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Mitochondrial Myopathy Studies on Permeabilized Muscle Fibers[1]

THIERRY LETELLIER, MONIQUE MALGAT, MICHELLE COQUET, BRIGITTE MORETTO, FRANÇOISE PARROT-ROULAUD, AND JEAN-PIERRE MAZAT

Université Bordeaux II and Institut de Biochimie Cellulaire et Neurochimie-CNRS, F-33076 Bordeaux-Cedex, France [T.L., M.M., B.M., J-P.M.], and Hôpital Pellegrin, F-33076 Bordeaux-Cedex, France [M.C., F.P-R.]

ABSTRACT. Respiratory parameters of skeletal muscle were determined in permeabilized muscle fibers by adapting a technique described by Veksler *et al.* for cardiac fibers (Biochim Biophys Acta, 892:191–196, 1987). This method consists of the permeabilization of muscle fibers by saponin by allowing respiratory substrates and inhibitors to reach the mitochondria. In this way, the mitochondria may be studied inside the fibers as if they were isolated. We have verified, using various techniques, that the mitochondria remain intact during this procedure. This method has been applied to the study of six newborn infants for whom a diagnosis of a mitochondrial defect was suspected. In all cases, the defect was to be found on the permeabilized fibers, and this was confirmed by an enzymatic study. The advantage of this new method, associated with the measurement of the enzymatic activities on a crude homogenate, is to enable a simple and rapid diagnosis on a small amount of sample without damaging the mitochondria during the isolation procedure. (*Pediatr Res* 32: 17–22, 1992)

Abbreviations

Cox, cytochrome *c* oxidase
UQ_1, ubiquinone 1
UQ_1H_2, ubiquinol 1
KCN, potassium cyanide

Mitochondrial disorders are a heterogeneous group of mostly inborn diseases with very diverse patterns of clinical expression from lactic acidosis, neonatal hypotonia, weakness, failure to thrive, and cardiomyopathy to complex disorders such as Leigh disease, Kearns-Sayre syndrome, MELAS (myopathy, encephalopathy, lactic acidosis, stroke-like episodes), and MERFF (myoclonic epilepsy, ragged red fibers) syndrome (1). One major subgroup in all of these clinical expressions is characterized by functional deficiencies of the mitochondrial respiratory chain.

The mitochondrial respiratory chain is a succession of enzymatic complexes catalyzing oxidoreduction reactions leading to the oxidation of a respiratory substrate and to the reduction of oxygen to water. The energy produced by these reactions is converted into a proton gradient between the matrix and the intermembrane space of the mitochondria (2), which is in turn used for ATP synthesis. Both the respiratory chain complexes and the mitochondrial inner membrane must be unimpaired for the proton gradient to be set up. In the analysis of mitochondrial myopathies, enzymatic assay of the different respiratory chain complexes is therefore not sufficient; the study of oxidative phosphorylation in whole mitochondria is also necessary. This may be followed by polarographic measurements of oxygen consumption by mitochondria isolated from a large tissue sample of at least 500 mg.

The necessity of obtaining large tissue samples for biopsy obviously somewhat limits such studies, especially in young children. For this reason, we decided to investigate the possibility of studying mitochondrial respiration directly on permeabilized muscle fibers, adapting a technique described by Veksler *et al.* (3) for cardiac fibers. One of the advantages of this technique is that it requires only a small amount of tissue. Another advantage is that it does not necessitate the isolation of mitochondria, which can be a damaging step especially when dealing with defective mitochondria.

We applied this new method to the study of several young children for whom a diagnosis of a mitochondrial defect was suspected. In all cases in which a defect was found on permeabilized muscle fibers, it was confirmed by enzymatic studies in muscle homogenate and, when possible, by polarographic measurements on isolated mitochondria.

MATERIALS AND METHODS

Muscle biopsy was performed on patients and controls (deltoid muscle) under general or local anesthesia (performed with informed parental consent). Control muscle was taken from patients who underwent muscle biopsy for neuromuscular symptoms but were ultimately found to be free of any muscular disease.

Morphology. For histochemical analysis, the muscle specimens were immediately frozen in isopentane cooled in liquid nitrogen. Muscle specimens were examined using routine procedures, including the modified Gomori trichrome stain and histochemical reactions for NADH dehydrogenase, cytochrome oxidase, and myofibrillar ATPase (4).

Specimens for electron microscopy were fixed using 2.5% glutaraldehyde and postfixed in osmium tetroxide; they were then embedded in epoxy resin. Ultra-thin sections were stained with saturated uranyl acetate and lead citrate.

Permeabilization of Fibers. Bundles of fibers between 10 and 20 mg were incubated for 20 min in 2 mL of solution A as described (3) (EGTA 10 mM, Mg^{2+} 3 mM, taurine 20 mM, DTT 0.5 mM, imidazole 20 mM, K^+2-[N-morpholino]ethane sulfonic acid 0.1 M, pH 7.0, ATP 5 mM, and phosphocreatine 15 mM) containing saponin 50 μg/mL. The bundles were then washed twice for 15 min each time in solution B (EGTA 10 mM, Mg^{2+} 3 mM, taurine 20 mM, DTT 0.5 mM, imidazole 20 mM, K^+2-

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Correspondence and reprint requests: Jean-Pierre Mazat, Université Bordeaux II and IBCN-CNRS, 146, rue Léo Saignat, 33076 Bordeaux-Cedex, France.
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[N-morpholino]ethane sulfonic acid 0.1 M, pH 7.0, phosphate 3 mM, and 5 mg/mL fatty acid-free BSA) to remove saponin. All procedures were carried out at 4°C with extensive stirring.

The extent of the permeabilization during the digestion was estimated by the determination of the activity of lactate dehydrogenase (5) and citrate synthase (6) in the medium and inside the fibers.

Respiration Measurements. The oxygen consumption rate was measured polarographically at 30°C using a Clark electrode connected to a computer that gave an on-line display of rate values. The respiratory rates were determined in an oxygraph cuvette containing one bundle in 1 mL of solution B. To express the respiration rates per mg of fibers after a respiration run, the fiber is dried and weighed. The respiratory activities are expressed in natom O/min/mg of fiber.

The bundles of fibers were kept in solution A and skinned in the solution with saponin just before the oxygraphic measurements were carried out. The preparations remain stable in the ice-cold solution A for at least 3 h.

Isolation of Mitochondria. When a larger amount of muscle, *i.e.* 500 mg or more, was available, purification of mitochondria was performed as described by Morgan-Hughes *et al.* (7). Muscle was collected in isolation medium I (mannitol 210 mM, sucrose 70 mM, Tris/HCl 50 mM, pH 7.4, and EDTA 10 mM), digested by trypsin (0.5 mg/g of muscle) for 30 min, and homogenized. The homogenate was centrifuged at 1000 × *g* for 5 min. The supernatant was strained on gauze and recentrifuged at 7000 × *g* for 10 min. The resulting pellet was resuspended in the ice-cold isolation medium II (mannitol 225 mM, sucrose 75 mM, Tris/HCl 10 mM, pH 7.4, and EDTA 0.1 mM), and a new series of centrifugations (1000 and 7000 × *g*) was performed. The last pellet of mitochondria was resuspended into a minimum volume of isolation medium II to obtain a mitochondria concentration lying between 20 and 40 mg/mL. Protein concentration was estimated by the tryptophan fluorescence (excitation: 295 nm; emission: 340 nm) of diluted mitochondria using BSA as standard.

The oxygen consumption rate was measured polarographically at 30°C in an oxygraph cuvette containing 0.25 mg of mitochondrial protein in 0.5 mL (0.5 mg/mL) of respiratory buffer (mannitol 75 mM, sucrose 25 mM, KCl 100 mM, Tris phosphate 10 mM, Tris/HCl 10 mM, pH 7.4, and EDTA 50 μM). The respiratory activities were expressed in natom O/min/mg of protein.

Preparation of Crude Homogenate. Twenty to 60 mg of the same muscle biopsy were homogenized in nine volumes of 0.15 M KCl, 10 mM Tris/HCl, pH 7.4, and centrifuged at 650 × *g*. Spectrophotometric assays were performed as described below.

Enzymatic Determination. Citrate synthase. Citrate synthase was measured as described by Sheperd and Garland (8) in the presence of 4% Triton (vol/vol).

Complex I (NADH ubiquinone reductase). The oxidation of NADH by complex I was recorded using the ubiquinone analogue UQ_1 as electron acceptor (9). The basic assay media (35 mM KH_2PO_4, 5 mM $MgCl_2$, and 2 mM KCN, pH 7.2) was supplemented with defatted BSA (2.5 mg/mL), antimycin (5 μg/mL), 65 μM UQ_1, and 0.13 mM NADH in a final volume of 1 mL. The enzyme activity was measured at 30°C, and the reaction was started with 50 μg of mitochondrial protein. The decrease in absorption due to NADH oxidation was measured at 340 nm both in the absence and in the presence of rotenone 5 μg/mL.

Complex III (ubiquinol cytochrome c reductase). The oxidation of UQ_1H_2 by complex III was determined using cytochrome *c* (III) as electron acceptor (9). The assay was carried out in basic medium supplemented with 2.5 mg/mL defatted BSA, 15 μM cytochrome *c* (III), and rotenone (5 μg/mL) in a final volume of 1 mL at 30°C. The reaction was started with 10 μg of mitochondrial protein, and the enzyme activity was measured at 550 nm.

Complex IV (Cox). The complex IV activity was measured by the method described by Wharton and Tzagoloff (10) using cytochrome *c* (II) as substrate. The oxidation of the cytochrome *c* was monitored at 550 nm at 30°C.

Complex II + III (succinate cytochrome c oxidoreductase). This activity was determined by observing the reduction of cytochrome *c* with succinate as substrate in the presence of rotenone and KCN. Rotenone is added to eliminate reduction of cytochrome *c* via the NADH dehydrogenase complex, and KCN is added to prevent further oxidation of cytochrome *c* by cytochrome *c* oxidase (11).

All the enzymatic activities are expressed in μmol of product formed per min and per g of tissue.

RESULTS

Permeabilization and Respiration of Fibers. Optimal fiber digestion time. Figure 1 shows the effect of saponin on the digestion time on three different control muscle biopsies. The release of lactate dehydrogenase (a cytosolic enzyme) and citrate synthase (an enzyme of the mitochondrial matrix) was measured

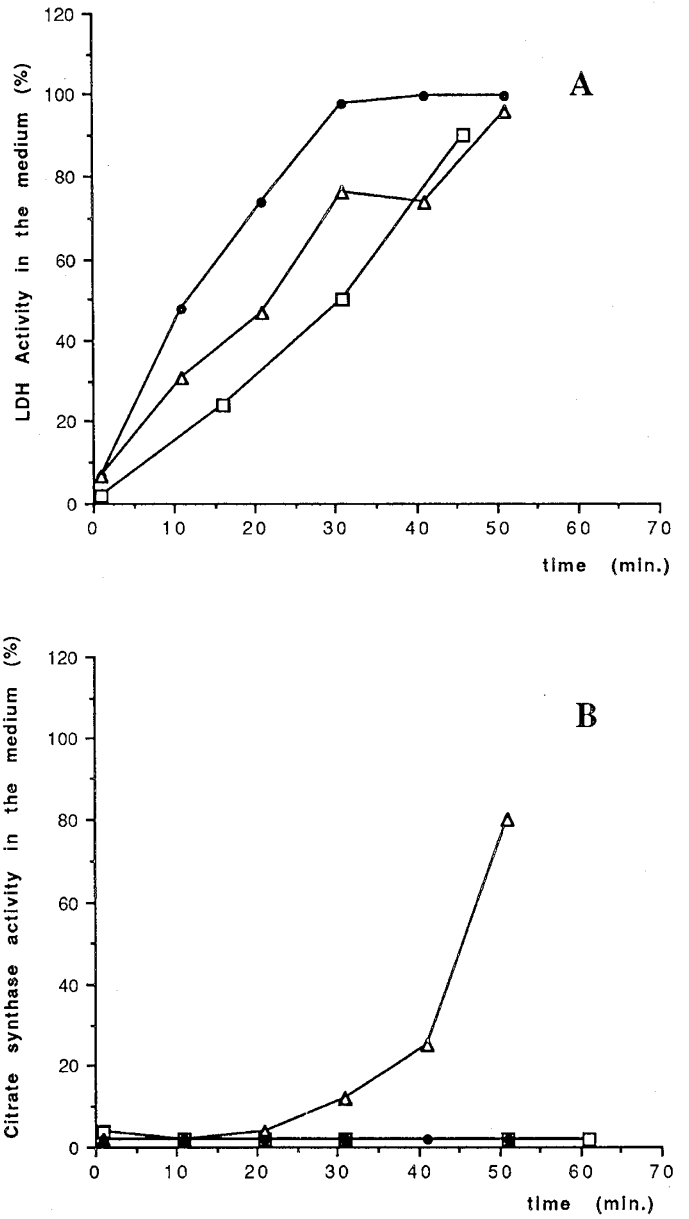
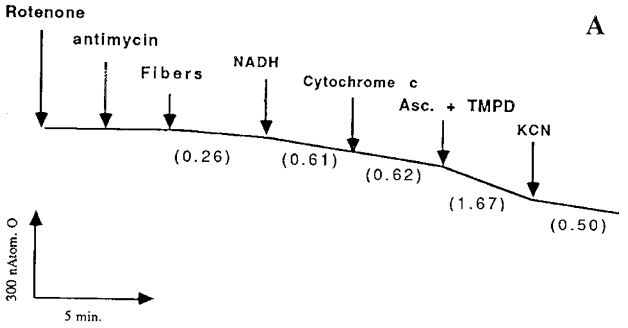
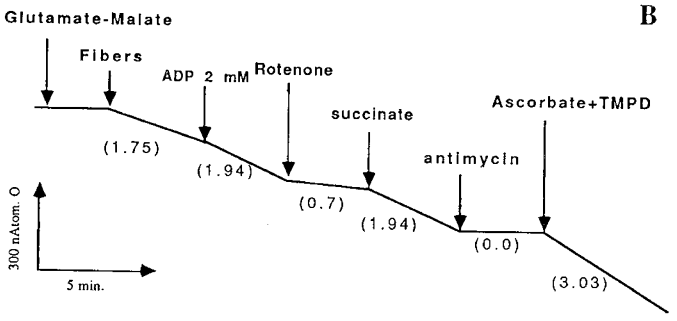


Fig. 1. Release of various enzymatic activities in the incubation medium during the permeabilization procedure. *A*, Release of lactate dehydrogenase (*LDH*) activity. *B*, Release of citrate synthase activity. The different symbols correspond to three different control biopsies at ages 18 y (•), 25 y (△) and 50 y (□).

in the medium. After 20 min, between 30 and 70% of the lactate dehydrogenase was found outside the fibers. This indicates a sufficient permeabilization of the cytoplasmic membrane to observe a mitochondrial respiration driven by external added substrates (see below). After 20 min, there was no citrate synthase activity observable in the medium. In one case only, citrate synthase began to appear outside the fiber after 30 min (10%); in the other two cases no citrate synthase was found outside the fibers even after 60 min. This indicates that the mitochondrial inner membrane is preserved intact. To verify that the outer mitochondrial membrane was intact, we used the well-known test with cytochrome *c* (12, 13): the oxygen consumption of permeabilized fiber incubated with antimycin A 20 μg/mL and rotenone 20 μg/mL was measured using NADH 2 mM and cytochrome *c* 25 μM as substrate (Fig. 2*A*). No increase in the respiration rate was observed for an incubation time with saponin ranging from 0 to 50 min. This indicates that cytochrome *c* cannot cross the mitochondrial outer membrane. It was verified that cytochrome *c* oxidase activity was stimulated by ascorbate-tetramethyl-p-phenylenediamine and inhibited by KCN. It follows from the above measurements that the optimal digestion time is 20 min, *i.e.* after 20 min the cytoplasmic membrane is sufficiently permeable and the mitochondria undamaged.

Electron microscopy. Electron microscopy studies have shown (Fig. 3) that the mitochondrial membranes remain intact during the 20 min of digestion of muscle fibers with saponin and after a further incubation in respiratory buffer in the oxygraph cuvette.





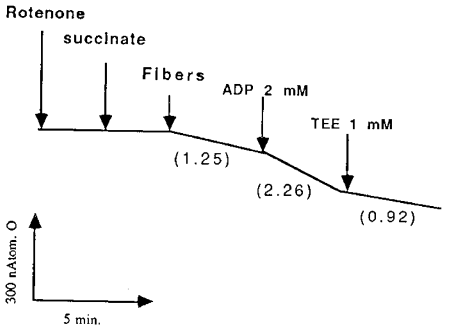


Fig. 2. Typical oxygraph runs of respiratory activities of mitochondria in permeabilized muscle fibers. The respiration buffer (1 mL) is described in Materials and Methods. *Numbers in parentheses* indicate the rate of oxygen consumption in natom O/min/mg of fiber at 30°C. The substrates and the inhibitors are added as indicated: NADH 2 mM, cytochrome *c* 25 μM, glutamate and malate 10 mM; ADP 2 mM; rotenone 20 μg/mL; succinate 10 mM, antimycin 20 μg/mL, triethyltin (TEE) 12 μM, ascorbate 4 mM, tetramethyl-p-phenylenediamine (TMPD) 0.3 mM, and KCN 2 mM (*A*, 15 mg; *B*, 17 mg; *C*, 20 mg).

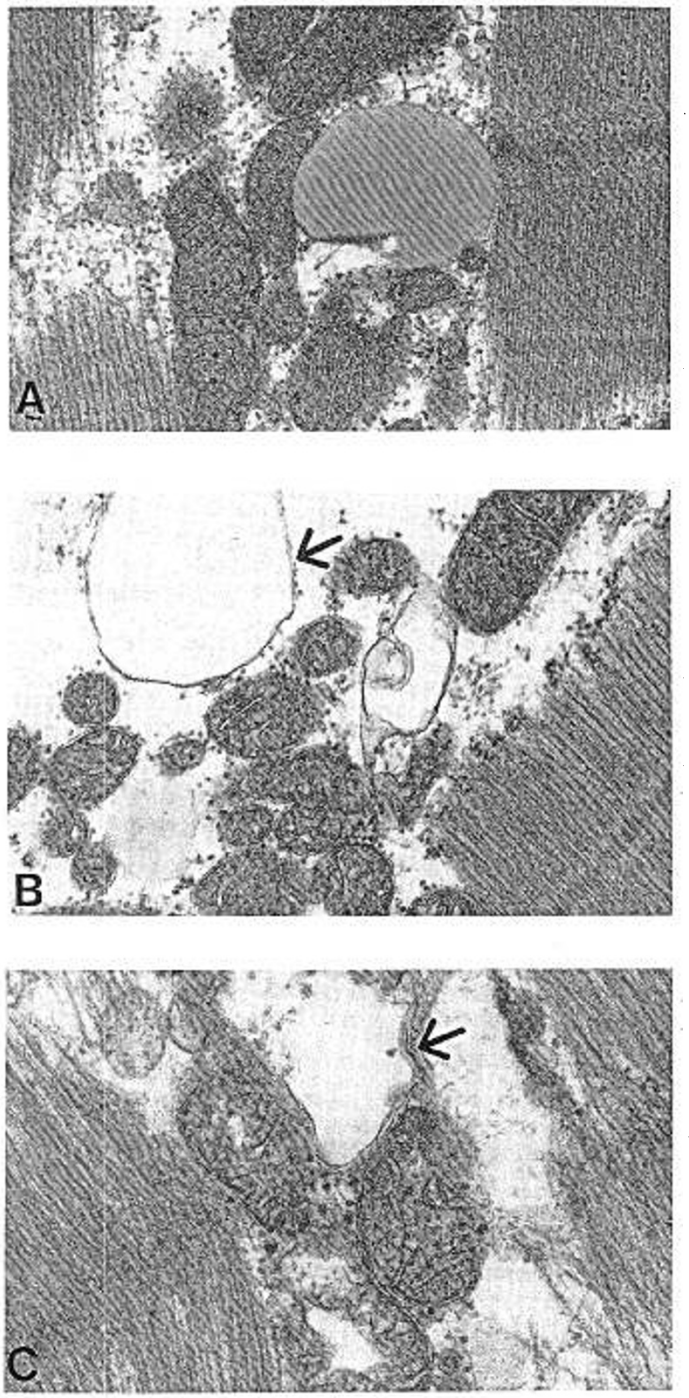


Fig. 3. Ultrastructural study, longitudinal sections. *A*, t_0. Cluster of mitochondria between two normal myofibrils can be observed (×30 000). *B*, t_0. Dilated sarcoplasmic reticulum (*arrow*) can be seen, but mitochondria are intact (×30 000). *C*, Myofibrils are disorganized, the sarcoplasmic reticulum is dilated (*arrow*), and one of the three mitochondria is clarified (×45 000).

At time zero (t_0), before the permeabilization treatment, there were no myofibril lesions or mitochondria lesions (Fig. 3*A*). After digestion (t_1), dilated sarcoplasmic reticulum was the only anomaly to be seen (Fig. 3*B*). After one respiration run (t_2), some segmentally hypercontracted muscle fibers were observed, sarcoplasmic reticulum was dilated, and mitochondria were sometimes enlarged and swollen (Fig. 3*C*).

Respiration of fibers. Figure 2*B* shows a typical run of oxygen consumption of permeabilized fibers. Even in the absence of ADP, a rather high respiratory rate is observed with glutamate or pyruvate (plus malate) or with succinate. This high rate is probably related to the endogeneous ATPase activities that regenerate ADP, as pointed out by Veksler *et al.* (3).

Addition of 2 mM ADP enhances the rate of respiration. This respiration is sensitive to the classical inhibitors of ATP synthase, namely oligomycin and triethyltin, and of respiratory chain (rotenone, antimycin, and cyanide), and also to uncouplers (carbonyl cyanide *m*-chloro-phenylhydrazone). This enables us to calculate a respiratory control of 2.64 ± 0.55 ($n = 4$), which gives an idea of the ability of mitochondria to synthesize ATP inside the fibers as shown in Figure 2*C*.

Enzyme Activities in Crude Homogenate. Control values were obtained from patients of the same age group (one at 2 mo, three at 3 mo, and one each at 8 mo and 5, 7, and 11 y) who appeared normal after examination. We decided to normalize the enzymatic activities connected with the respiratory chain with respect to an independent activity, the one of citrate synthase. With this normalization, the distribution of the control values appears to follow a normal distribution with an acceptable standard deviation. This enables us to distinguish which values are defective and which values are normal.

One problem remains, namely the case of complex I inhibition by rotenone. The extent of rotenone inhibition of complex I activity is highly variable. This is probably due to the mode of preparation and conservation of the crude homogenate. It has been shown by several authors (14, 15) that the rotenone sensitivity may be easily lost when the homogenate has been frozen or prepared from frozen or postmortem biopsies. For this reason, we have preferred to present both the total value and the rotenone-sensitive part of this activity. In the control, these values follow a normal distribution law.

Application to Patients. Six patients suffering from mitochondrial disease were studied. Details of their clinical case histories, together with the results of biologic and histologic investigations, are shown in Table 1 and 2.

Table 3 summarizes the polarographic study of the permeabilized fibers of the patients, and Table 4 summarizes the corresponding enzymatic activities measured on the crude homogenate.

Case H. Az. In this case, all the respiration rates of the fibers (Table 3) were uniformly low, suggesting a defect in complex IV. This was confirmed by the enzymatic study on the crude homogenate (Table 4), the enzymatic and polarographic studies on isolated mitochondria (data not shown), and the histologic determination of Cox activity (Table 2). We want to emphasize that although the NADH-UQ_1 reductase activity was rotenone insensitive the NADH-cytochrome *c* reductase activity was rotenone sensitive (not shown). For this reason, we retain only the defect in complex IV to explain the observations concerning this patient.

Case M. Az. This defect was found again in a younger brother of patient H. Az., although the Cox deficiency was less pronounced and, in fact, was situated within 2 SD of the mean. The histologic analysis also displayed a Cox deficiency.

Case L. N'F. Once more, all of the respiration rates of the fibers were uniformly low, but the enzymatic study did not seem to indicate a complex IV deficiency. In this case, the answer comes rather from the histologic examination, which indicated a clear glycogenosis, a normal Cox activity, and a low mitochondria content (Table 2), which explained the low respiration rates of the fibers. Nevertheless, the enzymatic study showed low complex I activity (normalized with respect to citrate synthase activity) associated with the absence of rotenone inhibition, which could indicate an associated defect in complex I. The same results are obtained from a postmortem biopsy. We isolated the mitochondria from this last biopsy. The measure of NADH-UQ_1 reductase activity on the mitochondria isolated from the second (postmortem) biopsy was also indicative of a complex I deficiency (data not shown). The fact that the oxidation of glutamate and pyruvate is not more severely affected than that of succinate (Table 3) might be explained by the low respiratory rates and also by the bad condition of the biopsy (full of glycogen with a low content in mitochondria).

Case B. La. Although this case did not present a clear clinical pattern suggesting a mitochondrial defect, a biopsy was nevertheless studied because of the weak hyperlactacidemia (after glucose load) and the fact that two sisters had died at 1 and 2 mo with more pronounced signs of mitochondrial myopathy. In this case, the fiber respiration rate was low with glutamate and pyruvate. Complex IV was normal (Tables 3 and 4), but the only indication of a complex I deficiency demonstrated on the enzymatic study was the absence of rotenone inhibition. These observations seem to suggest a defect in complex I. The same defect, although more pronounced, was found in the sister who had died at 2 mo.

Case A. Ge. The fiber respiration rate was low with glutamate and pyruvate and with succinate as respiratory substrates, even though it was within the range of 2 SD. This would tend to indicate a defect in complex III, which was indeed confirmed by the enzymatic study on crude homogenate (Table 4). The fact that there was no rotenone inhibition could also indicate an associated defect of complex I.

Case C. Sa. The fiber respiration rates were all within the 2 SD range (but low on glutamate or pyruvate and on succinate). The enzymatic study showed normal activity for all of the complexes. It was not possible to demonstrate the existence of a defect on the respiratory chain.

Table 1. *Case reports**

	H. Az	M. Az	L. N'F	B. La	A. Ge	C. Sa
Sex	F	M	F	F	F	F
Age at onset	24 h	24 h	24 h		48 h	1 mo
Age at death	1 wk	1 wk	6 mo	Alive	Alive	Alive
Age at study	1 wk	1 wk	2 wk and 6 mo	1 wk	4 mo	1 mo
Consanguinity	+	+	+	+	−	−
Failure to thrive	+	+	+	−	−	+
Hypotonia	+	+	+	−	+	+
Lactic acidosis	++	+	+	±	+	++
Tubulopathy	+	−	+	−	−	ND
Cardiomyopathy	−	−	−	−	+	ND
Hepatic dysfunction	+	+	+	−	−	+

* ND, not determined. + indicates the presence of the clinical feature; −, absence; ±, weak; and ++, strong.

Table 2. *Morphology and histoenzymology**

Patient	Ragged red fibers	Lipid storage	Cox	Other	U.S. mitochondrial anomalies
H. Az	–	–	–	Glycogen depletion	–
M. Az	–	–	–		–
L. N'F	–	–	+	Glycogen storage	–
L. N'F (postmortem)	–	–	+	Myophosphorylase deficit	–
B. La	–	–	+		–
A. Ge	–	–	+		–
C. Sa	–	±	+	Glycogen depletion	–

* Symbols are the same as in Table 1.

Table 3. *Fibers respiration (natom O/min/mg muscle)**

Patients	Respiratory substrate and studied complexes		
	Glu or pyr (I + III + IV)	Succinate (II + III + IV)	Ascorbate-TMPD (IV)
H. Az	0.37	0.29	0.68
M. Az (postmortem)	0.42	0.63	1.22
L. N'F	0.40	0.69	1.30
L. N'F (postmortem)	0.49	0.52	1.06
B. La	0.52	1.59	2.32
A. Ge	0.74	1.46	3.37
C. Sa	0.75	1.17	2.90
Control	1.55 ± 0.43 (15)	2.57 ± 0.73 (20)	3.80 ± 1.19 (12)

* Glu or pyr, glutamate or pyruvate; TMPD, tetramethyl-p-phenylenediamine. I, II, III, and IV denote complex I, II, III, and IV activities, respectively. For the control, the SD is given with the number of determinations in parentheses.

Table 4. *Enzymatic activities determined on a crude homogenate**

Patient	I/CS	Δ I/CS	(II + III)/CS (SCR/CS)	III/CS	IV/CS (Cox/CS)
H. Az	0.32	0.020	0.19	ND	0.28
M. Az	0.13	0.071	0.19	0.35	0.77
L. N'F	0.13	0.018	0.17	0.22	1.4
L. N'F (postmortem)	0.09	0	0.13	0.33	ND
B. La	0.48	0	0.18	0.87	1.04
A. Ge	0.33	0	0.05	0.11	ND
C. Sa	0.29	0.095	0.20	0.4	1.5
Control	0.26 ± 0.08 (6)	0.097 ± 0.05 (9)	0.23 ± 0.08 (10)	0.47 ± 0.15 (7)	1.09 ± 0.33 (8)

* All of the activities are normalized with respect to the citrate synthase activity (CS). I, II, III, and IV denote complex I, II, III, and IV activities, respectively. ΔI represents the rotenone-sensitive complex I activity; Cox is cytochrome *c* oxidase activity; and SCR is succinate cytochrome *c* reductase activity. ND, not determined. For the control, the SD is given with the number of determinations in parentheses.

DISCUSSION

We have shown that the method of Veksler *et al.* (3) can be applied to the respiration study of skeletal muscle fibers. The experiments described in this paper show that the incubation time of 20 min is enough to allow the smaller molecules of substrates and inhibitors to enter the fibers. We have verified that the mitochondria remain intact during this period using several techniques. Furthermore, we have observed that extending the digestion time can lead to fiber damage in some cases.

The respiratory pattern of permeabilized fibers (Fig. 2) is similar to that of isolated mitochondria and displays the same defects, when these occur. For a given biopsy, there seems to be a significant correlation between the same respiratory parameters measured on the permeabilized fibers and on isolated mitochondria when available (not shown). The same characteristics are found for the oxygen consumption in the fibers and in the isolated mitochondria, *e.g.* respiratory controls of the same order. The order of magnitude between the respiratory rates on the different substrates is also the same: the respiratory rate on the NADH-linked substrates is less than the respiratory rate on succinate, which is in turn less than the respiratory rate on ascorbate + tetramethyl-p-phenylenediamine. This reinforces our belief that this is a reliable method for the study of pathologic mitochondria *in situ*.

Although a polarographic study is essential, it does not always provide sufficient information to establish a clear diagnosis. So it is advisable to carry out an enzymatic study at the same time. In the present study, this enzymatic study was made on a crude homogenate. We have shown the activities normalized with respect to the citrate synthase activity are relevant parameters comparable to the polarographic ones. These two groups of parameters are used together to reveal pathologic defects.

The study of our six patients has shown that all sorts of respiratory chain defects can be demonstrated equally well on permeabilized fibers as on isolated mitochondria using polarographic measurements. Nevertheless, the interpretation of the rough results must take into account some particular features of the permeabilized fibers. As on isolated mitochondria, the presence of multiple defects cannot be found using polarographic measurements alone, *e.g.* as in case A. Ge. Furthermore, defects outside the respiratory chain (transport of metabolites, ATP synthesis, Krebs cycle, etc.) can also lead to an abnormal pattern of fiber respiration. In such cases, the results of the enzymatic study are normal, *e.g.* case C. Sa. Some specific problems arise from the polarographic measurements on permeabilized fibers.

The quantity of mitochondria in the fibers should be taken into account, because a low mitochondria content will automatically give a low respiration rate on all substrates. This observation could lead us wrongly to suspect the existence of a defect in complex IV, *e.g.* case N'F.

Another problem encountered in the fiber analysis arises from the simultaneous presence of normal and pathologic mitochondria inside a given fiber (heteroplasmy as described in Refs. 16–18). This can explain some of our results where some respiration rates, although low, were within the 2 SD range (cases A. Ge and C. Sa).

Conclusions. The polarographic studies on permeabilized muscle fibers give results similar to those found for isolated mitochondria and afford many other advantages. During the isolation of mitochondria, two artifacts can occur. One is damage of mitochondria that could be labile for other reasons. This could lead to the erroneous identification of a mitochondrial defect that is, in fact, an artifact of the preparation. A second type of artifact arises from the fact that several types of mitochondria can exist in the same tissue, particularly in muscle (19). Usually, all of these different types of mitochondria are isolated, presumably in given proportions for a given preparation. In the case of mitochondrial pathology, the proportions or the characteristics of the different populations of mitochondria can change, so a different proportion of these different mitochondria could be isolated with the isolation procedure that is adapted to normal mitochondria. In the extreme case, one can isolate only normal mitochondria. These possible artifacts are avoided by analyzing mitochondria respiration *in situ* in the muscle fiber.

Another advantage of this technique is that under these conditions the mitochondria are more in their normal cellular environment than when they are isolated in a respiratory buffer. The biggest advantage of this method is that it requires only a minute amount of muscle. Ten to 20 mg of fibers is enough to test the functioning of complexes I, III, and IV, complexes II, III, and IV, and, finally, complex IV alone. This last argument is of importance when dealing with newborn infants. Case C. Sa. illustrates this point very well. In this case, it was impossible to obtain more than 90 mg for the biopsy. Half of the biopsy was used to make a crude homogenate and the other half was permeabilized and studied polarographically.

Because the technique is relatively simple and rapid, it can now be used routinely. We are now able to examine an increasing number of young children in our laboratory.

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