Deficiency of the Reduced Nicotinamide Adenine Dinucleotide Dehydrogenase Component of Complex I of Mitochondrial Electron Transport

Fatal Infantile Lactic Acidosis and Hypermetabolism with Skeletal-Cardiac Myopathy and Encephalopathy

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Abstract

A mitochondrial defect was investigated in an infant with fatal congenital lactic acidosis (3–14 mM), high lactate-to-pyruvate ratio, hypotonia, and cardiomyopathy. His sister had died with a similar disorder. Resting oxygen consumption was 150% of controls. Pathological findings included increased numbers of skeletal muscle mitochondria (many with proliferated, concentric cristae), cardiomegaly, fatty infiltration of the viscera, and spongy encephalopathy. Mitochondria from liver and muscle biopsies oxidized NADH-linked substrates at rates 20–50% of controls, whereas succinate oxidation by muscle mitochondria was increased. Mitochondrial NADH dehydrogenase activity (complex I, assayed as rotenone-sensitive NADH oxidase, NADH-duroquinone reductase, and NADH-cytochrome c reductase) was 0–10% of controls, and NADH-ferricyanide reductase activity was 25–50% of controls in the mitochondria and in skin fibroblasts. Activities of other electron transport complexes and related enzymes were normal. Familial deficiency of a component of mitochondrial NADH dehydrogenase (complex I) proximal to the rotenone-sensitive site thus accounts for this disorder.

Introduction

The causes of congenital lactic acidosis are complex, frequently obscure, and usually difficult to distinguish in affected infants. A broad categorization of known genetic defects causing lactic acidosis includes deficiencies of enzymes specifically required for utilization of pyruvate and deficiencies of oxidation or electron transport affecting not only pyruvate but also other substrates such as fatty acids and amino acids. In recent years several defects of components of the mitochondrial electron transport chain have been described (1–3). Biochemical characterization of this group of genetic disorders remains at an early stage. Many of these disorders affect skeletal and cardiac muscle function and are variably associated with abnormal mitochondrial structure and number, lactic acidosis, and tissue accumulation of fat and glycogen. Some cases have been described of infants with severe systemic involvement and early death, whereas others have become evident later in life with limited dysfunction.

When first investigated, the infant described in this report was found to have severe lactic acidosis, skeletal and cardiomyopathy, abnormal mitochondrial structure, and decreased activity of complex I of the mitochondrial electron transport chain (4). Subsequently, we participated in the investigation of another fatal infantile case in which a defect of complex I was localized to the iron-sulfur clusters (5). Several other cases have been reported with apparent defects of complex I (6–12), most of which have been associated with juvenile or adult onset of relatively milder clinical symptoms of muscle weakness and neurological dysfunction.

Methods

Case summary

This infant boy was born after an apparently uncomplicated full-term pregnancy with a normal birth weight (3,010 g). Rapid breathing and poor feeding were noted in the newborn period, but he seemed well enough to be sent home at 1 wk of age. During the first month at home he continued to take formula poorly, vomited frequently, did not gain weight, and had noisy breathing. He was hospitalized at age 6 wk because of respiratory stridor, feeding difficulty, and failure to gain weight. He was found to have laryngomalacia, hypotonia, cardiomegaly, an enlarged liver, and metabolic acidosis. Blood lactate (14 mM), pyruvate (0.37 mM), and alanine (1.4 mM) were all increased. Other plasma and urine amino acids were not increased. Urine organic acid analysis showed intermittently increased amounts of lactic, succinic, fumaric, malic, aconitic, citric, adipic, suberic, and sebacic acids. Plasma free carnitine was decreased (6 μM vs. 32±7 SD for controls), plasma long-chain acylcarnitine was increased (5.0 μM vs. 1.0±0.4), and short-chain acylcarnitine was increased in the urine. The urine was appropriately acidic. Repeated measurements of venous and arterial oxygen content and cutaneous Po_2 monitoring showed that the persistent lactic acidosis was associated with normal peripheral oxygenation. Echocardiography showed progressively dilated and poorly contractile left and right ventricles. Standard and vector electrocardiography showed left ventricular hypertrophy and a conduction abnormality.

The infant continued to require hospitalization for medical support for the duration of his life. Sodium bicarbonate was administered in large quantities to control the acidosis (7 meq/kg/d). At age 3 mo a tracheostomy was performed and a biopsy of the sternocleidomastoid muscle was obtained that showed proliferation and abnormal structure of mitochondria. At age 3.5 mo a gastrostomy was placed because of difficulty with swallowing, and biopsies of skin, rectus abdominus muscle, and liver were obtained for special metabolic studies at that time. During recovery from surgery he developed intestinal obstruction and on repeat laparotomy was found to have intussusception; an additional incidental finding was malrotation of the intestines. After recovery he was fed a standard infant formula by gastrostomy and began to gain weight at a normal rate. There was no evidence of heart failure. The liver returned to normal size. However, metabolic acidosis and hypotonia persisted and mental development was not normal.

At age 6 mo he was transferred to a convalescent hospital and appeared

to be doing well. At age 7 mo he had a respiratory arrest 15 min after his condition had appeared satisfactory. He was unresponsive to prolonged resuscitation. Autopsy did not reveal the immediate cause of death. A possible cardiac arrythmia was suspected.

A sister had died 3 yr earlier at age 7 wk after a similar neonatal course wih poor feeding, lack of weight gain, and rapid breathing. During hospitalization, just before death, she was found to be in congestive heart failure with paroxysmal atrial tachycardia. She had an enlarged liver and an unexplained severe metabolic acidosis (pH 6.8). Postmortem examination showed cardiomegaly with fatty infiltration of the liver and heart. The parents and four other siblings showed no clinical evidence of a similar disorder.

Pathological findings

Muscle, liver, and skin biopsies and peripheral lymphocytes. The biopsy of the sternocleidomastoid muscle obtained at the time of tracheostomy showed moderate variation of fiber size with increased fat content (Fig. 1 A). Histochemical staining for NADH-tetrazolium reductase resulted in pronounced accumulation of the dye in many of the type I fibers. Electron microscopic examination showed extensive subsarcolemmal and intermyofibrillar accumulation of abnormal mitochondria. Many were large and had extensive condensations of parallel cristae. Others were rounded and contained concentric whorls of proliferated inner membranes or cristae (Fig. 1 B). Some had electron-dense osmophilic aggregates in the center. The liver biopsy showed cytoplasmic vacuolization due to microvesicular fatty infiltration, confirmed by Oil Red O staining of frozen sections and electron microscopy. Liver mitochondria were normal in structure. Light and electron microscopic examination of epidermis and dermis from a skin biopsy (obtained at surgery) did not reveal any abnormality; mitochondria appeared normal in structure and number. Likewise, there was no detectable electron microscopic abnormality of mitochondria in peripheral lymphocytes isolated from the patient's blood.

Autopsy of the patient. Marked cardiomegaly and biventricular hypertrophy were present. The heart was three times normal weight for age and had a distinct yellow color due to extensive fatty infiltration. Electron microscopic examination of heart mitochondria was hampered by postmortem changes, but mitochondria appeared essentially normal in number and structure. Numerous large and small lipid droplets were seen within and between cardiac muscle fibers. The liver was not enlarged but showed extensive large and small droplet steatosis. The brain showed a thin corpus callosum and a diffuse discoloration of the white matter, more pronounced on the left. On microscopic examination there was extensive spongy change of the deep layers of the neocortex. The globus pallidus, the white matter bundles traversing the putamen, and the hypothalamus were also involved, as were the red nuclei and pontine tegmentum. Throughout the white matter there were increased numbers of astrocytes, particularly in the corpus callosum where many reactive cells were found. Both astrocytic and neuronal cells were involved in vacuolation.

Autopsy of the patient's sister. There was a striking similarity of heart, liver, and brain findings in the postmortem examination of the sister who had died 3 yr earlier. The sister's heart showed the same globular cardiomegaly, ventricular hypertrophy, and extensive lipid deposition in myofibers. There was evidence of congestive heart failure with a pericardial effusion. The liver was yellow and showed extensive small droplet fatty infiltration. Microscopic examination of the brain revealed a pronounced spongy change in the lateral and posterior hypothalamus, lateral thalamic nuclei, pontine and medullary tegmentum, and basis pontis.

Methods

Clinical investigative procedures. The procedures employed in the investigation of the patient and controls were approved by the Institutional Review Boards of University Hospitals of Cleveland (patient and pediatric age controls) and the Veterans Administration Medical Center (adult controls) and were performed after obtaining informed consent from the parents or adult control subjects. Control rectus abdominus and liver biopsy samples were obtained from other infants with suspected metabolic disorders in whom the biopsies were performed as part of their diagnostic evaluation, in some cases at the time of abdominal surgery for other reasons. Control rectus abdominus muscle and liver biopsy samples were also obtained from consenting adults at the time of elective abdominal surgery.

Respiratory metabolism. Measurements of whole-body oxygen consumption and CO_2 production were carried out as previously described (13); respiratory quotients and rates of energy consumption were cal-
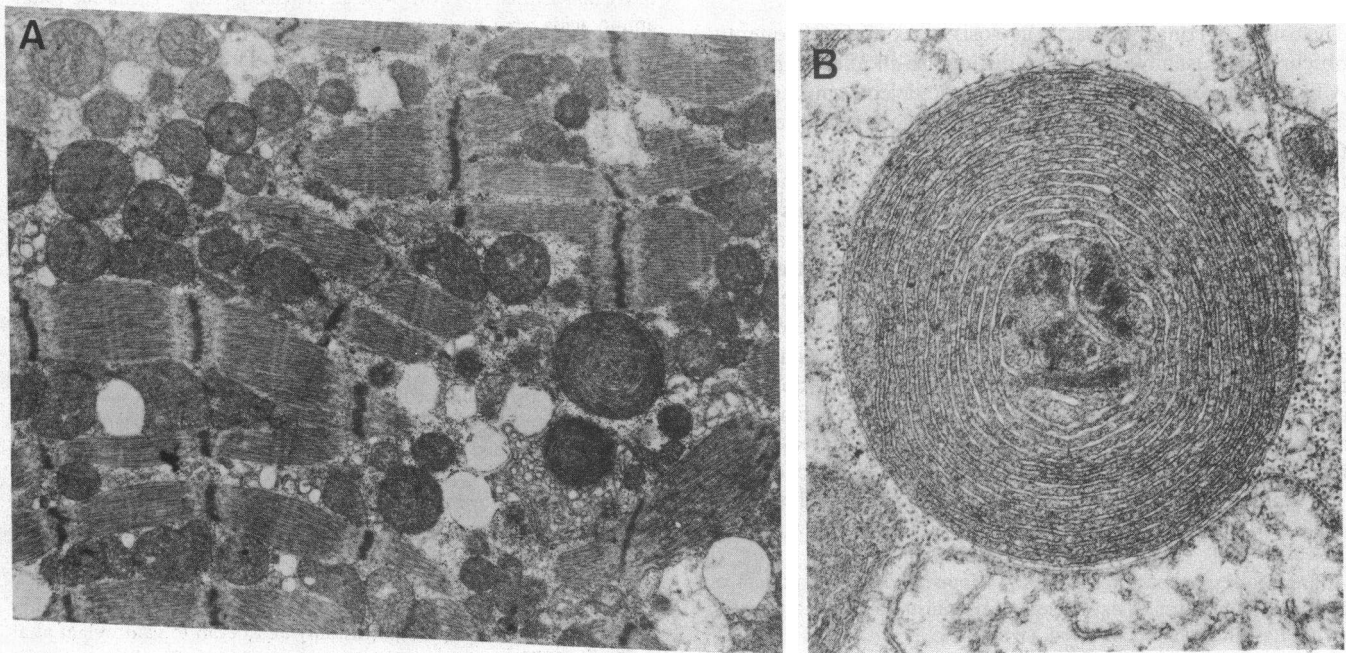

Figure 1. Electron micrographs of a biopsy of the patient's sternocleidomastoid muscle. (A) 6,000× magnification, showing accumulation of lipid droplets and increased numbers of abnormally enlarged mitochondria between muscle fibers. (B) 32,000× magnification, showing an abnormal giant mitochondrion filled with concentric whorls of cristae and electron-dense osmophilic granules in the center.

culated using standard assumptions of indirect calorimetry. Briefly, the patient's head was placed under a plastic hood through which air was withdrawn at a controlled flow rate and the mixed expired gases were sampled for oxygen and CO_2 analysis (Beckman Instruments, Inc., Fullerton, CA).

Isolation of mitochondria. Mitochondria were isolated from liver samples as previously described (14). Skeletal muscle biopsies were minced and mitochondria prepared by a modification of the nagarse method (15). The minced sample was suspended in 100 mM KCl, 50 mM Mops (morpholine propanesulfonic acid), pH 7.2, and 2 mM EGTA (10 ml/g wet wt), and 5 mg of nagarse/g wet wt was added. The suspension was stirred on ice for 10 min. An equal volume of suspension buffer containing 0.2% bovine serum albumin was added and the suspension homogenized with a loose fitting pestle. The homogenate was centrifuged at 7,000 g for 10 min, and the pellet was resuspended in the homogenization buffer (10 ml/g original wet wt). This was centrifuged at 300 g for 10 min and the supernatant was then recentrifuged at 7,000 g for 10 min. The pellet (mitochondrial fraction) was washed twice in 100 mM KCl, 50 mM Mops (pH 7.2), and 0.5 mM EGTA, and the final pellet was resuspended in this same medium.

Oxidative studies with intact mitochondria. Oxygen uptake was monitored as previously described in 0.5-ml reaction chambers at 30°C (14). Endogenous substrates were depleted by addition of small amounts (50 nmol) of ADP. Substrates were added, state 3 respiration (ADP-stimulated) and state 4 respiration (ADP-lacking) were measured, and respiratory control ratios (state 3/state 4) and the ADP/O ratio were calculated (16, 17).

Enzymes in mitochondrial extracts. For assay of mitochondrial enzymes, freeze-thawed mitochondria, or sonicated fibroblast suspensions were solubilized at a final protein concentration of 1 mg/ml in 1% cholate and 0.1 M potassium phosphate, pH 7.2. Oxidase assays were performed using an oxygen electrode as described above (18). The assay mixtures contained in a final volume of 0.5 ml: 20 mM potassium phosphate (pH 7.4), 0.1 mM EDTA, 0.2 mg of oxidized cytochrome c, mitochondria, and substrate (added last). NADH oxidase was measured with 2.8 mM NADH, with and without 7.5 μM rotenone. Succinate oxidase was measured with 0.8 mM duroquinone and 40 mM succinate, with and without 10 μg of antimycin A. Durohydroquinone oxidase was measured in the presence of 2 mM durohydroquinone, with and without 10 μg of antimycin A. Cytochrome c oxidase was measured in the presence of 0.24 mM $N'N'N'N'$ tetramethyl phenylene diamine and 7.2 mM ascorbate, with and without 2 mM sodium azide. Spectrophotometric assays were carried out at 37° according to published procedures for NADH–cytochrome c reductase (rotenone sensitive) (19), NADH-duroquinone reductase (rotenone sensitive) (20), NADH-ferricyanide reductase (20), succinate–cytochrome c reductase (21), succinate dehydrogenase (22), glutamate dehydrogenase (23), and citrate synthase (24). Pyruvate dehydrogenase complex was assayed by decarboxylation of [1-^{14}C]pyruvate after activation with dichloroacetate (25).

Other analytical methods. Blood lactate and pyruvate were measured spectrophotometrically with lactic dehydrogenase in supernatants of whole blood deproteinized immediately with 2 vol of 5% perchloric acid (26). Protein was determined by the Lowry and biuret methods (27). Plasma and tissue carnitine content was measured as total, free, short-chain acylcarnitine and long-chain acylcarnitine (28). Urinary organic acids were determined by capillary gas chromatography of the trimethylsilyl derivatives with confirmation of identity by gas chromatography-mass spectrometry (29, 30).

Results

Lactate and pyruvate (Fig. 2). Blood concentrations of both lactate and pyruvate were consistently higher than normal in numerous determinations under variable conditions. The ratio of lactate to pyruvate was usually above the normal range or at the upper limits of normal (range, 18–53; normal range for venous samples, 10–20). The elevated ratio of lactate to pyruvate could
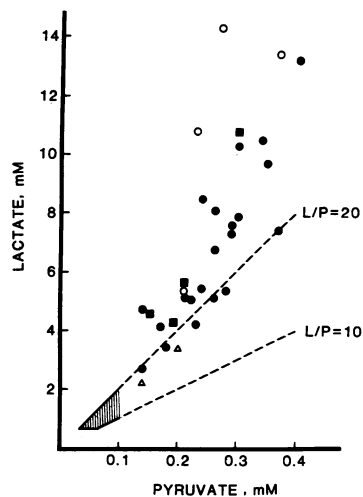

Figure 2. Relationship of blood lactate and pyruvate in multiple samples obtained from the patient. The shaded area corresponds to the normal range. The different clinical conditions under which samples were obtained are indicated by the following symbols: fed (o), fed + sodium bicarbonate (•), fed + sodium bicarbonate + O_2 (■), fasted + sodium bicarbonate (Δ).

not be accounted for by any detectable abnormality of circulation or respiration. Blood PO_2 and PCO_2 measurements were normal, and continuous cutaneous PO_2 monitoring failed to demonstrate any episodes of intermittent hypoxemia. Administration of 40% oxygen increased peripheral PO_2 but had no effect on the ratio of lactate to pyruvate. These observations suggested a peripheral defect in tissue oxidative metabolism.

Most blood samples were obtained during periods of administration of intravenous glucose or within a few hours of oral feeding. The lowest values for blood lactate and pyruvate were obtained after fasting for 9–10 h. The infant was not subjected to longer fasting. Hypoglycemia was never observed. Administration of sodium bicarbonate (5–7 meq/kg/d) appeared to be of some benefit clinically in reducing the severity of acidosis but did not result in any improvement in the lactate/pyruvate ratio.

Energy utilization (Table I). Rates of total energy utilization by the patient were estimated by respiratory gas exchange (indirect calorimetry) and by food intake. At two different times, ages 2 and 5 mo, resting oxygen consumption during intervals of minimal activity was 40–60% higher than the average integrated 24-h rate previously observed in control infants (13). The higher rate of oxygen consumption was associated with minimal activity without abnormal movements or fever. The respiratory quotient indicated that both carbohydrate and fat were being oxidized (13). Despite minimal activity, the amount of dietary energy intake consumed during periods of normal weight gain was greater than average for normal infants of this size. There was no evidence of malabsorption. These observations indicate that the resting metabolic rate was abnormally increased.

Oxidative metabolism of intact mitochondria (Table II). Intact mitochondria were isolated from liver and skeletal muscle biopsies. The yield of mitochondrial protein per gram wet weight in both tissues was greater than controls. Rates of oxidation were measured for various substrates that use different transport mechanisms, different dehydrogenases, and produce reducing equivalents that enter the respiratory chain at different points. The maximal rates of oxygen uptake in the presence of ADP (state 3) were lower than controls for all of the NADH-linked substrates tested with both types of mitochondria, and there was no evidence for respiratory control (no significant stimulation by addition of ADP or an uncoupler, dinitrophenol). The maximal rate of oxidation of succinate, on the other hand, was more rapid with the patient's muscle mitochondria than with controls,

Table I. Respiratory Metabolism and Energy Intake

Subject	Age	Time postprandial	Respiratory quotient	Resting O_2 consumption
	mo	h	CO_2/O_2	kcal/kg/d
A. Respiratory metabolism*				
Patient	2	9–10	0.89	130
Patient	5	2–3	0.78	112
Normal‡		0–24		79±9

Subject	Age	Integrated interval	Weight gain	Formula intake
	mo	d	g/kg/d	kcal/kg/d
B. Energy intake§				
Patient	4	12	6.7	126
Patient	5	13	7.3	133
Normal‖			8.1±1.8	116±17

* Each value for the patient is the average of three 5-min periods within the indicated interval.
‡ Normal average value (±SD) for infants in this age range (13).
§ Intake represents the average consumption during the entire interval.
‖ Normal average rates of weight gain and energy intake (±SD) for infants of the same size as the patient (32).

with a normal respiratory control ratio (+ADP/−ADP: 2.3 vs. 3.5±1.3 SD for controls). Succinate oxidation by liver mitochondria was slightly less than controls, but the respiratory control ratio was good (10 vs. 7.4±2.2 for controls). Oxidative phosphorylation, estimated by the ratio of ADP added/oxygen consumed, could not be estimated for NADH-linked substrates, because there was no stimulation by ADP, but was normal for succinate in both skeletal muscle and liver mitochondria (1.7 and 1.2 vs. 1.8±0.3 and 1.9±0.3, respectively, for controls).

Enzymes of oxidative metabolism in permeabilized mitochondria (Table III). Mitochondria that have been freeze-thawed and incubated in a hypotonic buffer readily oxidize a variety of

Table II. Oxidative Metabolism in Liver and Skeletal Muscle Mitochondria

Substrate	Liver		Skeletal muscle	
	Patient	Controls*	Patient	Controls‡
Pyruvate (10 mM)	7	28±13	9	31±10
+ malate (5 mM)		(14–56)		(18–56)
Glutamate (10 mM)	10	46±13	16	25±8
		(31–69)		(16–43)
Palmitoylcarnitine (40 μM)	14	50±17	16	26±9
+ malate (5 mM)		(25–83)		(18–51)
Succinate (10 mM)	32	89±33	76	52±5
+ rotenone (3.8 μM)		(35–139)		(44–60)
Yield mitochondrial	55	18±6	23	5.6±1.5
protein (mg/g wet wt)		(10–28)		(3.7–7.5)

State 3 oxidative rates (ADP stimulated) are expressed as ng atoms 0/min/mg mitochondrial protein, with controls as mean±SD and range in parentheses.
* Control liver mitochondria were isolated from biopsies of 10 infants. Data shown are from all 10 subjects, except for glutamate (n = 8).
‡ Control skeletal muscle mitochondria were isolated by the same method used for the patient from rectus abdominus biopsies of 10 adults undergoing laparotomy. Data shown are from all 10 subjects, except for palmitoylcarnitine (n = 9) and succinate (n = 6).

substrates. Liver and skeletal muscle mitochondria from the patient did not have detectable activity of rotenone-sensitive NADH oxidase, which is dependent on the combined activities of complexes I, III, and IV of the electron transport chain. In contrast, both types of mitochondria had activities of succinate oxidase within the range of controls; this activity depends on complexes II, III, and IV. This suggested that complexes III and IV were functionally intact, which was confirmed by finding normal activities of durohydroquinone oxidase (complexes III and IV) and reduced cytochrome *c* oxidase (complex IV). Therefore, these data from the activity of oxidases indicated that the defect was in complex I.

Enzymes in solubilized mitochondria (Table IV). The activity of mitochondrial NADH dehydrogenase (complex I) was estimated in solubilized mitochondria by measuring rotenone-sensitive NADH reduction of cytochrome *c* (dependent on complexes I and III) and reduction of duroquinone (complex I). The rotenone-sensitive portion of complex I transfers electrons from the iron-sulfur clusters to ubiquinone. Both of these rotenone-sensitive reactions of NADH were either absent or extremely low in the patient's liver and skeletal muscle mitochondria. On the other hand, succinate-cytochrome *c* reductase (including complexes II and III) and succinate dehydrogenase (complex II) activities were normal, indicating that complexes II and III were not involved in the defect.

The activity of complex I was further assessed by use of ferricyanide, which acts as an artificial electron acceptor from a site on complex I proximal to the rotenone-sensitive site. In crude extracts, ferricyanide can also accept electrons from a number of other dehydrogenases (21). NADH-ferricyanide reductase activity was one-half the average value of controls in the patient's liver and one-fourth as great in skeletal muscle mitochondria, and below the range of controls in both cases. This suggests that the defect in complex I involved a site proximal to the rotenone-sensitive site.

The activity of three other enzymes related to the Krebs cycle were estimated in mitochondria from the patient and controls, including glutamate dehydrogenases, pyruvate dehydrogenase complex, and citrate synthase (Table IV). There was no evidence for significant deficiency of any of these enzymes.

Enzyme activities in cultured skin fibroblasts (Tables III and IV). Activities of the oxidase and reductase reactions of the electron transport chain were also assayed in disrupted cultured skin fibroblasts, and the results were confirmatory of the defects found with isolated mitochondria. Homogenates of the patient's fibroblasts lacked activity of the rotenone-sensitive reactions with NADH, whereas the other reactions involving only complexes II, III, and IV had normal activity. Activities of the other Krebs cycle–related enzymes were also normal.

Trial of therapy with riboflavin. Because of evidence for a defect in activity of complex I in this infant, a trial of therapy with high doses of riboflavin was initiated to determine if provision of excess precursor of the cofactor for this complex (FMN) might be beneficial. The dose selected, 100 mg/d, is 100 times the recommended dietary allowance for this age. No toxicity has been described at this dose of riboflavin. The trial was continued for 4 wk, discontinued for 4 wk, and then resumed for 3 mo until the child died. Blood levels of lactate and pyruvate were compared before, during, and after the trial. The average concentration of lactate during riboflavin supplementation was 5.4 mM (range, 2.5–11.4; n = 7) with an average lactate-to-pyruvate ratio of 24 (range, 17–31), compared with average concentration of 5.1 mM (range, 4.1–6.5; n = 4) and ratio of 26

Table III. Mitochondrial and Fibroblast Oxidase and Reductase Activities

Activity assayed	Liver mitochondria		Muscle mitochondria		Fibroblasts	
	Patient	Controls*	Patient	Controls‡	Patient§	Controls§
NADH oxidase (rotenone sensitive)	0	42±14 (22–62)	0	100±14 (88–128)	0±0 (3)	3.9±0.6 (3.3–4.5)
Succinate oxidase	59	38±12 (19–51)	28	35±4 (31–42)	3.8 (1)	4.3±0.3 (4.0–4.5)
Durohydroquinone oxidase	300	132±31 (91–177)	201	200±6 (190–210)	16.6±1.3 (5)	17.1±2.3 (14.9–20.1)
Cytochrome c oxidase	291	507±81 (360–608)	266	212±12 (201–236)	15.8±0.9 (5)	16.5±1.0 (15.8–17.8)
NADH–cytochrome c reductase (rotenone sensitive)	9	108±36 (64–191)	5	76±13 (52–89)	0±0 (4)	1.8±0.5 (1.3–2.3)
NADH–duroquinone reductase (rotenone sensitive)			0	72±5 (64–77)	0.4±0 (4)	1.5±0.7 (0.8–2.4)
NADH–ferricyanide reductase	466	888±171 (550–1009)	169	762±39 (691–804)	57±2 (4)	183±7 (177–193)
Succinate–cytochrome c reductase	71	152±31 (107–207)	31	27±3 (24–31)	0.3±0.03 (4)	0.36±0.05 (0.31–0.42)

Activity expressed as nmol/min/mg mitochondrial protein; control data expressed as mean±SD, with range in parentheses. * Control values for liver mitochondria are from biopsies of eight adults, except for NADH-ferricyanide reductase (n = 5). ‡ Control skeletal muscle mitochondria were isolated from rectus abdominus biopsies of five adults. § Results shown for cultured skin fibroblasts are the mean±SD of several separate assays of cells from the patient (n in parentheses) and of control cell lines from 4 individuals. Assays were performed with freeze-thawed whole cells.

(range, 20–37) during the interval without riboflavin supplementation. Whereas these results do not exclude possible minor improvement, the concentration of blood lactate and the ratio to pyruvate remained well above normal and there was no discernible improvement in muscle strength or cardiac function. The patient was receiving riboflavin at the time of death.

Discussion

The clinical, pathological, and laboratory findings in this infant are due to an unusual profound systemic defect of mitochondrial electron transport. A congenital defect of oxidative metabolism was first suspected because of persistent lactic acidosis and an increased ratio of lactate to pyruvate which could not be attributed to inadequate oxygenation. The increased numbers of ab-

normal mitochondria and increased fat content in the initial muscle biopsy provided further evidence for a defect in mitochondrial function. Impaired NADH oxidation by intact liver and skeletal muscle mitochondria and abnormal enzyme activities in solubilized mitochondria established the presence of a defect in the NADH dehydrogenase component of complex I of the mitochondrial electron transport chain. The associated fatal systemic clinical syndrome of infantile onset of severe lactic acidosis, skeletal myopathy, cardiomyopathy, and encephalopathy is very different than most previously described defects of complex I, but is similar to another thoroughly studied infantile case (5).

Mitochondria from both muscle and liver oxidized NADH-linked substrates more slowly than controls but oxidized suc-

Table IV. Activity of Various Other Mitochondrial Enzymes

Activity assayed	Liver		Skeletal muscle		Fibroblasts	
	Patient	Controls*	Patient	Controls‡	Patient§	Controls§
Succinate dehydrogenase	88	55±40 (11–125)	91	129±3 (124–133)	1.2±0.1	1.3±0.1 (1.2–1.3)
Glutamate dehydrogenase	446	793±271 (335–1160)	12	19±2 (16–21)	6.2±0.2	7.7±0.4 (7.2–8.0)
Pyruvate dehydrogenase complex (DCA activated)	30	10.3±4.1 (4.2–18)			4.7	2.7±1.0 (1.1–6.7)
Citrate synthase	101	248±108 (73–419)	1031	1838±52 (1765–1912)	4.0±0.2	4.0±0.2 (3.9–4.2)

Activity expressed as nmol/min/mg protein, mean±SD (range). * Control values for liver mitochondria are from 10 children. ‡ Control skeletal muscle mitochondria were isolated from rectus abdominus biopsies of five adults. § Results shown for cultured skin fibroblasts are the mean±SD of four separate assays of cells from the patient and of control cell lines from four individuals, except 39 controls are shown for pyruvate dehydrogenase complex.

cinate normally with good respiratory control. Oxidation of succinate is mediated through complexes II, III, and IV and does not involve complex I. Using three different electron acceptors, the activity of rotenone-sensitive NADH dehydrogenase was extremely low in mitochondrial extracts and cultured fibroblast homogenates. Rotenone binds to complex I in such a way that it inhibits the transfer of electrons from the iron-sulfur centers to coenzyme Q. NADH oxidase (complexes I, III, and IV), NADH–cytochrome c reductase (complexes I and III), and NADH-duroquinone reductase (complex I) activities were markedly reduced. The activity of NADH-ferricyanide reductase in liver and muscle mitochondria as well as in cultured fibroblasts was consistently lower than controls. Ferricyanide can serve as an artificial electron acceptor from the flavoprotein of complex I at a point proximal to the rotenone-sensitive site. This compound also accepts electrons from a number of other cellular NADH-oxidizing systems and is therefore not specific to complex I. Inhibition by rotenone does not aid in differentiating reduction of ferricyanide by complex I vs. other NADH oxidizing systems. In mitochondria two NADH oxidizing systems are known to reduce ferricyanide, complex I, and the outer membrane NADH–cytochrome b_5 flavoprotein (21). The decreased mitochondrial NADH-ferricyanide reductase activity observed in this case can therefore be accounted for by a decrease in complex I, whereas the residual activity can be accounted for by the NADH–cytochrome b_5 flavoprotein. Complex I has been resolved into three major components (31). One of these, the flavoprotein fragment, is water soluble, contains flavin mononucleotide and three polypeptides, and is reactive with ferricyanide. Our data could be explained by a defect in the flavoprotein fragment of complex I.

This case is very similar but not identical to the infant described by Moreadith et al. (5). In that case, the NADH-ferricyanide reductase activity was normal. Mitochondrial particles obtained postmortem were analyzed by electron-spin resonance spectroscopy and showed a lack of one or more of the iron-sulfur clusters in complex I. Because there are multiple subunits in complex I, defects in different subunits could produce very similar pathophysiological consequences. Both cases were affected in early infancy with severe lactic acidosis, skeletal-cardiomyopathy, and similar pathological findings.

None of the other nine reported cases of complex I deficiency have been characterized biochemically in as great detail (7–12). Seven of these cases had onset beyond infancy, and in most cases the main clinical problem was skeletal muscle dysfunction with progressive exercise intolerance. The two other infantile cases were also fatal, but the clinical and pathological descriptions are limited; there is no mention of cardiomyopathy in either case, but one had a diffuse necrotizing encephalopathy, an unusual finding which could be similar to the present case (12). One older patient had evidence of cardiomyopathy (10). None of the older patients was described as having encephalopathy. In three of these previous cases, activity of NADH-ferricyanide reductase was assayed and was equal to reported controls (7, 10, 11).

The proliferation in number and size of the mitochondria in this case is consistent with adaptation to a relative deficiency of ATP and has been observed in other defects of electron transport. The concentric whorls of cristae seen in skeletal muscle in this case were described in 4 of the other patients with complex I deficiency (8, 9, 11), including the infantile case mentioned above (5). Other cases have shown paracrystalline structures of cristae stacked in long rows and/or prominent electron-dense

granules (6, 9, 10, 11). Concentric whorls of inner membrane have been described in several other mitochondrial myopathies and therefore provide a relatively characteristic, but not specific, marker of this disorder (1). Spongy degeneration in the central nervous system is also unusual but has been described in other types of mitochondrial encephalopathy (1). These lesions appear to be due to increased water content within cells and intercellular fluid, associated with astrocytic reaction to axonal loss. Fatty infiltration of skeletal muscle fibers is common to other cases of complex I deficiency as well as to other mitochondrial myopathies, but fatty infiltration of heart muscle to the degree seen in this index case and sibling is rare (5). The combination of fat deposition in tissues, increased urinary excretion of dicarboxylic fatty acids, and depletion of plasma carnitine emphasize the combined effect of this disorder which dramatically interferes with both carbohydrate and fat oxidation.

Because this defect produces a block in oxidative metabolism, it seems paradoxical to have found increased resting oxygen consumption and dietary energy utilization. One other case of complex I deficiency was found to have a hypermetabolic state, especially after exercise (8). We propose that the following considerations could account for these observations: (a) Increased yield of isolated mitochondrial protein and morphological evidence of increased numbers and size of mitochondria indicate the presence of more mitochondria per gram of tissue. (b) Increased mass of mitochondria could partially compensate for deficiency of complex I, permitting more rapid but uncontrolled oxidation of NADH. (The observed lack of stimulation of oxidation of NADH-linked substrates by intact mitochondria by ADP means that normal respiratory control was lost.) (c) ATP production from site I of oxidative phosphorylation (linked to complex I) would be very inefficient, whereas normal production of ATP would be expected from sites II and III. (Succinate oxidation by intact mitochondria showed good respiratory control.) (d) Therefore, in vivo oxidation of NADH would have resulted in production of at least two but less than three ATPs. This postulated degree of inefficiency of mitochondrial oxidative phosphorylation is consistent with the actual observed inefficiency of whole-body metabolism.

An alternate mechanism that might account for the observed metabolic inefficiency could be oxidation of NADH by the cytochrome b_5 flavoprotein or cytoplasmic diaphorase, with subsequent transfer of electrons back to the inner mitochondrial membrane, ultimately resulting in reduction of cytochrome c. Oxidation of reduced cytochrome c by complex IV, in turn, would produce only one ATP from NADH. However, there is no evidence for an intracellular carrier that could transport electrons from the outer mitochondrial membrane or cytoplasm to cytochrome c on the inner membrane. Therefore, this alternative mechanism seems less plausible.

Little is known about the genetics of complex I defects. The present patient's sister died of what appears to have been the same problem. The parents had several other normal children, and the infant described by Moreadith et al. (5) had a normal sibling. It seems probable that these are autosomal recessive disorders and unlikely that these defects are transmitted through mitochondrial DNA, which would affect all or nearly all offspring. Expression of complex I deficiency in fibroblasts from this case and a case described by Robinson et al. (12) offers a potential for further genetic studies and molecular characterization of the defect.

In conclusion, this case represents one of very few defects of electron transport in which defective component activity has

been localized. The defect has been shown in this case to involve the NADH-ferricyanide reductase component activity of complex I of electron transport. This or similar defects may be more common than we recognized, because in-depth investigation of such patients remains limited. Further characterization will depend on application of techniques for analyzing the many proteins and their genes involved in mitochondrial electron transport.

Acknowledgments

Note added in proof: Mordeadith et al. (1987. *J. Clin. Invest.* 79:463–467) have recently reported that on further investigation their patient (5) also had reduced activity of NADH ferricyanide reductase.

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