. All the siblings appeared mildly mentally subnormal, and 2 showed in addition signs of muscle weakness. These findings prompted a neuromuscular study of the whole family, leading in turn to the discovery that all the children had abnormal electromyograms or biopsies, or both, with morphological changes somewhat suggestive of a neuropathic process and ultrastructural evidence of a widespread and unusual mitochondrial abnormality.

Defects in both structure and function of mitochondria have been reported in cases of chronic neuromuscular disease of widely varying clinical expression,[9] but there appears so far to have been no correlative morphological and biochemical study on patients with cardiac and skeletal muscle disease.

In this article we present details of the structural changes encountered in (skeletal muscle) biopsies performed on the entire family of 8 patients, together with an evaluation of mitochondrial oxidative phosphorylation capacity.

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Materials and methods

Morphological studies

All patients were biopsied under local anaesthesia, and specimens taken from the quadriceps group of muscles (usually vastus lateralis) because relatively large tissue samples were required for biochemical studies. Specimens were taken separately for histochemical and ultrastructural examination, those for the latter being fixed for 2 hours in 2,5% buffered glutaraldehyde and embedded in araldite. Samples for histochemistry were frozen in liquid nitrogen, and cryostat sections were stained by the following techniques: haematoxylin and eosin (H and E), Gomori Trichrome (GT), acid and alkaline incubations for ATPase, myophorylase, nitroblue tetrazolium for reduced nicotinamide adenine dinucleotide (NADH), and oxidative enzymes including succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH), and Oil Red O and periodic acid-Schiff (PAS) for fat and glycogen respectively.

Mitochondrial studies

Biopsy samples were placed immediately in ice-cold isolation medium (0,18M KCl, 10 mM EDTA, pH adjusted to 7,4 with tris). Superficial fat and connective tissue were removed before weighing, and the muscle was minced finely with scissors and rinsed 3 - 4 times in the isolation medium. A 1:12 (w/v) homogenate was prepared in a Polytron PT 10 homogenizer, using 4-second bursts at maximum speed until intact fibres were no longer visible. Mitochondria were prepared by centrifugation at 700 *g* for 15 minutes.

After decanting of the supernatant, the nuclear pellet was resuspended in KCl-EDTA and centrifuged again. The two supernatant fractions were combined and centrifuged at 8 000 *g* for 10 minutes, and the final mitochondrial pellet was resuspended in KCl-EDTA at a final concentration of ±10 mg protein/ml. Mitochondrial oxidative phosphorylation was studied polarographically by the method of Sordahl *et al.*[10] The incubation medium consisted of 0,25M sucrose, 10 mM tris-HCl

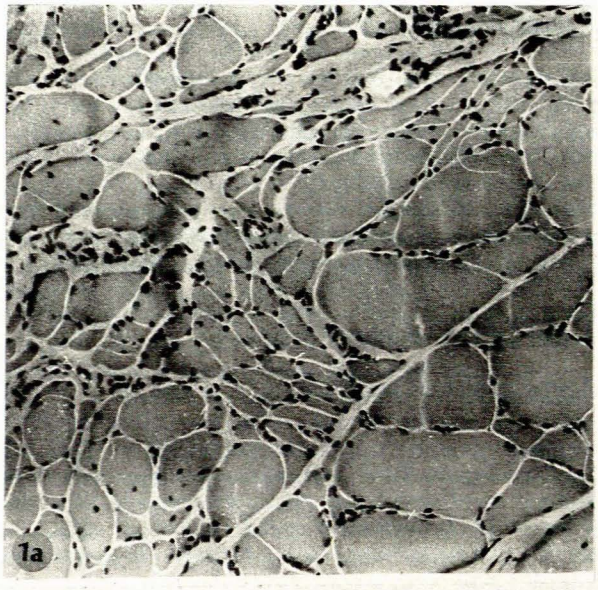
(pH 7,4), 8,5 mM K_2HPO_4 and 5 mM glutamate (tris-salt, pH 7,4). The amounts of ADP added varied between 450 and 550 nmol and the incubation temperature was 25°C. Mitochondrial protein content was measured by the method of Lowry *et al.*[11]

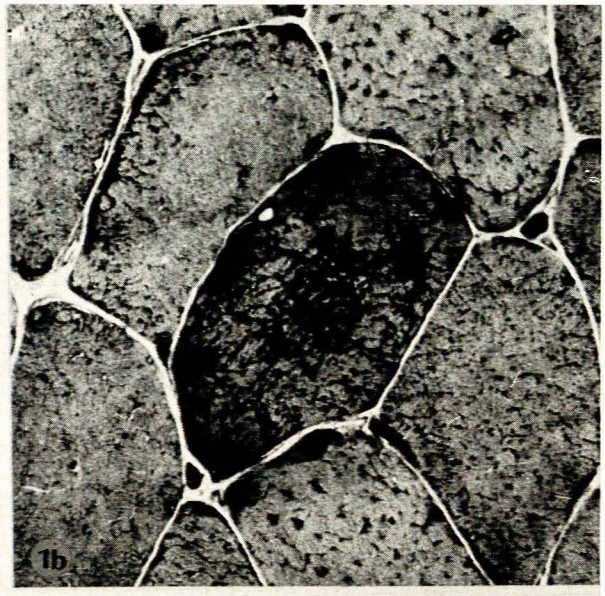
The following parameters of mitochondrial function were determined: ADP/O ratios (nmol ATP produced per natom oxygen consumed); mitochondrial oxygen uptake (Qo_2, state 3: natoms consumed in the presence of ADP/mg mitochondrial protein/min; Qo_2, state 4: natoms oxygen consumed after phosphorylation of ADP/mg mitochondrial protein/min.); respiratory control index (RCI; ratio of oxygen consumed in the presence of ADP to that after phosphorylation of ADP); oxidative phosphorylation rate (OPR; nmol ATP produced/mg mitochondrial protein/min). This parameter was calculated from the ADP/O ratio and Qo_2 (state 3) values.

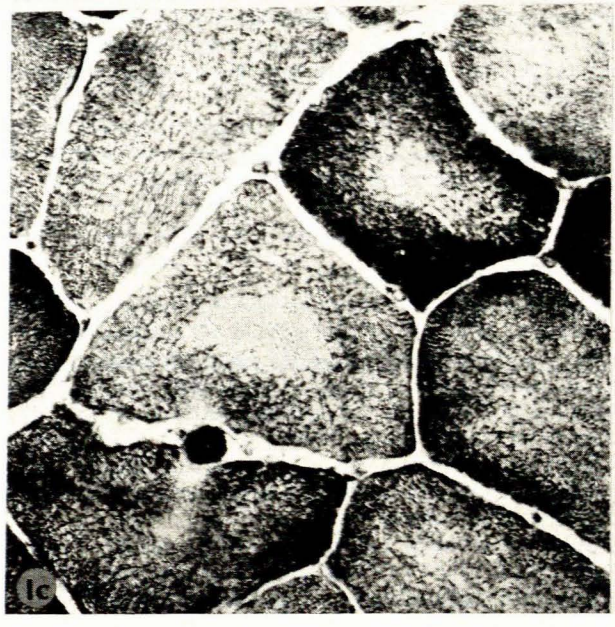
Cytochrome *c* oxidase and NADH-cytochrome *c* reductase were used as mitochondrial marker enzymes. All mitochondrial samples were treated with Triton X-100 (± 0,5 mg/mg mitochondrial protein) for maximal enzyme activity.

Cytochrome *c* oxidase activity was determined by measuring mitochondrial oxygen uptake polarographically using a Clarke electrode at 25°C, as described by Schnaitman *et al.*,[12] with an incubation medium of K-phosphate buffer, pH 7,2, 75 mM; cytochrome *c* 30 μM; sodium ascorbate 3,75 mM; and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD) 300 μM. The reaction was started by the addition of cytochrome *c*, and corrections were made for the rate of oxygen consumption prior to the addition of the substrate. The enzyme activity was expressed as natoms oxygen consumed/mg mitochondrial protein/min.

NADH-cytochrome *c* reductase activity was determined spectrophotometrically by measuring the reduction of cytochrome *c* in the presence of KCN by the method of Sottacasa *et al.*[13] The total (rotenone-sensitive and rotenone-insensitive)







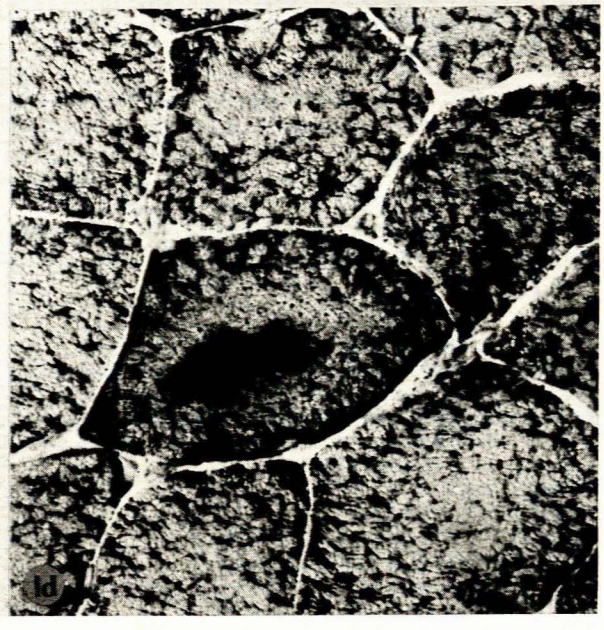


Fig. 1a. Group atrophy and hypertrophy of myofibres, indicative of neurogenic atrophy (case 7) (H and E x 200).

Fig. 1b. Myofibre showing dark, irregular patches of GT positivity, suggestive of ragged red cell formation; adjacent cells *(arrows)* show subsarcolemmal aggregates (GT x 400).

Fig. 1c. Central cores in myofibres (targetoid cells) (reduced NAD x 400).

Fig. 1d. Typical target cell (reduced NAD x 400).

NADH-cytochrome *c* reductase activity was expressed as nmol cytochrome *c* reduced/mg mitochondrial protein/min.

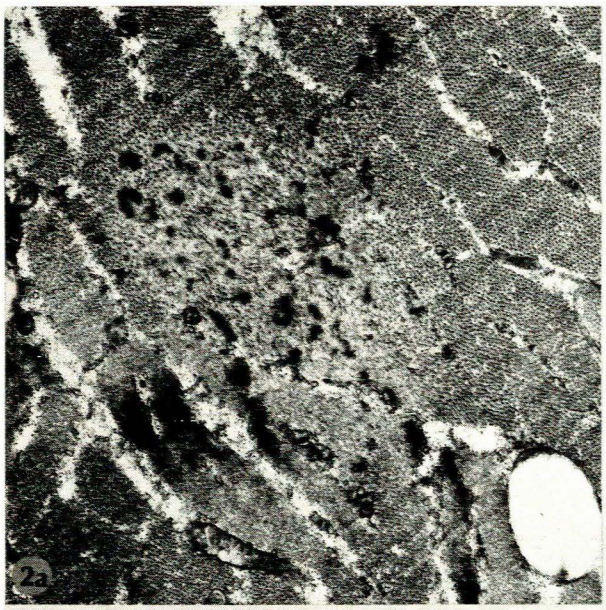
Tissue ATP and phosphocreatine (CrP) contents

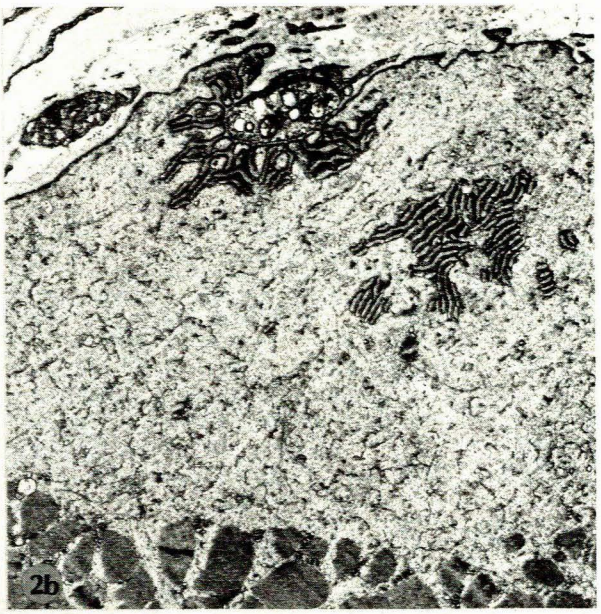
For the measurement of these substances, specimens were quick-frozen in liquid nitrogen and stored thus until extraction. Frozen tissue was pulverized in a stainless-steel mortar kept in liquid nitrogen. After weighing, a protein-free perchloric acid extract was prepared[14] and neutralized as follows: 40% KOH in saturated KCl (4 parts); 0,2M tris-HCl, pH 7,5 (6 parts). The ATP and CrP contents of the extract were determined within 24 hours (storage at —20°C). The ATP content was determined according to the method of Lamprecht and Trautschold,[15] and CrP was determined by the addition of ADP and creatine kinase to the same cuvette (results expressed as μmol/g wet weight).[16]

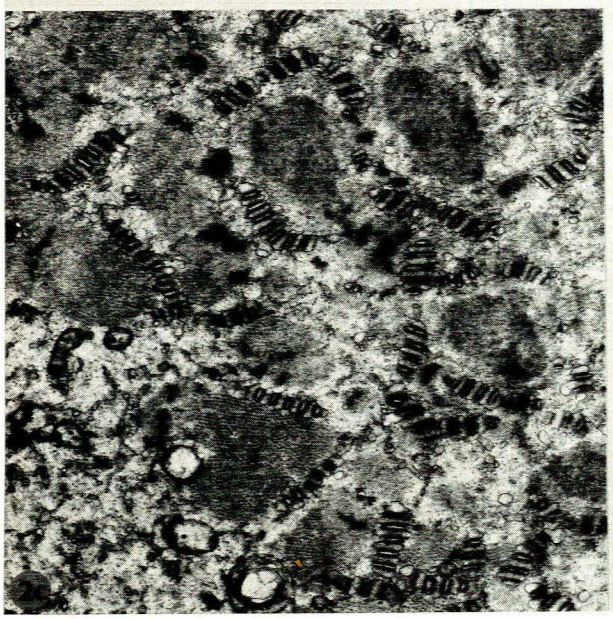
Results

Morphology

Light and electron microscopic features have been summarized in Table I. Both parents showed mild abnormalities, although the father's biopsy specimen (case 1) may be considered invalid, considering his poor physical condition.[8] Patient 7 merits immediate comment, for besides being the youngest and most normal-looking of the siblings, his biopsy specimen was the only one showing classic changes of denervation (Fig. 1a). The most severely affected patients were Nos. 3, 4 and 5, all of whose specimens exhibited occasional ragged red cells (Fig. 1b), generalized cytoplasmic 'untidiness' as demonstrated with the oxidative enzymes, and significant numbers of target and targetoid forms (Figs 1c, d). At an ultrastructural level, the typical, central myofibre degeneration of target cell formation was observed in case 4 (Fig. 2a), while a







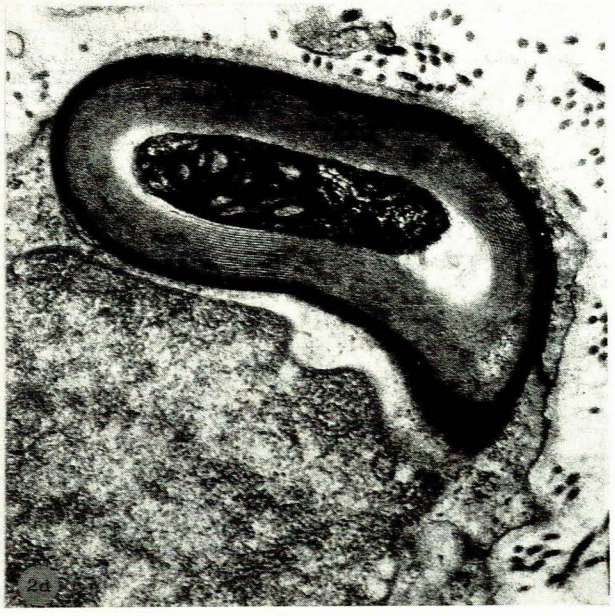


Fig. 2a. Central myofibrillar degeneration (target cell) (x 21 000).

Fig. 2b. T-tubule replication (x 20 000).

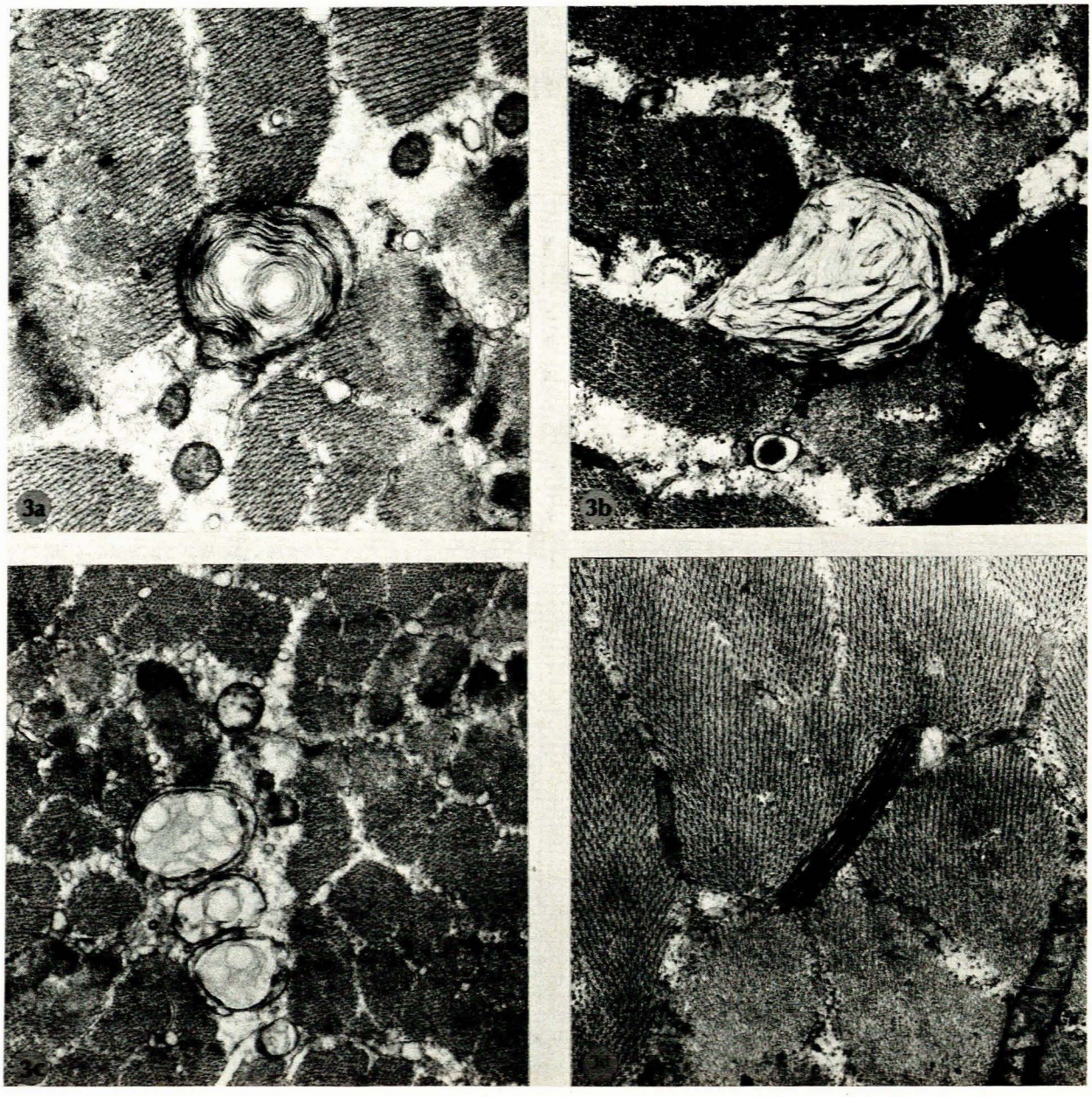
Fig. 2c. Abnormal motor end-plate (MEP), with axon hillock filled with tubular profiles and devoid of mitochondria. The synaptic complex fragmented (case 5) (x 8 300).

Fig. 2d. Shrunken, electron-dense intramuscular nerve fibre, with myelin sheath (case 5) (x 44 000).

peculiar degenerative phenomenon was seen in case 5, where clumps of electron-dense material (possibly Z-band protein) were associated with distinctive collections of stacked or replicated T-tubules (Fig. 2b). In such a focus, mitochondria were entirely absent. This change could be seen adjacent to an abnormal motor end-plate packed with tubular profiles suggestive of sarcoplasmic reticulum, devoid of mitochondria, and associated with a small, evidently fragmented, synaptic complex (Fig. 2c). The most consistent feature, seen in all the children's biopsies, was a mitochondrial abnormality; these organelles, besides their irregular size and shape (especially in cases 3, 4 and 5), exhibited poorly defined cristae, lamellated, membranous inclusions (Figs. 3a, b) and invaginated, feebly electron-dense, vacuolated material (Fig. 3c). Subsarcolemmal aggregates of mitochondria were common. These abnormalities were less pronounced in the specimens from patients 6, 7 and 8, although the last showed areas of T-tubule replication identical to those observed in case 5 (Fig. 2b). In the mother's biopsy specimen (case 2), a striking if infrequent change was focal dilatation of the sarcotubular system, containing parallel membranous arrays (Fig. 3d). The specimen from patient 5 also contained a number of terminal nerve fibres, all of which looked abnormal, such changes including contracted, sometimes electron-dense axons (Fig. 2d), and irregular, dilated microtubules.

Oxidative phosphorylation and enzyme activities of mitochondria

For comparison, skeletal muscle specimens were investigated from 6 patients aged 12 to 54 years undergoing surgery. Tissues were obtained from various sites including the rectus abdominis and psoas muscles. No significant differences were observed



Figs. 3a, b. Examples of membranous inclusions in mitochondria (x 40 000).

Fig. 3c. Mitochondria invaginated by vacuolated material (x 21 000).

Fig. 3d. Focal dilatation of sarcoplasmic reticulum containing parallel arrays (case 2) (x 44 000).

TABLE I. SUMMARY OF MORPHOLOGICAL FINDINGS

Case	Morphology (H and E, GT, oxidative enzymes)	Mosaic (ATPase at acid and alkaline incubations)	Ultrastructure	Pathological diagnosis
1	H and E normal; GT shows prominent red stippling and subsarcolemmal deposits; confirmed by oxidative enzymes, showing scattered cells containing black clumps of mitochondria	Relative 'loss' of type I cells, although these are scattered evenly through the sample. Type II fibres are therefore enclosed	Normal	Abnormal muscle — no diagnosis * GT stippling * Oxidative enzyme clumping (occasional)
2	Some fibre-size variation (± 30 - 90 μm) but small fibres rather few and not grouped. Small cells densely stained with the oxidative enzymes and larger cells show occasional central pallor or subsarcolemmal condensation	Normal	Basal laminae and myofibrils normal; occasional cytoplasmic filamentous body, surrounded by countless sarcoplasmic reticular profiles and a few T-tubules; mitochondria often small and forming collections below the sarcolemma, some showing abnormally straight borders without cristae. Occasional parallel arrays in focal dilatations of sarcoplasmic reticulum	Abnormal muscle — no diagnosis * Fibre size variation * Cytoplasmic degenerative changes and mitochondrial inclusions
3	H and E normal: oxidative enzymes show generalized cytoplasmic 'untidiness', especially of type II fibres, some of which progress to almost targetoid cores	Relative loss of type I cells, but spread evenly through the sample	Some cells show foci of disorganized (?degenerating) myofibrils, associated with sarcotubular profiles and populated with small mitochondria invariably devoid of cristae. Elsewhere, numerous abnormal mitochondria may be seen, either with multiple lamellated membranes, or containing (apparently) invaginated, feebly electron-dense vacuolated material	Abnormal muscle - possibly neuropathic * Targetoid cells * Abnormal mosaic — fall-out of type I cells * Myofibrillar degeneration * Abnormal mitochondria (lamellated inclusions)
4	Numbers of rounded cells; av. fibre diameter 80-90 μm; GT shows cytoplasmic untidiness progressing to occasional ragged red cells and cells with bright red cores; oxidative enzymes show generalized cytoplasmic disorganization and numerous well-formed targets and targetoid cells	Mild relative 'loss' of type II cells, with evidently enclosed type I fibres	Some cells show marked myofibrillar degeneration with Z-band streaming, characteristic of target formation. Other features include subsarcolemmal accumulation of mitochondria; mitochondria packed with membranous profiles (lamellated inclusions), occasional electron-dense cytoplasmic bodies surrounded by dilated sarcoplasmic tubules, and occasional ringbinden	Neuropathic * Ragged red cells * Target cells * Abnormal mosaic — enclosed type I fibres * Myofibrillar degeneration * Abnormal mitochondria (lamellated inclusions)

Continued overleaf

TABLE I. (continued)

Case	Morphology (H and E, GT, oxidative enzymes)	Mosaic (ATPase at acid and alkaline incubations)	Ultrastructure	Pathological diagnosis
5	H and E shows loss of cell angulation and significant numbers of internal nuclei. GT and oxidative enzymes confirm the presence of ragged red cells and target and targetoid fibres	Normal	Some foci of unusual myofibrillar degeneration containing round, moderately electron-dense but structureless masses, interspersed with numerous stacked T-tubules. Sometimes these foci are adjacent to MEPs which are characterised by complete absence of mitochondria, innumerable fragments of sarcotubules and (apparently) fragmented synaptic cleft complexes. Mitochondria elsewhere appear small, effete and often distended by vacuolated or membranous material	Neuropathic * Ragged red cells * Target/targetoid cells * Myofibrillar degeneration * Replicated T-tubules * Abnormal MEPs * Abnormal mitochondria (membranes, vacuoles).
6	Occasional small cells (± 35 μm) but average fibre size normal (± 75 μm). Occasional cells contain prominent GT-positive 'lumps' suggestive of abnormal mitochondria. Oxidative enzymes show cells with pale centres	Numerous enclosed type I cells — possibly early fibre type grouping	Basal laminae normal; some cells show areas of myofibrillar degeneration, with clumped Z-band material and numerous, stacked (or replicated) T tubules. Such areas are devoid of mitochondria so that it is assumed that Z-band protein is the cause of the GT positivity. Elsewhere, mitochondria are small, with ill-defined cristae, sometimes distended with membranous profiles, and sometimes forming subsarcolemmal accumulations	Abnormal muscle — no diagnosis * Abnormal GT staining * Abnormal mosaic * Myofibrillar degeneration and abnormal mitochondria
7	Normal, apart from one area of classic group atrophy (small cells ± 10 μm, normal fibres 70 μm). GT and oxidative enzymes normal	The fascicles which include the group atrophy also show fibre type grouping. Other fascicles are normal	Basal laminae and myofibrils normal; subsarcolemmal collections of small mitochondria; some mitochondria contain membranous profiles	Neuropathic * Neurogenic atrophy * Fibre type grouping * Abnormal mitochondria (membranous inclusions)
8	Some loss of angulation, but main feature is cell hypertrophy (± 120 μm). Also occasional atrophic cells (± 15 μm). Oxidative enzymes show targetoid forms	Normal	Basal laminae and myofibrils normal; numerous subsarcolemmal mitochondrial aggregates, some containing small dense structures suggestive of early cytoplasmic bodies, and tubular arrays. Mitochondria abnormally variable in size, with numerous examples of membranous inclusions	Abnormal muscle — no diagnosis * Hypertrophy * Targetoid forms * Abnormal mitochondria (membranous inclusions)

between different muscles, and results were therefore grouped together (Table II). The values obtained from human and rat skeletal muscle mitochondria compared well with each other.[17]

TABLE II. SKELETAL MUSCLE MITOCHONDRIA FROM CONTROL PATIENTS: OXIDATIVE PHOSPHORYLATION AND ENZYME ACTIVITY (MEAN ± SEM)

A. Oxidative phosphorylation

No. of patients	ADP/O	RCI	Qo_2 (state 3)	Qo_2 (state 4)	OPR
6	2,84 ±0,07	10,11 ±3,10	60,12 ±5,58	9,18 ±2,47	169,70 ±14,04

B. Enzyme activity

No. of patients	Cytochrome *c* oxidase	NADH-cytochrome *c* reductase
6	449,06 ±104,24	115,58 ±34,11

Abbreviations: see Materials and Methods
Cytochrome *c* oxidase: natoms oxygen uptake/mg mitochondrial protein/min.
NADH-cytochrome *c* reductase: nmoles cytochrome *c* reduced/mg mitochondrial protein/min.

Mitochondrial oxidative phosphorylation studies were performed on all members of the family, who, with the exception of case 6, all exhibited low Qo_2 values (both states 3 and 4) when compared with controls (Table III). Consequently, the rate at which ADP was phosphorylated (OPR) was also depressed in spite of a normal phosphorylation capacity (ADP/O). In this respect case 3 was the exception. It is of interest that the depressed rate of state 4 respiration observed in all members of the family apart from case 3 resulted in an elevation of RCI values, when compared with controls. In patient 3 all parameters of mitochondrial function studied as well as cytochrome *c* oxidase and NADH-cytochrome *c* reductase activities were most severely depressed; since no ADP/O ratios could be measured (oxygraph tracings showed no state 3 - 4 transition) the phosphorylation mechanism itself also appeared to be affected. On the other hand, mitochondria isolated from patient 6 yielded ADP/O, OPR and Qo_2 values approaching those of the control series, with normal mitochondrial enzyme activities.

It thus appears as if the brothers can be divided into two groups: patients 3, 4 and 5 exhibited lower Qo_2, OPR and ADP/O values as well as depressed NADH-cytochrome *c* reductase and cytochrome *c* oxidase activities. In contrast to these, patients 6, 7 and 8 showed higher values. Of the parents (father, case 1, and mother, case 2), the latter had better mitochondrial function, with exceptionally high RCI values and cytochrome *c* oxidase activity.

In marked contrast to the evidence of depressed mitochondrial function, tissue ATP and CrP contents in this family (Table IV) appeared normal, and in some cases even higher than those of the controls.

TABLE III. OXIDATIVE PHOSPHORYLATION AND ENZYME ACTIVITY OF SKELETAL MUSCLE MITOCHONDRIA

A. Oxidative phosphorylation

Case	ADP/O	RCI	Qo_2 (state 3)	Qo_2 (state 4)	OPR
!	2,60	11,5	42,99	3,74	111,77
2	3,03	13,5	42,59	3,15	129,05
3	—*	2,8	5,27	2,88	—*
4	3,98	6,67	15,34	2,30	61,05
5	3,06	6,0	20,93	3,49	64,05
6	2,82	14,50	57,93	4,00	163,36
7	4,40	6,88	30,02	4,37	132,09
8	2,82	10,67	34,78	3,26	98,08

B. Enzyme activity

Case	Cytochrome *c* oxidase	NADH-cytochrome coxidase
1	100,93	72,60
2	974,80	100,96
3	62,22	55,90
4	91,26	65,73
5	167,44	28,60
6	465,12	244,36
7	408,73	168,29
8	482,39	38,17

* Measurements could not be made with accuracy.

TABLE IV. ATP AND CrP (μmol/g WET WEIGHT) IN TISSUE SPECIMENS FROM FAMILY AND CONTROLS

Case	ATP	CrP
4	4,87	13,93
5	3,56	9,20
6	2,42	7,00
7	3,08	9,02
8	2,04	11,37
Mean ± SEM	3,19 ± 0,49	10,10 ± 1,18
Controls (4)	1,96 ± 0,18	6,44 ± 1,14

Discussion

It has been established that both primary myopathic and neuropathic disorders of skeletal muscle may be associated with pathological changes in the myocardium.[1-3] However, in those patients presenting with a primary cardiomyopathy, and in whom skeletal muscle involvement is clearly a subordinate clinical or electrophysiological finding, the question still remains whether the pathological process starts within the muscle cell or as a result of defective innervation. The cardiac and skeletal muscle syndromes so far described are summarized in Table V, and if one accepts the neuropathic implications of type-specific atrophy, fibre type grouping, target cells and Z-band streaming, then the overall impression is one of disordered nerve function or tropism. Our own series serves to confirm these findings, particularly with regard to target or targetoid cell forms, abnormalities of the fascicular mosaic, and group atrophy. Some of the 'ragged red cells' described (Fig. 1b) did not have an exact counterpart in serial sections stained for oxidative enzymes, nor were these fibres sufficiently numerous to be identified with the electron microscope, so that their relationship to the accepted form of this abnormality is unclear. We ourselves, however, have encountered similar changes in muscle specimens from patients with chronic, unclassified neuropathy on many occasions. If such distinctive structural abnormalities (target cells, group atrophy, etc.) were all features of a neuropathic process, the converse did not apply, and overtly myopathic changes such as hyalinization, necrosis and phagocytosis were entirely absent.

TABLE V. SUMMARY OF CASES DESCRIBED IN THE LITERATURE

Authors	Clinical features	Biochemical investigations	Electrophysiology	Morphology	Conclusions
Shafiq *et al.*[4] (6 patients)	**Clinically detectable weakness in 4 patients**		**Neurogenic pattern in 1 patient, myopathy in 2**	* Type II fibre atrophy (4) * Myopathic changes (1) * Type I hypotrophy (1) * Cytoplasmic bodies (2) * Ringbinden (1)	**Patients with primary cardiomyopathy assumed to have more generalized disorder; 1 patient diagnosed as a neuropathy**
Isaacs and Munke[5] (3 patients)	**Symptomatic muscle weakness in all 3 subjects**	**CPK mildly elevated, and abnormal ischaemic exercise test in 1 patient**	**Myopathic EMG in 2 patients, neuropathic in 1 patient**	* Subsarcolemmal mitochondrial accumulations (1) * Variably sized mitochondria (1) * Group atrophy and fibre type grouping (2) * Target cells (2) * Abnormal MEPs (2) * Vacuolated cells (3) * Abnormal MEPs (3) * Target cells (3)	**One patient diagnosed as neurogenic atrophy, 1 as a mitochondrial myopathy and 1 as a vacuolar myopathy**
Smith *et al.*[6] (11 patients)	**Only 1 patient had symptomatic muscle weakness**	**Mild rise in CPK in 3 patients**	**All patients showed brief, low amplitude potentials; 9 (out of 11) had increased short duration polyphasics**	* Central cores (4) * Subsarcolemmal mitochondrial accumulations (6) * Mitochondrial abnormalities including paracrystalline structures (6)	**All patients asymptomatic; electrophysiological or functional abnormalities (or both); uncertain whether changes myopathic or neuropathic**
Darsee *et al.*[7] (19 patients)	**No patient had muscle weakness, but 6 patients showed evidence of peripheral neuropathy**	**Moderate rise in 2 patients**	**8 patients showed minimal to moderate degrees of peripheral neuropathy**	* Neurogenic atrophy (2) * Fibre type grouping (1) * Type II fibre atrophy (2)	**Neurogenic abnormalities of skeletal muscle either from denervation or other acquired disorders**

The structured degenerative changes observed electron microscopically, including Z-band streaming, cytoplasmic bodies, T-tubule replication and intrasarcoplasmic reticular arrays (Fig. 3a) have all been observed in neuropathic conditions,[18,19] although it is not suggested that these are specific. The mitochondrial membranous inclusions, however, are of particular interest because they were observed in specimens from all 6 brothers. These structures at times resembled the ubiquitous 'myelin figure', but could always be seen to be originating from mitochondria, and their association with a metabolic state of tightly coupled oxidative phosphorylation led us to hope for a causal relationship — particularly in view of the complete absence of the popular paracrystalline inclusions so often described by others,[9,20,21] including Luft *et al.*[22] in his original case of hypermetabolism. Membranous profiles showing varying degrees of compaction similar to the organelles in our cases have been described in association with experimental denervation[23] and in a congenital myopathy exhibiting loosely coupled oxidative phosphorylation;[24] so these too have no specificity.

The functional aspects of those conditions associated with abnormal skeletal muscle mitochondria have been the subject of a number of reports, recently reviewed by Bethlem,[9] from which one predominant fact has emerged: the patients appear to have a pattern of mitochondrial metabolism in which oxidative phosphorylation is loosely coupled, so that there is a high respiratory rate in the absence of ADP, little or no stimulation after addition of ADP and consequently very low RCI values ($\pm$ 1). Other parameters of mitochondrial function varied, e.g. mitochondrial oxygen uptake was reported to be normal,[22,25] increased[26,27] or decreased,[28] while the capacity to phosphorylate ADP (ADP/O ratios) was found to be normal[22] or depressed.[26]

In addition these patients showed significantly increased mitochondrial ATPase activity, with 2,4-dinitrophenol effecting insignificant stimulation[22,26,29] in contrast with the marked elevation of ATPase activity in controls. In summary, mitochondria isolated from hypermetabolic and /or myopathic patients can, despite their defective capacity for respiratory control, still produce ATP when phosphate acceptor is present, i.e. the mitochondria are 'loosely' coupled but not uncoupled.

Although our patients displayed varying degrees of depressed mitochondrial function (patients 3, 4 and 5 being most severely affected), the metabolic pattern was completely different from those in previous studies: mitochondria isolated from skeletal muscle specimens from the family we investigated had a metabolic pattern of tightly coupled oxidative phosphorylation, despite the low respiratory rates: addition of ADP resulted in marked stimulation of oxygen uptake. Of particular interest was the almost three-fold reduction in state 4 respiration (i.e. respiration in the absence of ADP), resulting in a relatively high RCI (10 - 14) in cases 1, 2, 6 and 8. Since mitochondrial respiratory rates are regulated by several factors (e.g. substrate availability, intactness of the respiratory chain, or ATP utilization) it is clear that further studies will be required in order to elucidate the exact mechanism(s) of depression of mitochondrial oxygen uptake in our patients. Despite the depression in the rate of ATP synthesis, tissue ATP and CrP levels in all the patients studied were found to be higher than in controls (Table IV); since these levels depend on relative rates of synthesis and degradation, the possibility exists that ATP breakdown rates might be low (allowing for the fact that tissue specimens were taken at rest). Unfortunately, owing to the small size of the specimens, further studies to evaluate sarcoplasmic reticulum function as well as other properties of the contractile mechanism were not possible.

It has to be pointed out that the experimental techniques employed in the present study differ from those referred to above,[22,26,27] all of which used the same method with KCl-tris-ATP-EDTA[30] as the mitochondrial isolation medium, and a Warburg respirometer to study oxidative phosphorylation. The

present study employed a polarographic technique with a relatively simple incubation medium and short incubation times; however, since control studies were performed in each case, differences in techniques should not affect the results obtained. Furthermore, Gimeno *et al.*,[28] using the polarographic technique, also reported very low RCI values in 5 cases of human neuromuscular disorders.

A certain clinicopathological correlation is evident in the series and is summarized in Table I. Patients 3, 4 and 5 were the most severely affected from all aspects of the combined investigation, while patients 7 and 8 (who also appeared clinically normal) were the least affected. Although the data undoubtedly lend added significance to the assumed relationship between disordered structure and function in the skeletal muscle samples, we cannot claim to have resolved the problem of cause and effect.

The combination in these patients of a peculiar habitus, intellectual subnormality, cardiomyopathy and skeletal muscle weakness also raises the important issue of a multisystem disorder. This subject has been adequately discussed by McLeod *et al.*,[31] who concluded that the frequent clinical and pathological involvement of tissues other than muscle in the mitochondrial myopathies, made it likely that muscle disease was only one manifestation of a more widespread disturbance of tissue metabolism. Pathological studies of cardiac and skeletal muscle in our series, together with a brain biopsy performed on one patient (case 6), failed to demonstrate a uniform or commonly recognized abnormality of mitochondria — whatever such an observation might be worth. However, the well-known paracrystalline mitochondrial inclusions were seen in muscle biopsies by Isaacs and Munke,[5] Smith *et al.*[6] and Fisher and Danowski,[32] whose patients all had overt cardiac disease. Similarly, these inclusions have been found in muscle and cerebellum in a case of Kearn-Sayre syndrome,[33] so that their absence in three different tissues studied by us, together with a distinctive skeletal muscle metabolic picture, makes it likely that we have encountered a different pathological entity. We therefore feel justified in proposing the following hypothesis: the siblings in this family have an inherited form of cardiac and skeletal muscle disease whose morphological expression includes such neuropathic features as target cells, cores, replicated T-tubules and abnormal myoneural junctions. These changes may be the consequence of chronic, partial denervation and reinnervation implied in the 'sick motor neuron' theory of McComas *et al.*,[34] mitochondrial structure and function being affected on the proven basis of neurotropic regulation.[35,36] This would also be consistent with the findings that experimentally induced denervation atrophy and disuse atrophy can bring about depressed mitochondrial function;[37] in our cases the mitochondrial lesion may thus be entirely secondary.

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