Respiratory Chain Defects in the Mitochondria of Cultured Skin Fibroblasts from Three Patients with Lacticacidemia

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Abstract

The cultured skin fibroblasts from three patients with lacticacidemia were found to have low rates of 1-[^{14}C]pyruvate oxidation in the face of normal pyruvate dehydrogenase activity. After incubation with 1 mM glucose, these three cell strains also exhibited lactate/pyruvate ratios which were three times greater than those of controls. In two of the patients, both ATP and oxygen consumption in fibroblast mitochondrial preparations was deficient with NAD-linked substrates but normal with succinate and ascorbate/*N'N'N'N'* tetramethyl phenylene diamine. In the third patient, ATP synthesis in mitochondrial preparations was deficient with all substrates tested. Measurement of Rotenone-sensitive NADH-cytochrome c reductase in mitochondrial preparations from skin fibroblasts showed that two of the patients had 14 and 18%, respectively, of control activity. In the third patient, cytochrome oxidase activity was 15% of that in controls. We conclude that respiratory chain defects can be demonstrated in cultured skin fibroblasts with consistency using a number of different techniques.

Introduction

Reported defects in the mitochondrial respiratory chain both in children and in adults have often been associated with chronic lacticacidemia. These defects include deficiencies of NADH-Coenzyme Q reductase (1–7), cytochrome b (8–10), succinate cytochrome c reductase (11, 12) and cytochrome oxidase (13–25). Many of these defects are reported as being tissue-specific in that only certain tissues, usually muscle, exhibit the enzyme deficiencies; but except for two sibs with cytochrome oxidase deficiency (24), the deficit in enzyme activity is not demonstrable in fibroblasts. Clinical phenotypes vary from muscular weakness in adults to overwhelming lactic acidosis in infants, with a spectrum of neurological and neuromuscular presenting features in between, and it is not clear why such basic defects should exhibit such heterogeneity.

In the course of screening a population of pediatric patients with chronic lacticacidemia for observable defects in cultured skin fibroblasts, we found a number of cell strains that exhibited low rates of 1-[^{14}C]pyruvate oxidation to $^{14}CO_2$ in the face of normal activity of the pyruvate dehydrogenase complex. In following up the etiology of this malfunction we have shown in a series of well-documented steps that three of these cell strains have defects in the mitochondrial respiratory chain. In one of these patients we have published a preliminary investigation suggesting the respiratory chain as the site of the enzyme defect (7).

Case reports

Patient 1. K.V. was a small-for-gestational-age infant who, after a term delivery, fed poorly for the first week of life. At 10 d of age the infant developed an acute acidotic episode with tachypnea. By the 12th d the serum lactate was 30 mM with a serum pyruvate of 0.22 mM. Serum 3-hydroxybutyrate was 1.5 mM and acetoacetate 0.04 mM. On day 13 the child expired due to overwhelming acidosis. Two previous sibs had died in the neonatal period after a similar course.

Patient 2. I.W. presented at 15 d with anorexia, weakness, irritability and hypertonic posturing. An electroencephalogram showed focal seizure discharge indicative of diffuse encephalopathy. In the next two days he deteriorated with periods of apnea and then total respiratory failure for which he was intubated. He was found to have lacticacidemia with a blood lactate of 7.3 mM and a blood pyruvate of 0.12 mM. The patient expired at 1 mo of age, and at post-mortem the central nervous system was seen to be rarefied with diffuse necrosis of the subcortical white matter and dysmyelination. The parents were first cousins.

Patient 3. W.F. was healthy at birth except for the presence of hypospadias and a micropenis. He had only one testicle, which was undescended. The frontal bones of the forehead were underdeveloped, giving a narrow forehead. At 8 mo the patient was readmitted to hospital with hypoglycemia and acidosis after a urinary tract infection. He was also not thriving and had increased blood lactic acid. Blood lactic acid continued to be elevated at levels between 3.0 and 6.9 mM. By 18 mo he could sit up supported, but his development, both physical and mental, was delayed.

Methods

Chemicals. All chemicals used were analytical grade. Cytochrome e and TMPD were obtained from the Sigma Chemical Co. (St. Louis, Missouri).

Skin fibroblast cultures. Human skin fibroblasts were grown from explants of forearm skin biopsies taken with informed parental consent and cultured in α-minimal essential medium supplemented with 10% fetal calf serum. It is a characteristic of the respiratory chain–deficient cell strains that cell death occurs very rapidly after the glucose in the culture medium has been used up. We therefore had to refeed the cultures at 5-d intervals to keep them growing. In the later investigations, when large numbers of cells had to be grown for isolation of mitochondria, the α-medium was supplemented with an additional 5 mM glucose to give a final concentration of 10.5 mM glucose in the culture medium.

Determination of lactate/pyruvate ratios. Confluent skin fibroblast cultures (1 mg cell protein) were drained of culture fluid and the medium replaced with a Krebs phosphate buffer (2 ml). The fibroblast cultures were then incubated for 1 h at 37°C to deplete substrate and glycogen reserves. At this point the buffer was replaced by 2 ml of Krebs phosphate buffer containing 1 mM glucose. After 1 h, 0.1 ml 1.6 M perchloric acid

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was added. The cells were scraped from the dish with the acidified buffer, centrifuged, and separated into supernatant and pellet. The supernatant was assayed for lactate and pyruvate (26, 27) and the pellet for protein content.

Determination of enzyme activity. Pyruvate dehydrogenase was measured by the method of Hyland and Leonard (28) and whole cell 1-[^{14}C]pyruvate oxidation by the method of Robinson, Taylor, and Sherwood (29). Rotenone-sensitive NADH-cytochrome c reductase was measured in sonicated mitochondrial preparations as described by Moreadith et al. (6), except that sodium azide (5 mM) rather than cyanide was used to inhibit reoxidation of cytochrome c by cytochrome oxidase. Cytochrome oxidase was determined in mitochondria by the methods described by DiMauro et al. (19). Succinate cytochrome c reductase was measured by following the reduction of cytochrome c at 550 nm (30).

Measurement of ATP synthesis in fibroblast mitochondria and digitonin-treated fibroblasts. The ability of fibroblast mitochondria to synthesize ATP was tested in two systems, one using isolated mitochondria prepared by the method of Millis and Pious (31) and the other using digitonin-treated fibroblasts (7). In the latter procedure, confluent skin fibroblast cultures in 9-cm Petri dishes were drained of culture medium and the medium replaced by 1 ml of a solution containing 0.25 M sucrose, 20 mM morpholinopropane sulphonate (MOPS), pH 7.4, and 0.8 mg/ml digitonin. 3 min later this was removed and replaced with 1 ml of a buffer containing 0.25 M sucrose, 20 mM EDTA, and 20 mM MOPS, pH 7.4. After 5 min this medium was replaced with 1 ml of a medium containing 0.25 M sucrose, 25 mM MOPS, pH 7.4, 1 mM EDTA, 5 mM potassium phosphate, 1 mM ADP (no-substrate medium). To assess the potency of various substrates with respect to ATP production, various substrates were added to this basic medium. After 1 h at 37°C, 0.05 ml 1.6 M perchloric acid was added, the solution centrifuged to remove protein, and the resulting extract assayed by enzyme fluorimetric methods for ATP content (32). In the case of isolated mitochondria, 30 μg mitochondrial protein were added to 200 μl of no-substrate medium or substrate containing medium. Incubation was carried out for 1 h at 37°C, and 10 μl 1.6 M perchloric acid added to stop the reaction. Again, after centrifugation to remove precipitated protein, the supernatants were assayed for ATP content.

Measurement of oxygen consumption. We have found that a preparation of cultured skin fibroblasts, treated with low concentrations of digitonin to release cytoplasmic constituents, can be monitored with an oxygen electrode in an analogous fashion to a preparation of isolated mitochondria. To assess the respiration of individual cell strain mitochondria the procedure was carried out as follows: five Petri dishes of confluent fibroblasts (total protein 5 mg) were drained of culture medium and incubated with 1 ml of a solution containing 0.25 M sucrose, 20 mM MOPS, 0.05 mg/ml digitonin, pH 7.4, for 5 min. At this time this solution was removed and replaced by 1 ml of a solution containing 0.25 M sucrose 20 mM MOPS 1 mM EDTA (SME medium). The permeabilized cells were then scraped off the dishes into the 1-ml aliquots of sucrose medium, centrifuged at 13,000 rpm in an Eppendorf microfuge for 1 min and the pellets combined and resuspended in 1.5 ml SME medium. This was added to the chamber of a Clark Oxygen Electrode (Yellow Springs, Texas) and oxygen utilization was measured in response to ADP with a variety of substrates. This technique, though it does not give as high state 3/state 4 ratios as conventionally prepared mitochondria, routinely gives ratios of 3.5 for glutamate plus malate as substrate and 2.2 for succinate as substrate. Since in this type of investigation the rates of oxygen consumption with ADP and phosphate are the most important parameter to measure rather than coupling efficiency, this technique lends itself to study of respiratory chain defects.

Results

We have reported previously that cell strains from patients with chronic lacticacidemia exist which in spite of having normal activity of the pyruvate dehydrogenase complex have low rates of 1-[^{14}C]pyruvate oxidation to $^{14}CO_2$ and an abnormal NAD/NADH ratio, determined either by direct measurement (33) or from the lactate/pyruvate ratio in cell cultures (7). Three cell strains were selected for this study which had rates of 1-[^{14}C]pyruvate which were 38, 16, and 27% of control rates, respectively (Table I). The activities of the pyruvate dehydrogenase complex were 88, 93, and 57%, respectively, of that in controls. For purposes of comparison, a pyruvate dehydrogenase–deficient cell strain is included that has 2% of the normal activity of the pyruvate dehydrogenase complex and 20% of the normal rate of 1-[^{14}C]pyruvate oxidation.

Confluent cultures of skin fibroblasts of the patients being studied were incubated for 1 h in glucose-free phosphate-buffered saline (PBS). Following a further 1-h incubation in PBS with 1 mM glucose, the accumulation of lactate and pyruvate was measured in the incubation medium. Control cells strains produced lactate at a rate of about 600 nmol/h per mg cell protein, and this increases somewhat in the presence of the respiratory inhibitor rotenone. Cell strains from the patients, while giving slightly but not significantly increased rates of lactate production, gave much lower rates of pyruvate production. The resulting lactate/pyruvate ratio in the medium for these patients were some three times greater than in control cell strains. Both the amounts of lactate and pyruvate produced and the ratio of lactate to pyruvate was normal in the pyruvate dehydrogenase deficient cell line. The respiratory inhibitor rotenone reduced the amount of pyruvate produced in a control cell strain and elevated the lactate/pyruvate ratio by a factor of five.

ATP production and oxygen consumption in mitochondria. Mitochondrial ATP production was assessed in two different preparations, firstly in digitonin-treated fibroblast preparations, and secondly in conventionally prepared mitochondrial preparations. In the digitonin-treated fibroblasts ATP production could be stimulated adequately in control cell strains by the addition of either pyruvate plus malate, isocitrate plus malate, succinate plus rotenone, or ascorbate plus *N'N'N'N'* tetramethyl phenylene diamine (TMPD) (Table III). In patients 1 and 2 ATP synthesis was not stimulated by pyruvate or isocitrate plus malate but was adequate for either succinate or ascorbate/TMPD. In patient 3 ATP synthesis could be stimulated by all substrates, but at reduced rates compared with controls. The isolated mitochondrial preparations, because of their greater pu-

Table I. Enzyme Activities in Control and Respiration-deficient Cell Strains

	Pyruvate dehydrogenase complex		Whole cell 1-[^{14}C]pyruvate oxidation
	Native	Dichloroacetate activated	
	nmol/min per mg	*nmol/min per mg*	*nmol/h per mg*
Controls	0.451±0.051 (9)	0.600±0.063 (9)	24.3±4.7 (6)
Patient 1 1680	0.476±0.066 (3)	0.551±0.155 (1)	3.9±0.9 (7)
Patient 2 1895	0.567±0.121 (5)	0.530±0.144 (5)	9.3±0.1 (11)
Patient 3 1909	0.251±0.066 (6)	0.343±0.068 (6)	6.8±1.6 (4)
PDH deficient 1373	0.015±0.008 (9)	0.012±0.008 (9)	4.9±1.7 (5)

Determinations were carried out as described in the Methods section. Values are expressed as the mean±SEM with the number of determinations shown in parentheses.

1. *Abbreviations used in this paper:* L/P ratio, lactate to pyruvate ratio; MOPS, morpholinopropane sulphonate; TMPD, *N'N'N'N'* tetramethyl phenylene diamine.

Table II. Production of Lactate and Pyruvate from Glucose by Cultured Skin Fibroblasts in a 1-h Incubation

Cell strain no.	Lactate produced	Pyruvate produced	Lactate/pyruvate ratio
	nmol/h per mg cell protein	*nmol/h per mg cell protein*	
Control			
1685	620±58 (9)	25.0±4.7 (9)	24.8±2.7 (9)
1206	586±92 (5)	20.7±4.0 (5)	28.3±3.1 (5)
1228	569±53 (3)	33.6±3.2 (3)	16.9±3.7 (3)
PDH Deficient			
1373	710±58 (8)	33.4±4.7 (6)	21.2±3.8 (6)
Respiration Deficient			
Patient 1 1680	753±137 (8)	10.5±1.9 (8)	71.7±5.3 (8)
1685 + Rotenone	827±51 (5)	6.2±1.7 (5)	133±23 (5)
Patient 2 1895	862±53 (5)	9.4±1.7 (5)	93.5±8.8 (5)
Patient 3 1909	777±96 (4)	6.0±1.4 (4)	137.1±14.3 (4)

Determinations were carried out as described in the Methods section to document the amount of pyruvate and lactate produced by incubating cell cultures (~1 mg protein) with 1 mM glucose. Values are given as the mean±SEM, the number of determinations being given in parentheses.

rity, had a much higher specific activity of ATP production. Patients 1 and 2 again had a greatly compromised ability to synthesize ATP with NAD-linked substrates, while patient 3's ability to synthesize ATP appeared to be about 20% of normal with all substrates tested.

Oxygen consumption was measured in preparations of mitochondria obtained by mild digitonin treatment of cells. These preparations behaved in an analogous fashion to isolated mitochondrial preparations, but had the advantage that experiments could be performed without having to grow large numbers of cells for mitochondrial preparation. The oxygen consumption of these mitochondria measured polarographically showed that in patients 1 and 2 oxygen consumption was very low with NAD-linked substrates, but was normal with succinate or ascorbate/TMPD (Table IV). In patient 3, oxygen consumption was slow with NAD-linked substrates, slow with succinate as substrate in state 4 and state 3, almost up to 50% normal with succinate in the uncoupled state (plus 2,4 dinitrophenol), and about 20% of normal with ascorbate/TMPD. 2,4 dinitrophenol addition gave only a poor stimulation of oxygen consumption (<50%) with NAD-linked substrates.

Assay of respiratory chain components. In order to obtain reproducible measurements of the respiratory chain components present in cultured skin fibroblast mitochondria, we found that it was necessary first to prepare isolated mitochondria by the protease digestion technique (31). A suspension of purified mitochondria (0.3–0.5 mg protein) in 0.5 ml of a solution containing 2.5 M sucrose, 20 mM tris HCl (pH 7.4), 1 mM EDTA was made 10 mM with respect to Mg^{++} and then sonicated to disrupt the mitochondria. 25-μl aliquots of this preparation were used for the assay of NADH cytochrome c reductase (rotenone-sensitive), succinate cytochrome c reductase, and cytochrome oxidase, as described in the Methods section (Table V).

Rotenone-sensitive NADH–cytochrome c reductase was 14% of controls in mitochondria from patient 1 and 18% in patient 2, while patient 3 showed levels not significantly different from

Table III. Production of ATP by Isolated Mitochondria with Different Substrates

Cell strain no.	No substrate	Pyruvate malate	Isocitrate malate	Succinate rotenone	Ascorbate/TMPD
	nmol/h per mg mitochondrial protein				
Conventional					
Controls	34±10 (7)	459±47 (8)	633±70 (8)	960±71 (8)	517±74 (4)
Patient 1 1680	19±3 (4)	33.7±2 (4)	58.3±8 (4)	802±41 (4)	304±54 (4)
Patient 2 1895	19±2 (3)	22±3 (3)	51±3 (3)	670±54 (3)	277±54 (3)
Patient 3 1909	20±1 (4)	113±36 (4)	118±36 (4)	142±21 (4)	99±2 (4)
	nmol/h per mg digitonin fraction protein				
Digitonin					
Controls 1685	49±5 (10)	162±20 (6)	201±23 (4)	176±16 (8)	98±12 (4)
1228	51±8 (5)	142±29 (4)	133±20 (4)	176±17 (3)	—
1206	44±10 (5)	149±27 (5)	151±20 (4)	200±21 (3)	—
Patient 1 1680	29±2 (6)	34±4 (8)	30±3 (6)	154±21 (7)	86±6 (4)
Patient 2 1895	45±7 (5)	42±1 (3)	52±7 (3)	142±10 (4)	69±10 (3)
Patient 3 1909	28±2 (4)	78±5 (4)	66±14 (4)	76±10 (4)	49±2 (3)

Mitochondrial fractions prepared by either conventional or digitonin fractionation techniques were incubated as described in the Methods section with a buffer containing either no substrate, 5 mM pyruvate and 1 mM isocitrate and 0.1 mM malate, 5 mM succinate and 1 μM rotenone, or 2 mM ascorbate and 0.1 mM TMPD. After 1 h, perchloric acid extracts were prepared and ATP content was determined. Values are given for the mean±SEM, the number of determinations being given in parentheses.

Table IV. Oxygen Utilization by Digitonin Mitochondria from Cultured Skin Fibroblasts

	Glutamate/Malate		Succinate			Ascorbate/TPMD
	State 4	State 3	State 4	State 3	Dinitrophenol	Dinitrophenol
	ng atoms 0/min per mg protein					
Controls						
1268	1.18±0.44 (4)	3.45±0.33 (5)	2.96±0.60 (5)	7.01±0.77 (4)	9.3±0.7 (3)	—
1206	1.06±0.23 (8)	3.67±0.53 (4)	3.52±0.97 (4)	7.4±1.1 (3)	11.7±1.8 (4)	34.2±7.3 (3)
Respiration deficient						
Patient 1 1680	0.07±0.05 (3)	0.39±0.09 (3)	3.62±0.13 (4)	6.13±0.35 (4)	9.99±0.45 (4)	32.3±6.3 (4)
Patient 2 1895	0.52±0.07 (4)	0.88±0.19 (4)	2.45±0.65 (3)	5.66±0.45 (5)	8.0±0.67 (3)	22.6±6.9 (2)
Patient 3 1909	0.27±0.20 (3)	0.73±0.39 (3)	0.56±0.36 (2)	1.66±1.1 (2)	5.36±0.97 (5)	5.7±1.7 (5)

Mitochondria prepared by digitonin fractionation were added to the chamber of a Clark oxygen electrode in a sucrose buffer and the oxygen consumption measured as described in the Methods section. For each substrate, the rate was measured in the absence of added ADP (state 4), in the presence of ADP (state 3), or in the presence of 0.1 mM dinitrophenol. The three substrate categories used were: 5 mM glutamate with 1 mM malate, 5 mM succinate with 1 μM rotenone, and 2 mM ascorbate with 0.2 mM TMPD. Values are given as the mean±SEM, the number of determinations being given in parentheses.

normal. Succinate cytochrome c reductase had normal activity in patients 1 and 2, but was 54.3% of normal in patient 3. Cytochrome oxidase was 181% of the control values in patient 1, 108% in patient 2, and 15.2% in patient 3.

Discussion

The initial observation that cell strains with low 1-[^{14}C]pyruvate oxidation rates and normal activities of the pyruvate dehydrogenase complex had an abnormal lactate/pyruvate (L/P) ratio gave the first indication that a respiratory chain defect might be operative. Cells with a pyruvate dehydrogenase or pyruvate carboxylase defect do not have this type of abnormality. The increased L/P ratio is a reflection of the redox state of the cytosolic compartment (34), such that an L/P ratio of 25 indicates an NAD/NADH ratio of 360:1 and an L/P ratio of 75 indicates an NAD/NADH ratio of 120:1. This more reduced cytosolic redox state could have in these cases been due to a defect in the system that transports reducing equivalents into the mitochondria (35) or to a defect in the oxidation of intramitochondrial NADH. In this latter case there would have been a decreased intramitochondrial NAD/NADH ratio that by equilibrium would be reflected in a more reduced cytosolic redox state.

Table V. Activity of the Components of the Respiratory Chain in Cultured Skin Fibroblast Mitochondria

Cell line	Succinate cytochrome c reductase	NADH–cytochrome c reductase (±rotenone)	Cytochrome oxidase
	nmol/min per mg mitochondrial protein		
Controls	88.0±7.1 (8)	54.2±5.1 (10)	39.9±6.3 (8)
1680 Patient 1	104.1±12.8 (4)	7.6±1.6 (4)	72.5±8.5 (4)
1895 Patient 2	113.0±14.1 (4)	10.0±2.3 (5)	42.9±4.1 (4)
1909 Patient 3	47.8±6.8 (4)	42.9±4.1 (4)	7.7±1.2 (9)

Determinations were carried out as described in the Methods section using mitochondria prepared by the protease digestion technique (31) which had been sonicated in the presence of 10 mM Mg^{++} in a medium containing 0.25 M sucrose, 20 mM tris HCl (pH 7.4). Values are given as the mean±SEM, the number of determinations being given in parentheses.

The decreased ability of mitochondrial preparations to synthesize ATP with specific substrates can be used not only to document the presence of a defect in the respiratory chain but also to locate the site of the defect. Thus, in patients 1 and 2, the inability to make ATP from NAD-linked substrates while having normal ATP synthesis with succinate and ascorbate TMPD places the defect between NADH and CoQ in the respiratory chain. In patient 3 the decreased ATP synthesis with all respiratory substrates would indicate a number of possible problems to include (*a*) a defect in the translocation of ADP and ATP into the mitochondria, (*b*) a defect in the oligomycin-sensitive ATPase, (*c*) a defect in the coupling of electron transport to oxidative phosphorylation, and (*d*) a defect in cytochrome oxidase.

The measurement of polarographic oxygen consumption in mitochondrial preparations from these patients was also instrumental in documenting the site of the lesions. Patients 1 and 2 again showed poor rates of oxygen consumption for NAD-linked substrates with normal rates for succinate and ascorbate/TMPD. The block in respiration could not be released by the addition of dinitrophenol, indicating a block in electron transport between NADH and CoQ. With patient 3, oxygen consumption, like ATP synthesis, was markedly decreased with all substrates. Dinitrophenol gave a good stimulation of succinate-driven oxygen consumption, but not with NAD-linked substrates. This at first led us to investigate a possible defect in ADP translocation or coupling, but experiments measuring dinitrophenol-stimulated ATPase activity in whole mitochondria gave only normal values for patient 3 (results not shown). Measurement of the respiratory chain components, however, demonstrated unequivocally that the defect in patient 3 resided in the cytochrome oxidase complex and that the defects in patient 1 and 2 resided in the NADH–CoQ reductase enzyme complex. The explanation for the rather misleading stimulation of succinate oxidation with dinitrophenol probably lies in the availability of reducing equivalents to drive the cytochrome oxidase reaction, the flow of electrons from succinate dehydrogenase being faster in the presence of an uncoupling agent.

An examination of the cases of muscle NADH–CoQ reductase deficiency in the literature reveals six cases, all but one having a late onset and muscle weakness in childhood (1–5). The case described by Moreadith et al. (6) is of an infant who died at a few months of age with severe lacticacidemia. The cases we present here are very similar to this latter case in terms of their clinical progress and severity of lactic acidosis. The locality of the defect in the above-mentioned case (6) was centered in one of the iron sulphur proteins in the NADH–CoQ reductase complex by measurement of abnormal electron paramagnetic resonance spectroscopy in submitochondrial particles. The amount of material required precludes this type of measurement in fibroblast mitochondria at this time. Since there are at least 25 different polypeptide components in the NADH–CoQ reductase complex (36), it is quite possible that heterogeneity exists among the different patients with an overall defect in this complex. We suggested that some of these polypeptides might be encoded by the mitochondrial genome (7) a fact that has since been elegantly shown for six of them by Chomyn et al. (37). Since there is no evidence of maternal inheritance of the defect in either of our patients, it is unlikely that the defect we describe resides in a mitochondrially encoded protein.

The clinical presentation of patients with muscle cytochrome oxidase deficiency is again heterogenous. Many of the patients have lacticacidemia, but the age of onset of problems divides the patients into two groups, with presentation either in childhood or in infancy. One group with onset in infancy has Fanconi syndrome (15–18, 20). Others with an early onset have a combination of growth retardation and neurological problems (13, 14, 19, 22). The initial presenting features of our patient 3 of lacticacidemia and micropenis have been added to now by a continuing delayed physical and neurological development. This does not seem to fit exactly with any described cases in the literature, but might have been similar to the group with growth retardation and neurological problems (above). Cytochrome oxidase again is an enzyme complex with 13 subunit polypeptides, three of which are encoded by the mitochondrial genome. Deficiencies in the complex are obviously going to be heterogenous and in the absence of any evidence maternal transmission will be in the nucleus-encoded polypeptides (38). Since the three mitochondrially encoded proteins are the actual catalytic subunits of the complex which carry out the electron transport function (38), it will be interesting to find out exactly how a defect in one of the regulatory proteins can reduce the activity to 20% of normal.

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