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Fatal Lactic Acidosis in Infancy with a Defect of Complex III of the Respiratory Chain

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ABSTRACT. We report our studies on the metabolic defects which caused a newborn infant to present with a severe lactic acidemia (25 mM) and to die on the 3rd d after birth despite intensive supportive measures. The mitochondrial fractions prepared from skeletal muscle and liver oxidised NAD^+-linked substrates and succinate slowly. Spectrophotometric assays for complexes I, II, and III of the respiratory chain demonstrate a specific defect of complex III in the skeletal muscle and liver mitochondrial fractions. The concentrations of cytochrome *b* were 75% lower in the skeletal muscle and heart mitochondria than in control preparations. The amount of non-heme iron sulphur protein of complex III was low in skeletal muscle, liver, and heart. This case differs from previous reports of complex III deficiency in three respects: the patient presented in the neonatal period, the defect was expressed in several tissues, and it was fatal. (*Pediatr Res* 25: 553–559, 1989)

Abbreviations

DNP, dinitrophenol
RCR, respiratory control ratio
UQ_1, ubiquinone-1
UQ_1H_2, ubiqinol-1

Lactic acidosis is an important clinical problem which can be caused by one of several biochemical lesions (1) including inborn errors of one or more of the complexes of the mitochondrial respiratory chain (2). In patients with the latter abnormality, the impaired flux of electrons along the respiratory chain to oxygen limits the oxidation of pyruvate. This is converted to lactate so maintaining the cytosolic $NADH/NAD^+$ ratio and allowing glycolysis to continue. We describe the clinical and biochemical investigations of a neonate who died with severe lactic acidosis due to a defect of complex III of the respiratory chain.

The respiratory chain is divided into four complexes: complex I (NADH-ubiquinone reductase), complex II (succinate-ubiquinone reductase), complex III (ubiquinol-cytochrome *c* reductase), and complex IV (cytochrome *c* oxidase). These complexes, each of which contains several polypeptide subunits span the mitochondrial inner membrane. The respiratory chain transfers electrons from reduced cofactors to molecular oxygen. The passage of electrons along the respiratory chain is used to generate a proton gradient across the inner mitochondrial membrane which is used for the synthesis of ATP (3). Complex III consists of 11 subunits; core proteins 1 and 2, a nonhem FeS centre (nFeS, Rieske protein), cytochrome *b*, cytochrome c_1 and six additional peptides (4). Cytochrome *b* has 2 iron-porphyrin prosthetic groups with characteristic absorption spectra and oxidation reduction potentials (cytochrome b_i [b_{562}] and cytochrome b_o [b_{566}]) bound to a single protein. Ten peptides of complex III are coded for by nuclear DNA, synthesized on cytoplasmic ribosomes and imported into the mitochondrial inner membrane; only the apoprotein of cytochrome b is coded for and synthesized by the mitochondria.

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CASE REPORT

The patient was male, the 3rd baby born to healthy, unrelated parents. The pregnancy was normal until the 3rd trimester when fetal growth was noted to be slow. Because of this, delivery was induced at 38 wk of gestation. During labor, there was no fetal distress, and the baby was delivered vaginally. The two previous pregnancies were uneventful and the siblings are alive and well. There is no family history of neurologic or metabolic disease.

The baby's wt (2020 g) was below the 3rd percentile for gestational age, and there was wasting of the buttocks and thighs compatible with intrauterine growth retardation. The circumference of his head was 30.5 cm, and his anterior fontanelle was normotensive. He was irritable but otherwise neurologically normal. He had a mass palpable in the left hypochondrium due to a polycystic kidney.

At four h of age, the baby started to hyperventilate and became hypotonic. He was hypoglycemic (blood glucose 0.7 mM) with a severe lactic acidosis (blood lactate concentration 25 mM; pyruvate 185 μM; bicarbonate 6 mM; arterial blood pH was 7.13). There was generalized aminoaciduria compatible with renal tubular dysfunction. Aminoaciduria has previously been described in cytochrome *c* oxidase deficiency (5).

He was given a bolus of dextrose intravenously (0.2 $g \cdot kg^{-1}$) and was started on a continous infusion of 10% dextrose at a rate of 6 $mg^{-1} \cdot kg^{-1} \cdot min$. His blood glucose concentration returned to normal, and he subsequently remained normoglycemic. A bicarbonate infusion was given, but this failed to improve his condition and acidosis. Peritoneal dialysis was then commenced, and his blood pH increased to 7.31, and his blood lactate concentration fell to 18.5 mM. He was given thiamine, riboflavin, and biotin (100 mg, 100 mg, and 30 mg, respectively).

The patient remained conscious with normal tone, although he was iritable on handling. At 36 h of age he developed dystonic posturing, seizures, and coma and died at 53 h despite peritoneal dialysis and vitamin therapy. Consent for an urgent postmortem examination was given and tissues (skeletal muscle, liver, heart, and kidney) were removed within one h of death and placed in ice-cold isolation media (see below). Mitochondrial fractions were prepared within 2–4 h of removal of the tissues.

MATERIALS AND METHODS

Materials. UQ_1 was a gift from Takeda Chemical Industries, Osaka, Japan. Nitrocellulose filters (0.45-μm pore size) were obtained from Anderman and Co., London, UK. Other biochemicals were obtained from the Sigma Chemical Co. (St. Louis, MO) or from Boehringer Corp., (Indianapolis, IN).

Cytochemistry. Muscle obtained by biopsy was frozen in dichlorodifluoromethane (Arcton 12, ICI) and cooled to −150°C in liquid nitrogen. Routine cytochemical screening using frozen sections (6) included demonstration of cytochrome *c* oxidase activity (7).

Preparation of mitochondrial fractions. Skeletal muscle mitochondrial fractions were prepared from 0.4–2.0 g of quadriceps from infant and adult controls (patients with no evidence of neuromuscular pathology) and from 5 g of the patient's muscle as described by Watmough *et al.*, (8). We were unable to obtain human heart suitable for oxidative studies or for assay of the individual complexes. The liver and kidney mitochondrial fractions were prepared as described by Gatley and Sherratt (9) except that the medium used was 250-mM-sucrose, 10-mM Hepes, 1-mM EGTA, pH 7.2. Defatted BSA (5 $mg \cdot mL^{-1}$) was added during homogenization. No human kidney controls were available; the control liver samples were obtained from children or adults undergoing diagnostic laparotomy who were found to have no detectable metabolic disease. Protein was determined by a modified Lowry method (10).

Determination of mitochondrial oxidations. Substrate oxidations by mitochondrial fractions (0.2–0.4 $mg \cdot ml^{-1}$ final assay protein concentration) were recorded spectrophotometrically using an Hitachi 557 dual-wavelength spectrophotometer by following the reduction of ferricyanide at 420 nm with 475 nm as a reference wavelength (11). Oxygen consumption was measured polarographically at 30°C in a final vol of 750 μl; RCRs and ADP/O ratios were calculated as described by Sherratt *et al.* (10).

Determination of cytochrome redox spectra. The low temperature reduced minus-oxidized spectra of mitochondrial fractions after reduction with succinate, and after further reduction with dithionite, were recorded (10). The wavelengths and extinction coefficients quoted by Bookelman *et al.* (12) and an intensification factor of 7 for the medium (13) was used to calculate the cytochrome concentrations.

Spectrophotometric assay of individual respiratory chain complexes. Before determining the activity of the individual complexes, the mitochondrial membranes were disrupted by freeze thawing three times. A total of 0.6 mg of mitochondrial protein was required to determine the activity of complexes I–IV.

Complex I (NADH ubiquinone reductase). A modification of the method described by Fischer (14) was used. The oxidation of NADH by complex I was recorded using the ubiquinone analogue UQ_1 as the electron acceptor. The assay medium (35-mM KH_2PO_4, 5-mM $MgCl_2$, 2-mM KCN, pH 7.2) was supplemented with defatted BSA (2.5 $mg \cdot mL^{-1}$), antimycin (2 $\mu g \cdot mL^{-1}$), 65-μM UQ_1 and 0.13-mM NADH in a final vol of 1.0 mL. The enzyme activity was determined at 30°C and the reaction started with 60–90 μg mitochondrial protein. The decrease in absorption due to NADH oxidation was measured at 340 nm with 425 nm as a reference wavelength. As NADH cytochrome b_5 reductase in the outer mitochondrial membrane also oxidizes NADH (15), complex I activity was calculated from the difference in rate before and after the addition of rotenone (2 $\mu g \cdot mL^{-1}$), a specific inhibitor of complex I.

Complex II (succinate ubiquinone reductase). The oxidation of succinate by complex II was recorded with UQ_1 as an electron acceptor. The assay medium (35-mM KH_2PO_4, 5-mM $MgCl_2$, 2-mM KCN, pH 7.2) was supplemented with 65-μM UQ_1, antimycin (2 $\mu g \cdot mL^{-1}$), rotenone (2 $\mu g \cdot mL^{-1}$), and 60–90 μg of mitochondrial protein in a final vol of 1.0 mL at 30°C. The reaction was started by addition of 8-mM succinate and the decrease in absorbance due to the reduction of UQ_1 to ubiquinol followed at 280 nm with 465 nm as a reference wavelength. A reduced minus-oxidized extinction coefficient for UQ_1 of 13 $mM \cdot^{-1} cm^{-1}$ was used to calculate the rates (16). Complex II activity was inhibited 65–85% by 50-μM-thenoyltrifluoroacetone.

Complex III (ubiquinol-cytochrome c reductase). This procedure is a modification of the method of Nelson and Gellerfors (17). The oxidation of UQ_1H_2 by complex III was determined using cytochrome *c*(III) as the electron acceptor. To prepare UQ_1H_2, 7-mM UQ_1 in ethanol was reduced with excess solid sodium borohydride; reduction was assumed to be complete after a few minutes. The addition of 12 μL of 3-M HCl (final concentration 0.1 M) stabilizes the UQ_1H_2 and destroys the residual borohydride. Insoluble material was removed by centrifugation at 12000 × g_{av} for 4 min. The UQ_1H_2 can be stored in the dark for up to 24 h. Before each experiment, the characteristic UV spectra of UQ_1 and UQ_1H_2 were determined to confirm that none of the ubiquinol had been oxidized. The assay medium (35-mM KH_2PO_4, 5-mM $MgCl_2$, 2-mM KCN, pH 7.2) was supplemented with defatted BSA (2.5 $mg \cdot mL^{-1}$), 15-μM-cytochrome *c*(III) and rotenone (2 $\mu g \cdot mL^{-1}$) in a final vol of 1.0 mL at 30°C. UQ_1H_2 (15 nmol) was added, and the nonenzymatic reduction of cytochrome *c*(III) was recorded (550 nm with 580 nm as the reference wavelength) for 1 min. The reaction was started with 15–30 μg of mitochondrial protein, and the enzyme activity is expressed as an apparent first order rate constant after the remaining cytochrome *c* was reduced by a few grains of ascorbic acid. Antimycin inhibited this activity by 95%.

Complex IV (cytochrome c oxidase). Cytochrome oxidase activity was determined spectrophotometrically as described by Smith (18) using cytochrome *c*(II) as substrate and is expressed as an apparent first order rate constant with respect to cytochrome *c*(II) concentration. A final concentration of mitochondrial protein of 15–30 $\mu g \cdot mL^{-1}$ was used in the assay.

Duroquinol-ubiquinone transhydrogenase activity. The assay is based on the studies of Boveris *et al.* (19). The transhydrogenase reaction involves reduction of cytochrome b_i by duroquinol, which then reduces ubiquinone. The assay medium contained in a total vol of 1.0 mL at 30°C: 100-mM KCl, 1-mM EGTA, 2-mM KCN, 50-mM Hepes (pH 7.0), 0.1-mM duroquinol (prepared in the same way as UQ_1H_2) and 0.2–0.4 mg of mitochondrial protein. The reaction was started with 8 μM UQ_1 and the decrease in absorbance was followed using the wavelength pair 284–305nm. Sp act was defined as an apparent first order rate constant. This includes both the enzyme catalyzed rate, which was inhibited by antimycin, and a spontaneous chemical reaction. It was confirmed that enzyme activity at cytochrome b_i was unaffected by myxothiazol (2 $\mu g \cdot mL^{-1}$), which inhibits electron transport at cytochrome b_o (20).

Immunoblot analysis of peptide subunits of complex III. Mitochondrial fractions were solubilized by incubation with 8-M urea, 2% (vol/vol) mercaptoethanol, 5% (wt/vol) SDS at 30°C for 1 h. Peptides were separated by SDS-PAGE as described by Fuller *et al.* (21); both the stacking gel (5% polyacrylamide) and the separating gel contained 6-M urea. Antisera were raised in rabbits against holobeef heart complex III and subunits of the complex (22). A 15% separating gel was used for immunoblotting using antisera to the holocomplex III and the nFeS protein and a 10% separating gel for the core proteins. The proteins were transferred electrophoretically from the gel to a nitrocellulose

filter as described by Towbin *et al.* (23) with the addition of 1% SDS to the transfer buffer. Immunoreactive peptides were visualised using the immunoperoxidase method with 4-chloro-1-naphthol as substrate (24).

RESULTS

Cytochemistry. Skeletal muscle appeared histologically and histochemically normal for a child of this age. Deficiency of the respiratory chain including complex III deficiency usually causes morphologic changes, notably the accumulation of abnormal mitochondria. However, several patients have been described without such changes (25), and it should be emphasized that normal muscle morphology does not exclude a respiratory chain defect. The histologic demonstration of cytochrome *c* oxidase activity excluded the benign form of cytochrome *c* oxidase deficiency (2).

Substrate oxidations. Oxidations by mitochondrial fractions were measured within 8 h of death. The maximum rates of succinate and of glutamate plus malate oxidation by muscle mitochondria from the patient, measured using ferricyanide as acceptor, were about 20–30% of those from mitochondrial fractions from children and adults (Table I). We have no control data from normal human neonates. Cardiac mitochondria were tightly coupled with an unimpaired capacity for oxidative phosphorylation (not shown). Kidney mitochondria were also coupled with an RCR of 3.5 and ADP/O ratio of 3.4 with glutamate plus malate as substrate. Muscle and liver mitochondria were poorly coupled (RCR 1.5), although it is not known if this was due to postmortem changes in the tissue. The maximum rates of oxygen uptake recorded (ng atoms $O \cdot min^{-1} \cdot mg\ protein^{-1}$) were: skeletal muscle; succinate 64, glutamate plus malate 49 (controls [glutamate plus malate], 116 ± 31; $n = 4$), heart; glutamate plus malate 146, liver; succinate 86, glutamate plus malate 76, kidney; succinate 54, glutamate plus malate, 25.

Cytochrome concentration. Reduced minus oxidized spectra were determined for skeletal muscle mitochondrial fractions (Fig. 1; Table 2) and cardiac muscle mitochondrial fractions (Table 2). The cytochrome aa_3 and cytochrome *b* concentrations determined both after reduction with succinate or dithionite were respectively about 50% and 75% lower than in normal mitochondria (Table 2). The lower concentration of cytochrome *b* was confirmed from the decrease in absorption at 428 nm in the Soret regions of the spectra (not shown). The absorption due to cytochrome b in skeletal muscle and heart mitochondria after reduction with succinate was approximately doubled by further reduction with dithionite with a peak shift from 562 nm to 566 nm (Fig. 1) showing the presence of both cytochromes b_o and b_i. When the ratio of cytochrome aa_3 to cytochrome *c* was calculated, it was found to be close to control values, whereas the cytochrome *b* to either cytochrome aa_3 or cytochrome *c* was only

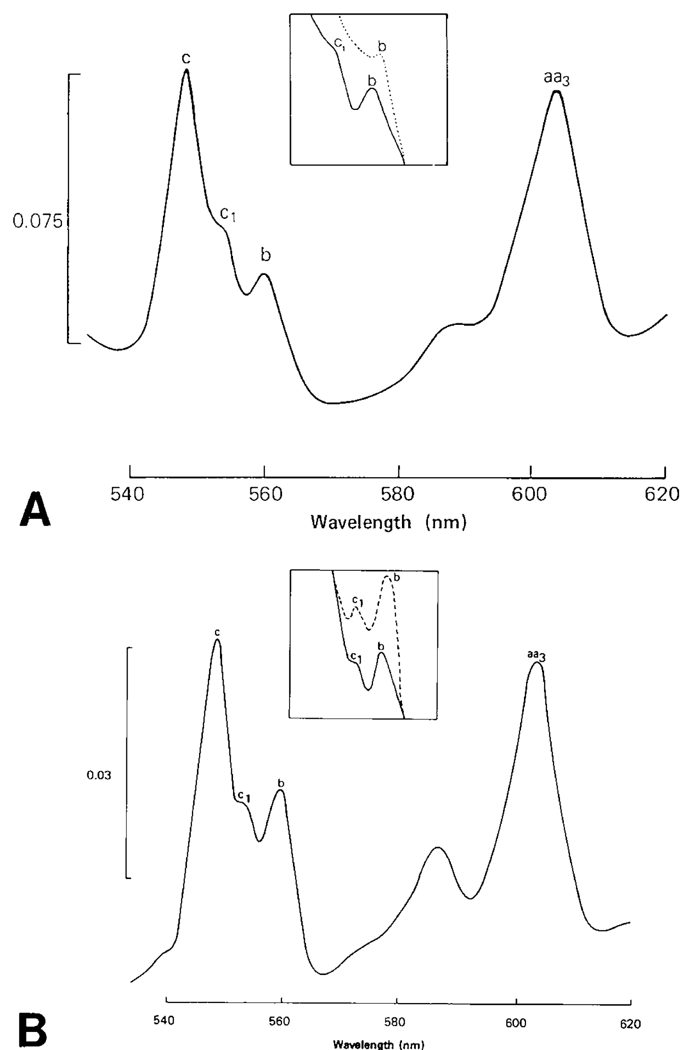

Fig. 1. Reduced minus oxidized difference spectrum of skeletal muscle mitochondrial fraction from the patient and a control. The spectra were recorded at −190°C. The sample cells were reduced with 10-mM succinate and 0.1-mM KCN. *Inserts* shows that further reduction with dithionite causes increased absorbance by cytochrome b with a peak shift from 562 nm to 566 nm (same scale, *dotted line* after dithionite). *A*, the spectra from the patient's mitochondria; each cell contained 1.8 mg mitochondrial protein. *B*, shows spectra from control mitochondria; each cell contained 1.5 mg mitochondrial protein. Very similar spectra to those shown in *A* were obtained with heart mitochondria from the patient.

Table 1. *Rates of substrate oxidations by mitochondrial fractions**

Tissue	Substrate	Controls	Patient A.S.
Skeletal muscle	10-mM-succinate	234 ± 20 (12)	48
	10-mM-glutamate + 1-mM-malate	88 ± 20 (12)	26
Heart	10-mM-succinate		226
	10-mM-glutamate + 1-mM-malate		48
Liver	10-mM-succinate	329 ± 13 (5)	88
	10-mM-glutamate + 1-mM-malate	126 ± 29 (5)	21
Kidney	10-mM-succinate		164
	10-mM-glutamate + 1-mM-malate		36

* Rates are expressed as nmols of ferricyanide reduced (in the presence of 10-mM-ADP) $min^{-1} \cdot mg\ protein^{-1}$ as means ± SD, with the number of observations in parentheses, or as individual values.

Table 2. *Cytochrome concentrations in mitochondrial fractions from skeletal muscle and cardiac muscle**

	Controls		Patient A.S.	
	Succinate	Dithionite	Succinate	Dithionite
Skeletal muscle				
Cytochrome aa_3	0.26 ± 0.10	0.27 ± 0.06	0.14	0.13
Cytochrome b	0.16 ± 0.06	0.25 ± 0.04	0.04	0.08
Cytochrome c	0.22 ± 0.10	0.28 ± 0.06	0.14	0.16
Cytochrome aa_3/cytochrome c	1.14 ± 0.14	0.97 ± 0.13	1.00	0.82
Cytochrome b/cytochrome c	0.68 ± 0.11	0.92 ± 0.18	0.27	0.50
Cytochrome b/cytochrome aa_3	0.62 ± 0.08	0.92 ± 0.14	0.27	0.62
Cardiac muscle				
Cytochrome aa_3	0.40	0.37	0.21	0.29
Cytochrome b	0.22	0.31	0.04	0.07
Cytochrome c	0.44	0.48	0.22	0.28
Cytochrome aa_3/cytochrome c	0.91	0.76	1.00	1.02
Cytochrome b/cytochrome c	0.45	0.63	0.16	0.35
Cytochrome b/cytochrome aa_3	0.54	0.83	0.17	0.34

* Cytochrome concentrations were determined from low temperature redox spectra with succinate or dithionite as reductants. The results are expressed as nmol mg protein^{-1} and are mean ± SD for eight controls or individual values. The concentration of cytochrome a is expressed as $a + a_3$; however, as cytochrome c oxidase contains both heme groups in one complex, the concentration of cytochrome c oxidase is one-half that of cytochrome aa_3 reported above (37). The values are expressed as cytochrome aa_3 to allow comparison with results from other investigators.

Table 3. *Activities of individual respiratory chain complexes and determination of duroquinol-ubiquinone transhydrogenase activity**

	Skeletal muscle		Cardiac muscle		Liver	
	Controls	Patient A.S.	Controls	Patient A.S.	Controls	Patient A.S.
Respiratory chain complexes						
Complex I	40 ± 14	30	62, 36	48	20 ± 5	15
Complex II	35 ± 3	31	45, 49	25	47 ± 7	35
Complex III	0.69 ± 0.06	0.26	0.81, 0.94	0.49	0.13 ± 0.02	0.05
Complex IV	0.67 ± 0.04	0.33	0.53, 0.69	0.59	0.13 ± 0.02	0.14
Duroquinol-ubiquinone						
+ Antimycin	0.11, 0.11	0.09	0.11	0.09	0.06	0.05
− Antimycin	0.22, 0.24	0.11	0.27	0.14	0.10	0.05

* The activities of the complexes were measured at 30°C as described in "Materials and Methods" and are expressed as nmol substrate transformed min^{-1}·mg mitochondrial protein^{-1} (complexes I and II) or as first order rate constants (s^{-1} mg protein^{-1}) (complexes III and IV; duroquinol-ubiqunone transhydrogenase). The figures shown are the mean ± SD or individual values.

about 50% of controls (Table 2). This contrasts with cytochrome c_1, which, although difficult to estimate because of the large overlapping peak of cytochrome c, appears normal relative to cytochrome c and to cytochrome aa_3. The recovery of mitochondria from skeletal muscle was similar to that from controls (not shown).

Activities of individual complexes. The activities of complexes I and II were normal in liver and muscle mitochondrial fractions from the patient compared with the controls (Table 3), but the activity of complex III was low in both tissues (38% of controls). Complex III activity was also low in the patient's cardiac mitochondria, but no adequate control data are available for the kidney mitochondrial fraction. The activity of cytochrome c oxidase in the skeletal muscle mitochondrial fraction from our patient was about 50% of the control values. The concentration of cytochrome aa_3 determined spectroscopically is also 50% of controls; however, the ratio of cytochrome aa_3 to cytochrome c is normal (Table 2). Immunoblotting with antibodies to holocytochrome c oxidase indicated that the relative amounts of the cytochrome c oxidase subunits were normal (not shown). This suggests that the low activity of complex IV in skeletal muscle may be accounted for by the lack of heme rather than the absence of one of the subunits.

Duroquinol-ubiquinone transhydrogenase activity. There was no significant antimycin-sensitive activity in skeletal muscle and liver mitochondria from the patient. In heart mitochondria, the apparent rate constant was about 30% of control (Table 3).

Immunoblot analysis of subunits of complex III. The skeletal muscle, liver and heart mitochondrial fractions from the patient were immunoblotted using antibodies to the nFeS peptide (Fig. 2), the core proteins (Fig. 3) and holocomplex III (Fig. 4). Mitochondrial fractions from muscle, liver, and heart had markedly lower amounts of the nFeS peptide (Fig. 2). Antibody to the holoenzyme only reacted well with muscle mitochondria and showed the apparently normal amounts of the core proteins, cytochrome c_1, and subunit VI. In addition, antibody to the core proteins showed a normal amount of these proteins in liver and heart mitochondrial fractions.

DISCUSSION

Patients previously described with complex III deficiency have presented with a variety of symptoms. In some patients, the defect is apparently confined to skeletal muscle; in others it is generalized. In the former group, exercise-related problems predominate, including muscle discomfort and breathlessness (26). These patients are often weak, and their weakness is exacerbated

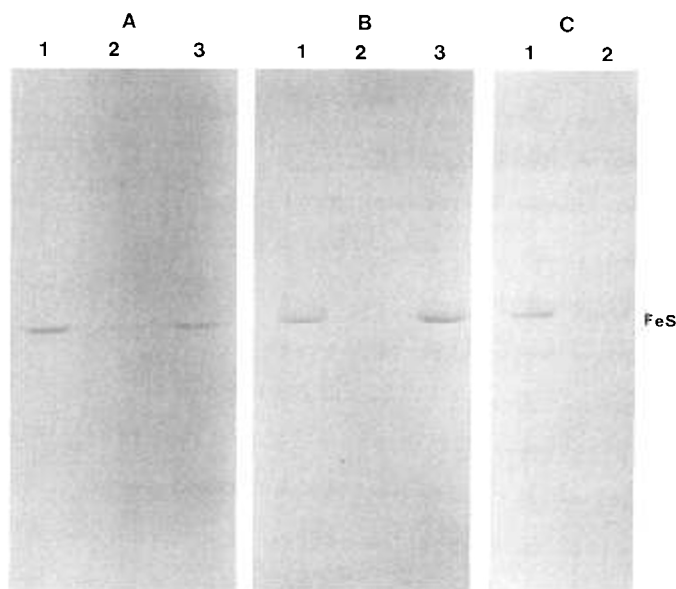

Fig. 2. Immunoblot analysis of nFeS peptide. Human tissue mitochondrial fractions (75 μg of protein) were loaded on the gel. The separated proteins were subsequently blotted onto nitrocellulose paper which was incubated with antibodies to the nFeS peptide. *A*, human skeletal muscle mitochondria from: *lane 1*, adult male; *lane 2*, patient; *lane 3*, adult female. *B*, human liver mitochondrial fractions from: *lane 1*, 4-mo-old infant; *lane 2*, patient; *lane 3*, 13-yr-old girl. *C*, human heart mitochondrial fractions from: *lane 1*, adult male; *lane 2*, patient.

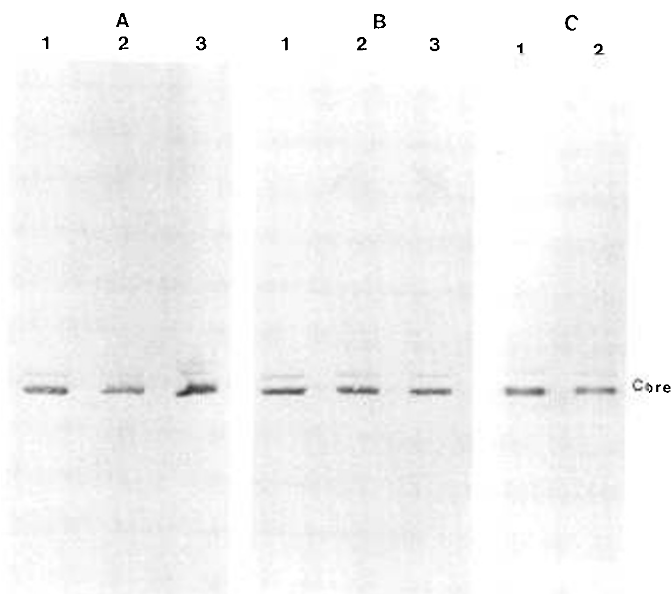

Fig. 3. Immunoblot analysis of the core proteins. *A*, human skeletal muscle mitochondrial fractions (15 μg of protein) from: *lane 1*, adult male; *lane 2*, patient; *lane 3*, adult female. *B*, human liver mitochondrial fractions (25 μg of protein) from: *lane 1*, 4-mo-old infant; *lane 2*, patient; *lane 3*, 13-yr-old girl. *C*, human heart mitochondrial fractions (25 μg of protein) from: *lane 1*, adult male; *lane 2*, patient.

by exertion. Patients with multisystem involvement have presented in their teens or in adult life with muscle weakness, intellectual impairment, ataxia and chorioretinitis (27, 28). The presentation of our patient with fatal lactic acidosis in infancy was very different.

The most striking biochemical feature in this patient was the very high blood lactate concentration. Marked lacticacidemia has previously been described in a variety of metabolic conditions

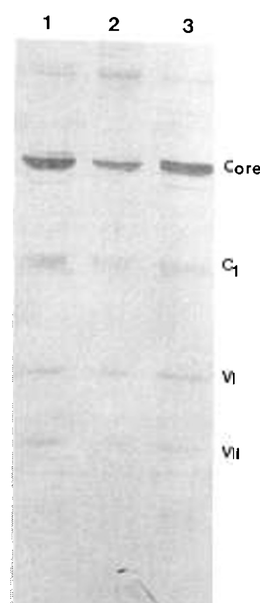

Fig. 4. Immunoblot analysis of holo-complex III. Skeletal muscle mitochondrial fractions (100 μg of protein) from: *lane 1*, adult male; *lane 2*, patient; *lane 3*, adult female.

including deficiency of the pyruvate dehydrogenase complex (29), pyruvate carboxylase (30), biotinidase (31), holocarboxylase synthetase (32), fructose-1,6-bisphosphatase (33), glucose-6-phosphatase (34), and cytochrome *c* oxidase (35). None of these possibilities would explain the impaired oxidations of succinate and glutamate in the liver and muscle mitochondria using ferricyanide as an electron acceptor.

Although complex IV was low compared with controls as determined both by direct enzyme assay and spectrophotometrically, the relative amount was normal. However, not only was the absolute amount of complex III low compared with controls, but the there was a proportionally low amount of cytochrome *b* relative to cytochrome *c* and cytochrome aa_3. These results led us to assume that a generalized defect specific to complex III in this patient would explain the metabolic disturbances found. The fact that these were more severe than has been reported previously may have been due either to the impaired energy supply due to the respiratory chain lesion or to the secondary hyperlacticacidemia.

Impaired electron transport would inhibit pyruvate oxidation by decreasing the mitochondrial NAD^+/NADH ratio. The anaerobic oxidation of pyruvate produces lactate, which is exported into the blood and normally cleared by the liver where it is used as a gluconeogenic precursor. Impaired gluconeogenesis associated with impaired fatty acid oxidation secondary to the respiratory chain disorder (36) would account for the hypoglycemia and lacticacidemia observed in this child.

Previous diagnoses of patients with complex III deficiency have been based on slow rates of oxygen uptake or of succinate-cytochrome *c*(III) reductase and rotenone sensitive NADH-cytochrome *c*(III) reductase activities in mitochondrial fractions prepared from muscle (27, 37, 38) or heart (39). In our case, the oxidation of both succinate and NAD^+-linked substrates was low in liver and muscle mitochondrial fractions (Table 1). This could be explained by a defect of complex III or a combined defect of complexes I and II. Direct assay showed the defect to be of complex III (Table 4). Further confirmation was provided by the duroquinol-ubiquinone transhydrogenase assay which measures the reactivity of the b_i site of complex III. This activity was low in heart and undetectable in skeletal muscle and liver mitochondria (Table 3).

Immunochemical and spectroscopic studies of complex III demonstrated that there were small amounts of the nFeS peptide

and of cytochrome *b* compared with controls. However there were normal amounts of core proteins, cytochrome c_1, and subunit VI. Antibodies were not available to the other subunits of complex III, so it could not be established whether any other subunits were deficient. In another patient with complex III deficiency, Darley-Usmar *et al.* (22) found very low amounts of the core proteins, nFeS protein, and subunit VI but a normal amount of cytochrome c_1, and there are several reports of low amounts of cytochrome *b* (27, 28, 39, 40).

The child's death from severe acidosis was secondary to a defect in complex III of the respiratory chain, characterized by a partial deficiency of cytochrome *b* and nFeS. Ten of the 11 peptides including nFeS, are coded for by nuclear DNA, and the cytochrome *b* apoprotein is coded for by the mitochondrial DNA. The defect, if genetically determined, could be caused by an abnormality of the nuclear or mitochondrial DNA, and two broad possibilities may be outlined.

First, a mutation affecting one of the nuclear-coded subunits could cause impaired incorporation of cytochrome *b* into an abnormal complex or decreased stability of the complex. However, there are differences in the expression of the defect between skeletal muscle, liver, and heart. The ADP/O ratio was normal in heart and kidney, and the duroquinol-ubiquinol transhydrogenase was less affected in heart than in muscle and liver. This could be caused by mutations of tissue-specific isoforms of one or more of the nuclear-coded subunits of complex III. Possibly a mutation of a single intron causing differently spliced mRNAs might generate different abnormal forms of that subunit in different tissues to account for the range of phenotypic variation observed (41). There are tissue-specific isoforms of nuclear coded, but not mitochondrially coded, subunits of complex IV (42). Alternatively, two isoforms of a single subunit may be expressed in heart, giving a mixed population of complex III oligomers (either in the same or different mitochondria). If only the affected isoform was expressed in liver and skeletal muscle, then these tissues would be more severely affected.

Second, the defect might be caused by a mutation of the mitochondrial DNA. Such defects have recently been described in some mitochondrial myopathies associated with complex I, with heteroplasmy of normal and abnormal DNA in skeletal muscle (43). A mutation in the mitochondrial DNA affecting the cytochrome *b* apoprotein may have segregated preferentially during early embryogenesis into muscle and liver rather than heart. Tissue-specific differences in expression may have been modified by a background of different isoforms of the nuclear-coded subunits.

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