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MINI-REVIEW

Defects in the Cytochrome bc_1 Complex in Mitochondrial Diseases

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Abstract

The clinical and biochemical findings of 14 patients with an isolated defect of the bc_1 complex have been summarized. The heterogeneity of this group of disorders reflects the severity and tissue specific expression of the defect and the complexity of this multisubunit protein with components that are coded on both nuclear and mitochondrial DNA. The data on several patients with a combined defect of cytochrome oxidase and the bc_1 complex or with multiple respiratory chain defects have also been presented and discussed in relation to our knowledge of the biosynthesis and assembly of the respiratory chain complexes. The severity of the defect *in vivo* is illustrated in one patient with isolated complex III deficiency by measurement of O_2 consumption and CO_2 production following exercise, or by ^{31}P-NMR. The latter also provides a means by which response to therapy can be followed.

Key words: Mitochondrial myopathy; mitochondrial encephalomyopathy; complex III; bc_1 complex; cytochrome *b*; ubiquinol : cytochrome *c* reductase; succinate : cytochrome *c* reductase; electron transport; respiratory chain; ^{31}P-NMR.

Introduction

The major function of mitochondria in aerobically active tissues such as skeletal muscle or brain is to catalyze the terminal reactions of fuel utilization and to produce sufficient ATP to meet the high energy demands of these tissues. Within the last few years, a group of disorders of mitochondrial function has been recognized. These include: (1) defects of substrate utilization

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such as pyruvate dehydrogenase deficiency (reviewed elsewhere in this issue) and disorders of β-oxidation or the carnitine system (Angelini, 1984; Vianey-Liaud *et al.*, 1987); (2) disorders of the respiratory chain; and (3) disorders of ATP synthesis or the coupling mechanism (DiMauro *et al.*, 1985). Disorders of the respiratory chain are usually associated with structural as well as functional abnormalities of mitochondria and, not surprisingly, affect those tissues with a high demand for ATP such as skeletal muscle, brain, heart, and retina. The clinical expression of these disorders varies according to the major target organ involved, usually skeletal muscle and/or the CNS.

The proteins of the mitochondrial electron transport chain catalyze the oxidation of NADH-linked substrates, or of succinate, coupled to the synthesis of ATP. These proteins are assembled into five major complexes which span the mitochondrial inner membrane, comprising NADH : ubiquinone reductase (complex I), succinate : ubiquinone reductase (complex II), ubiquinol : cytochrome *c* reductase (complex III or the bc_1 complex), cytochrome *c* oxidase (complex IV), and the ATP synthase (complex V), as shown schematically in Fig. 1. Disorders of each complex have been described (DiMauro *et al.*, 1985). Each complex is made up of a number of distinct subunits, eleven in the case of the bc_1 complex in mammals. Moreover, tissue-specific or developmentally regulated forms of some of these subunits may exist. Any of these individual proteins could be the site of a genetic defect, so the potential for genetic heterogeneity is enormous. This is particularly true if one includes the additional possibility of defects of regulation, transport, assembly, or processing of these proteins.

Most proteins of the respiratory chain are coded on nuclear DNA, synthesized on cytoplasmic ribosomes, and then transported into the mitochondria. However, 13 structural proteins, all components of complexes I, III, IV, and V, are coded on mitochondrial DNA (mtDNA) and synthesized within the mitochondria (Anderson *et al.*, 1981; Chomyn *et al.*, 1985). This includes apocytochrome *b* of complex III. Thus there is also the potential for defects of mtDNA in this group of diseases. Such defects would be expected to cause a multisystem disease with variability of expression within families, or among different tissues of one individual, according to the distribution of normal or mutant mitochondria during embryogenesis. Moreover, since mitochondria appear to be inherited exclusively from the mother, these disorders should have a maternal inheritance pattern (Giles *et al.*, 1980). A few such families have now been described (Wallace, 1986).

This review is restricted to disorders of the bc_1 complex. However, since there is little to differentiate the different disorders of the respiratory chain at the clinical level, and the diagnostic evaluation is similar, these aspects, which are discussed below, are applicable to all of the disorders.

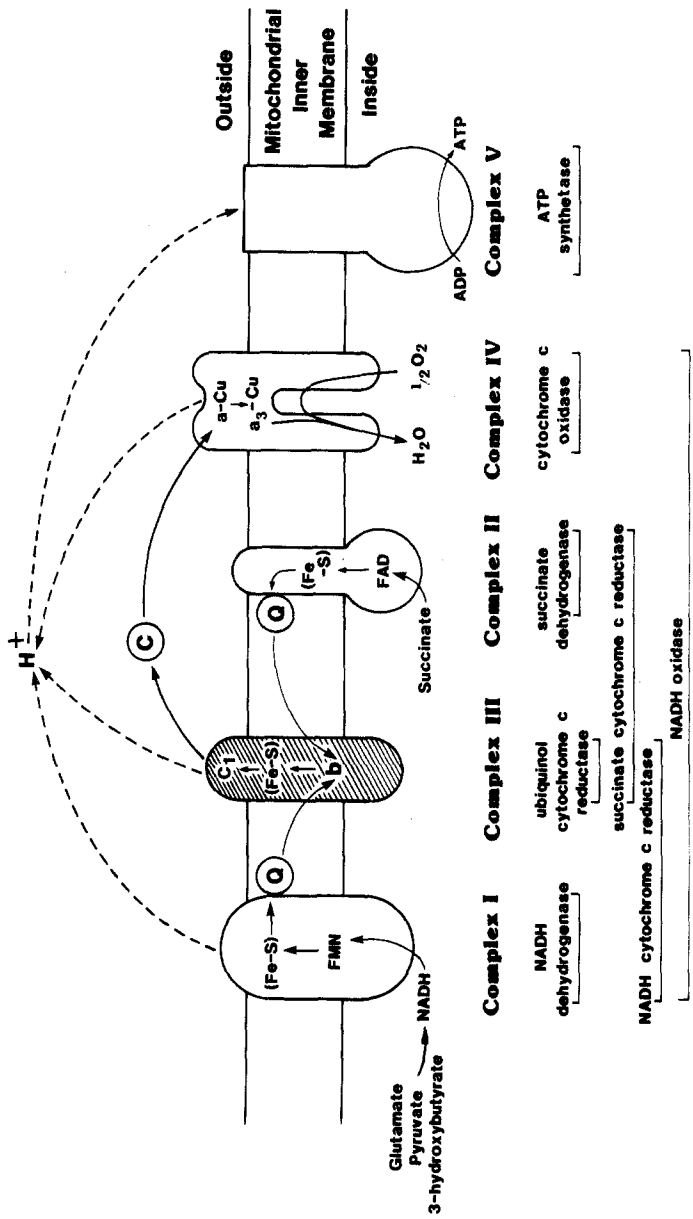

Fig. 1. Schematic representation of the mitochondrial electron transport chain. Shaded area represents bc_1 complex. Q = ubiquinone or ubiquinol.

Clinical Presentation of Patients with Disorders of the Respiratory Chain

The clinical presentation of patients with disorders of the electron transport chain depends on the severity and tissue distribution of the defect but can be divided into four main groups: (a) mitochondrial myopathy, with predominant or exclusive involvement of skeletal muscle; (b) mitochondrial encephalomyopathy, or multisystem disease, with major involvement of the central nervous system but usually with evidence of skeletal muscle disease and possibly other tissue involvement; (c) infantile mitochondrial encephalomyopathy, including Leigh's syndrome, with much earlier onset and a more severe course; and (d) mitochondrial cardiomyopathy, in which the heart may be the sole organ involved.

There are also several syndromes which may fall into the above classification, or may in some cases be distinct. These include chronic progressive external ophthalmoplegia (CPEO) in which the muscle weakness predominantly affects the extraocular muscles which have a very high energy requirement. A series of multisystem disorders includes: Kearns–Sayre syndrome or "ophthalmoplegia plus" in which retinopathy and cardiac conduction defects occur in addition to mitochondrial myopathy and CPEO (Berenberg *et al.*, 1977); MELAS syndrome, an acronym for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (Pavlakis *et al.*, 1984); MERRF syndrome, an acronym for myoclonic epilepsy and ragged red fibers (Fukuhara *et al.*, 1980; Rosing *et al.*, 1985); and some multisystem endocrinopathies. These varied presentations may be caused by lesions of the respiratory chain, but the biochemical etiology cannot be differentiated on clinical grounds. It is also not clear whether some defects may be caused by other genetic or environmental factors; for example, the Kearns–Sayre syndrome is almost always sporadic.

Diagnostic Evaluation of Patients with Disorders of the Respiratory Chain

Lactic acidosis is frequent in these patients; it may be severe, causing profound metabolic acidosis, or may only be demonstrable following exercise. The plasma lactate/pyruvate ratio may also be elevated, as may the β-hydroxybutyrate/acetoacetate ratio, reflecting an increased $NADH/NAD^+$ in both the cytoplasm and mitochondria and suggesting a defect of the respiratory chain.

In most cases, histochemical examination of skeletal muscle, often performed on small samples obtained by needle biopsy, is the initial study which confirms a mitochondrial myopathy and leads to detailed biochemical evaluation. On histochemistry, the tissue frequently shows ragged red fibers, so

called because of the granular red material seen with the modified Gomori trichrome stain. This material, which is comprised of collections of mitochondria, is found most commonly just under the cell membrane but may extend throughout the muscle fiber. It also shows increased staining for mitochondrial activities such as succinate dehydrogenase or NADH-tetrazolium reductase. Ragged red fibers may constitute a major proportion of the muscle fibers, may occur infrequently, or occasionally may be absent. Their number does not seem to correlate with the clinical severity and they may represent one stage of the evolution of the disease in a given fiber. Immunocytochemistry, using antibodies to components of the respiratory chain, now permits documentation of certain polypeptide deficiencies in some patients (Bresolin *et al.*, 1985). On electron microscopy, aggregates of mitochondria can usually be found, predominantly in the subsarcolemmal region of the fiber; these mitochondria may be greatly enlarged, of bizarre shapes, and frequently contain densely packed cristae, sometimes arranged in concentric whorls. In other cases, crystalline inclusions are seen in the intracristal or intermembrane space (Stadhouders and Sengers, 1987). Neutral fat and glycogen are often increased. Immunoelectron microscopy may be diagnostically valuable, especially when limited sample is available (Kim *et al.*, 1987).

Biochemical Studies in Muscle

A variety of methods have been applied to pinpoint the site of the defect in patients with disorders of the respiratory chain. Measurement of oxygen consumption in isolated mitochondria, incubated with a variety of substrates, provides valuable information on the integrity of transport systems for uptake of substrates into mitochondria, reactions of the citric acid cycle, the electron transport chain, and the capacity for oxidative phosphorylation. Unfortunately it requires fairly large amounts of fresh tissue and is subject to a number of errors if only small samples are available (Scholte *et al.*, 1985). Production of $^{14}CO_2$ from a variety of substrates, in the presence and absence of ADP or uncoupler, is a more sensitive approach that has been investigated in fresh mitochondria and slow-speed supernatants or homogenates (Bookelman *et al.*, 1978b; Gabreëls *et al.*, 1984).

The quantities of reducible cytochromes b, $c + c_1$, and aa_3 can be obtained from reduced–oxidized spectra, reduction being carried out either with dithionite or with succinate in the presence of cyanide or antimycin A, the latter blocking electron flow between cytochromes b and c_1. With dithionite, reduction is complete but spurious results can be caused by the presence of hemoglobin or myoglobin (Bookelman *et al.*, 1978a). With succinate, however, reduction is not as complete but there is no interference from

hemoglobin or myoglobin. Another potential problem is loss of cytochrome *c* from mitochondria or tissues which have been frozen.

A number of activities, as indicated in Fig. 1, can be measured in mitochondria or in slow-speed supernatants of fresh or frozen tissue. Theoretically, NADH dehydrogenase and NADH : ubiquinone reductase should be normal in patients with a defect of the bc_1 complex whereas rotenone-sensitive NADH : cytochrome *c* reductase and NADH oxidase should be reduced. However, rotenone-insensitive activity which arises predominantly from outer membrane NADH : cytochrome b_5 reductase activity (Sottocasa *et al.*, 1967) may constitute as much as 90% of total NADH : cytochrome *c* reductase; its presence can therefore mask a defect of the inner membrane activity, especially in impure preparations. Succinate dehydrogenase is usually measured in the presence of 2,6-dichlorophenol indophenol (DCIP) with or without phenazine methosulfate (PMS). In the absence of PMS, DCIP is believed to accept electrons at the level of coenzyme Q, thus providing a measure of succinate : ubiquinone reductase activity (Fischer *et al.*, 1985).

Finally, with the availability of subunit specific antibodies, it is now possible to probe the subunit composition of respiratory chain complexes by immunoprecipitation and SDS-polyacrylamide gel electrophoresis, or by Western blot analysis (Darley-Usmar *et al.*, 1983; Bresolin *et al.*, 1985; Tanaka *et al.*, 1986).

Disorders of the bc_1 Complex

Patients with isolated complex III deficiency have been grouped according to the clinical classification outlined in Table I. This division is somewhat arbitrary in that there is considerable clinical overlap between these groups. However, until these defects can be defined at the molecular level, there is no better classification for patients with a disorder of the bc_1 complex. Table I lists the salient features of 14 patients on whom detailed information is available. Tables II and III provide data on the biochemical findings in muscle from these patients. Later sections deal with partial deficiency of the bc_1 complex, combined defects of cytochrome oxidase and the bc_1 complex, and multiple respiratory chain defects and/or secondary defects of the bc_1 complex.

Mitochondrial Myopathy

Fatigue, shortness of breath, and severe exercise intolerance dominate the clinical picture in these patients. Onset is in childhood or adolescence. There may be associated weakness, and an oculoskeletal myopathy with

CPEO and/or ptosis may be present. Muscle pain or cramps occur infrequently and myoglobinuria has not been reported. Lactic acidosis may be mild or severe, and in some patients may only be demonstrated following exercise. As far as we can tell, the disease is restricted to skeletal muscle. On microscopy, the muscle invariably shows ragged red fibers and accumulations of structurally abnormal mitochondria, typical of mitochondrial myopathies. Family history has been negative in all cases, except patient 4 whose daughter was similarly affected.

In patients 1–4, the diagnosis of a defect of the bc_1 complex was based on the markedly reduced respiratory activities with succinate as well as NADH-linked substrates, especially in comparison to activities with ascorbate + TMPD in most of these patients (Table II). In cases 2 and 3, state 3 oxygen consumption was not increased by uncoupler (FCCP), indicating that the low repiratory activities were not due to a deficiency of the mitochondrial ATPase. In support of this conclusion, respiratory activities with pyruvate + malate and succinate + rotenone increased more than 3-fold in patient 3 and more than 15-fold in patient 4 with the addition of TMPD which is believed to transfer electrons directly from complex I or II to cytochrome *c*, thus bypassing a block in the bc_1 complex (Lee *et al.*, 1967). Low levels of cytochrome *b* in patient 2 and cytochromes *b* and c_1 in patient 3 confirm the defect of complex III. The apparent deficiency of cytochromes aa_3 in addition to *b* and c_1 in patient 3 is hard to reconcile with the relatively normal respiratory activity measured with ascorbate + TMPD as substrate. Two additional patients with CPEO and exercise intolerance, not listed in Tables I and II, had normal levels of cytochromes (Morgan-Hughes *et al.*, 1987) as did patients 1 and 4. In cases 5 and 6 (Table III), a defect of complex III was established on the basis of markedly reduced activities of rotenone-sensitive NADH : cytochrome *c* reductase and succinate : cytochrome *c* reductase while succinate dehydrogenase and cytochrome oxidase were normal. Low levels of reducible cytochrome *b* in patient 6 confirmed this diagnosis.

Mitochondrial Encephalomyopathy

Four patients have been described in whom multisystem disease, starting either in childhood or in adult life, was present (Table I). Ataxia and intellectual deterioration occurred in three patients; myoclonus (involuntary jerking movements) was present in two, preceding other symptoms by 9 years in patient 9. Pigmentary degeneration of the retina was present in two cases. Ragged red fibers were usually seen on muscle biopsy but structural changes in mitochondria were not always present (Spiro *et al.*, 1970). Liver biopsy was said to be histologically normal in case 9.

Table I. Clinical and Associated Findings in Disorders of the bc_1 Complex

Case	Age/sex	Age of onset	Clinical and associated findings	Blood lactate (mM)[a]	Reference
			Mitochondrial myopathy		
1	40 yr M	15 yr	CPEO, severe exercise intolerance	Elevated[b]	Morgan-Hughes *et al.*, 1984a, 1987
2	38 yr M	Childhood	Weakness, progressive exercise intolerance	11.9[b]	Morgan-Hughes *et al.*, 1977; 1987
3	20 yr F	Childhood	Weakness, ptosis, exercise intolerance	4.5[b]	Hayes *et al.*, 1984
4	38 yr F	24 yr	CPEO, ptosis, exercise intolerance, double vision	na	Morgan-Hughes *et al.*, 1985, 1987
5	19 yr M	Childhood	Muscle pain, severe exercise intolerance	12.0[b]	Reichmann *et al.*, 1986
6	17 yr F	9 yr	Severe exercise intolerance; weakness	3.4; 13.2[b]	Darley-Usmar *et al.*, 1983; Kennaway *et al.*, 1984
			Mitochondrial encephalomyopathy		
7	46 yr M	33 yr	Weakness, ataxia, memory deterioration, slurred speech, absent reflexes	na	Spiro *et al.*, 1970
8	16 yr M	8 yr	Weakness, ataxia, intention tremor, myoclonus, intellectual, speech, and visual deterioration	na	Spiro *et al.*, 1970
9	48 yr F	33 yr	Weakness, myoclonus ataxia, dementia, deafness; RP, Died at 49 yr.	3.1; 9.5[b]	Morgan-Hughes *et al.*, 1982, 1987
10	43 yr F	Childhood	CPEO, weakness, RP, severe exercise intolerance	13[b]	Morgan-Hughes *et al.*, 1984a, 1987

			Infantile mitochondrial encephalomyopathy		
11	Infant M	Birth	Severe hypotonia, vomiting, lethargy, severe acidosis, decreased reflexes. Died at 5 mo.	12.3	Behbehani *et al.*, 1984
12	2.8 yr M	Infancy	Hypotonia, failure to thrive, coordinative movement disorder; severe mental retardation	na	Scholte *et al.*, 1987; Przyrembel, 1987
13	4.5 yr M	2 yr	Hypotonia, weakness, ptosis, exercise intolerance, cerebellar atrophy, abnormal EEG	3.8; 4.3	Sengers *et al.*, 1983
			Mitochondrial cardiomyopathy		
14	3 wk F	Neonatal	Histiocytoid cardiomyopathy, failure to thrive. Died at 4 wk.	na	Papadimitriou *et al.*, 1984

[a]Normal resting values up to 1.8 mM; [b]following exercise; na = not available; RP = pigmentary changes in retina.

Table II. Respiratory Activities and Content of Cytochromes in Skeletal Muscle Mitochondria in Eight Patients

Case[a]	State 3 respiratory activities[b]				Cytochrome spectra[c]		
	Pyruvate (+ Malate)	Glutamate (+ Malate)	Succinate (+ Rotenone)	Ascorbate + TMPD	*b*	$c + c_1$	$a + a_3$
			Mitochondrial myopathy				
1	0	28	0	261	Normal	Normal	Normal
2	18	20	13	nd	180	1230	1060
3	29	13	32	115	170	840[d]	190
4	10	0	6	449	Normal	Normal	Normal
			Mitochondrial encephalomyopathy				
7	nd	nd	nd	nd	Low	na	? Low
8	nd	30% of normal	25% of normal	nd	Low	na	? Low
9	34[e]	25[e]	15[e]	123[e]	170	3070	560
10	24	15	31	75	Normal	Normal	Normal
Controls[f]	90–149	68–147	55–185	177–493	na	na	na
Disease controls[g,h]	55; 75	72	51; 99	84; 251	530 (290–790)	1640 (1040–2300)	810 (300–1560)

[a] References as in Table I; [b] natoms O/min/mg mitochondrial protein; [c] Picomol/mg mitochondrial protein; [d] cytochrome c_1 undetectable; [e] measured in the presence of uncoupler FCCP; [f] combined ranges from Morgan-Hughes *et al.*, 1982, and Clark *et al.*, 1983; [g] Respiratory activities from Hayes *et al.*, 1984; [h] Mean and range of cytochromes from two disease controls and seven patients with NADH : ubiquinone reductase deficiency in whom mitochondrial cytochromes were considered normal (Hayes *et al.*, 1984); TMPD = tetramethylphenylenediamine; na = not available; nd = not determined.

Table III. Activity Measurements and Content of Cytochromes in Muscle in Six Patients

Case[a]		NADH: cytochrome *c* reductase[b]	Succinate: cytochrome *c* reductase	Succinate dehydrogenase	Cytochrome oxidase	Cytochrome spectra[c]		
						b	$c + c_1$	$a + a_3$
				Mitochondrial myopathy				
Skeletal muscle	5	9[d] (122 ± 45)	30[d] (260 ± 40)	100[d] (170 ± 50)	1820[d] (1350 ± 370)		"Appeared normal"	
	6	0[d] (136 ± 43)	8[d] (340)	57[d] (94)	1222[d] (945)	100 (630)	660 (660)	600 (1000)
				Infantile mitochondrial encephalomyopathy				
Skeletal muscle	11	nd	0[d] (100)	nd	nd	nd	nd	nd
	12	nd	0.70[e] (3.99 ± 0.43)	402[f] (723 ± 40)	54[f] (90 ± 7)	215 (213–343)	128 (311–524)	216 (225–353)
	13	nd	5.0[f] (90–356)	414[f] (193–463)	90[g] (73–284)	250[g] (290 ± 71)[h]	574 (648 ± 187)[h]	248 (374 ± 117)[h]
				Cardiomyopathy				
Skeletal muscle	14	Normal	540[f] (1160 ± 470)	nd	Normal	nd	nd	nd
Cardiac muscle	14	0[d] (165)	25[d] (154–236)	1.14[f] (1.14–3.24)	228[d] (257–405)	16 (419–577)	139[f] (428–638)	660 (643–775)

[a] References as in Table I: normal reported values are shown below in parentheses; [b] Rotenone-sensitive; [c] pmol/mg mitochondrial protein; [d] nmol/min/mg mitochondrial protein (biopsy); [e] U/g wet weight, measured in homogenate; in previous biospy at 8 mo., activity was undetectable; [f] mU/g wet weight, measure in homogenate; [g] mU/mg protein, measured in 600 g supernatant; [h] from Bookelman *et al.*, 1978a; [i] low value probably due to leakage of cytochrome *c* during freezing of mitochondria or tissue; [j] nmol/min/g wet weight in 600 g or 1000 g supernatant.

The first two patients with complex III deficiency who were investigated at the biochemical level were cases 7 and 8. Although respiratory activities were not measured in case 7, it was assumed he had the same biochemical defect as his son (case 8). Low levels of cytochrome *b*, reduced with succinate in the presence of antimycin A, in both father and son confirmed a defect of the bc_1 complex in these two patients. Low levels of reducible cytochromes *a* and a_3 were reported but are hard to interpret in view of the apparent contamination of the samples with hemoglobin (Bookelman *et al.*, 1978a). Thus these patients could have had a combined defect of complexes III and IV.

In cases 9 and 10, the respiratory activities suggested a defect at the level of complex III, and this was confirmed by the low (but detectable) levels of reducible cytochrome *b* in mitochondria from patient 9. In this patient, the absorption maximum of cytochrome *b* was shifted from 562 to 558 nm, suggesting either an abnormality in its structure or membrane environment. In contrast, in patient 10 and in one other patient with multisystem disease (Morgan-Hughes *et al.*, 1987), mitochondrial cytochromes were normal.

Infantile Mitochondrial Encephalomyopathy

In these patients the early onset of severe hypotonia, developmental delay, failure to thrive, and lactic acidosis may be overwhelming, leading to death at an early age. Both brain and muscle appear to be involved. In case 13 onset appeared to be later and the course milder, although this patient was weak as an infant and motor milestones were delayed. Family history was negative in all cases. Pathologically, muscle from these patients showed the typical histochemical and electron microscopic appearance of ragged red fiber myopathy. In contrast to the picture in skeletal muscle, liver from patient 11 showed no microscopic abnormalities.

In cases 11–13 (Table III) a severe deficiency of succinate: cytochrome *c* reductase activity was found in muscle, consistent with a defect of complex II or complex III. In patient 11, an elevated lactate/pyruvate ratio in blood was felt to indicate impaired oxidation of NADH and therefore a defect at the level of the bc_1 complex, rather than complex II. The defect also appeared to be tissue specific in that succinate : cytochrome *c* reductase was normal in liver.

In case 12, mitochondria prepared from muscle biopsy at the age of 8 months also showed low oxygen consumption with pyruvate + malate, succinate + rotenone, and duroquinol, and relatively normal respiration with ascorbate + TMPD, consistent with a specific defect of complex III (data not shown). Succinate : cytochrome *c* reductase in this biopsy was undetectable. Spectral analysis of cytochromes showed a slow rate of reduction of aa_3 by succinate and marked deficiency of $c + c_1$; in view of the normal

oxidation of ascorbate, which required endogenous cytochrome *c*, it was deduced that cytochrome c_1 was deficient. In a repeat biopsy at 2.8 years of age, respiration with ascorbate/TMPD had fallen to about 50% of normal and was no longer stimulated by ADP. It was suggested that this might reflect a secondary effect of accumulated long-chain acyl-CoA on the adenine nucleotide carrier (see later discussion). Very low activity of succinate: cytochrome *c* reductase in liver documented the defect in this tissue also; however, the activity was normal in cultured skin fibroblasts.

In patient 13, in addition to the reduced activity of succinate: cytochrome *c* reductase in muscle, $^{14}CO_2$ production from [1-^{14}C] pyruvate + malate or carnitine and [U-^{14}C] malate + pyruvate or acetylcarnitine was markedly reduced, consistent with a disorder of the bc_1 complex. Cytochrome spectra were normal, suggesting a defect in electron transport between ubiquinone and cytochrome *b*. There was no evidence for involvement of tissues other than muscle and brain in this patient, the elevated levels of lactate and pyruvate in CSF suggesting that the defect was indeed expressed in brain.

Mitochondrial Cardiomyopathy

In case 14, the clinical picture was dominated by massive enlargement of the heart due to hypertrophy of both ventricles, leading to cardiac failure and death at four weeks of age. At autopsy, cardiac muscle fibers were enlarged (histiocyte-like cells) and contained only remnants of myofibrils, large accumulations of mitochondria, slightly increased fat, and decreased glycogen. Other tissues showed only nonspecific changes. Family history was negative. Severe deficiency of both rotenone-sensitive NADH: cytochrome *c* reductase and succinate: cytochrome *c* reductase, with normal succinate dehyrogenase and cytochrome oxidase, in heart mitochondria localized the defect to the bc_1 complex, and the low levels of reducible cytochrome *b* were consistent with this. Normal activity of succinate: cytochrome *c* reductase in both muscle and liver confirmed that the disorder was tissue specific.

Partial Deficiency of the bc_1 Complex

Mitsumoto *et al.* (1983) studied 13 patients with CPEO alone or "ophthalmoplegia plus" syndrome, eight of whom had ragged red fibers on muscle biopsy (group 1) and five of whom did not (group 2). In group 1, the mean state 3 respiration was 32% of controls with glutamate + malate ($p < 0.001$) and 46% of controls with succinate ($p < 0.005$). Uncoupled respiration was also significantly reduced. Cytochrome spectra and cytochrome oxidase activity were normal. In group 2, mean state 3 respiration was 57% of controls with glutamate + malate ($p < 0.01$) and 68% of controls with succinate (not significant). However, uncoupled respiration was

significantly reduced with both substrates. More interestingly, mitochondrial cytochromes showed low levels of reducible cytochrome *b* (73% of controls $p < 0.01$) and cytochromes $c + c_1$ (69% of controls $p < 0.005$) in group 2 patients. Cytochrome oxidase activity was normal. These data suggest a partial defect of the bc_1 complex, especially in group 2 patients, but possibly also in group 1, although not directly affecting the bc_1 site. In view of the clinical heterogeneity within each group, a secondary effect on the bc_1 complex cannot be ruled out.

Succinate : cytochrome c Reductase Deficiency in the Absence of a Complex III Defect

Sperl *et al.* (1988) described a 3-month-old girl with severe developmental delay and lactic acidosis from 2 days of age who is unique in that she developed the DeToni–Fanconi–Debré Syndrome of renal tubular dysfunction, together with signs of liver dysfunction, suggesting that the metabolic defect was expressed in several tissues. A similar presentation occurs in some cases of cytochrome oxidase deficiency (DiMauro *et al.*, 1985). The pathology of muscle was also unusual in that no ragged red fibers or mitochondrial abnormalities were found. Succinate : cytochrome *c* reductase activity was markedly reduced in muscle (2.0 mU/mg protein; controls 10–33 mU/mg protein) and was also low in liver (4.4 mU/mg protein; controls 6.0–50.0 mU/mg protein). Cytochromes in muscle were normal apart from an apparent loss of cytochrome *c* during freezing of the tissue. Cytochrome oxidase activity, and succinate dehydrogenase, measured both with and without PMS, were normal in both liver and muscle. The latter presumably rules out a defect of complex II. Normal NADH oxidase activity ruled out a defect of complex III. The authors suggested that the data could best be explained by a defect in a (protein) component in the microenvironment of coenzyme Q.

Two siblings, aged 7 and 9 years respectively, with mitochondrial encephalomyopathy were reported by Riggs *et al.* (1984). Rotenone-sensitive NADH : cytochrome *c* reductase, succinate dehydrogenase, and cytochrome oxidase were normal in muscle from both patients; however, succinate : cytochrome *c* reductase was reduced in both (0.43 and 0.13 μmol/min/g tissue compared to 1.11 ± 0.43 μmol/min/g tissue in controls). These data were interpreted as indicating a defect of complex II of the respiratory chain, involving the segment between the flavoprotein of succinate dehydrogenase and coenzyme Q. Unfortunately insufficient material was available for measurement of succinate : ubiquinone reductase or the mitochondrial cytochromes.

Classification and Tissue Specificity of Complex III Defects

Ideally the classification of respiratory chain disorders should be based on a knowledge of the primary molecular defect which has not yet been

established in any patient. Classification on the basis of clinical findings alone is obviously unsatisfactory, as illustrated by patient 6 who has had several episodes of significant neurological symptoms following what for her is excessive exercise leading to severe lactic acidosis. These episodes usually start with visual hallucinations comprising bizarre complex patterns and, if untreated, may progress to frank seizures. Nonetheless this patient normally had no central neurological symptoms and is therefore classified as a myopathy rather than an encephalomyopathy. It seems likely that the neurological symptoms in some patients may be caused by acute major distortions in the levels of metabolites in the circulation, rather than by expression of the underlying defect in the nervous system.

Attempts to classify these patients on the basis of tissue specificity or specific biochemical abnormalities present a very confusing picture: muscle is affected in most patients but not all; heart is rarely affected; brain appears to be involved in about 50% of cases; occasionally the defect is expressed in liver but only once was it reported to be expressed in cultured skin fibroblasts (Scholte *et al.*, 1987). It is clear that there is a great deal of heterogeneity within this group of disorders, hardly surprising in view of the complexity of this multisubunit protein complex.

On the basis of the cytochrome spectra, these patients can be divided into at least three distinct groups: (1) *normal cytochromes*, suggesting a defect at the level of coenzyme Q or the Reiske iron-sulfur centers; interestingly, the serum level of ubiquinone was reduced in patient 10 although it was normal in four other patients with normal levels of cytochromes (Morgan-Hughes *et al.*, 1987); (2) *low cytochrome b*, presumably interrupting electron flow at that site; (3) *low cytochromes b and* c_1, again interrupting electron transport at the bc_1 site. The pattern of cytochromes does not appear to correlate with either the clinical presentation or tissue-specific expression of the disease. The basis of the tissue specificity is not clear either, although, in analogy with what has been reported for cytochrome oxidase (Kadenbach *et al.*, 1982; Kuhn-Nentwig and Kadenbach, 1985), it seems likely that some of the nuclear coded subunits of complex III may be tissue specific. This topic is addressed in a separate review in this issue. The occurrence of combined deficiencies of more than one of the respiratory chain complexes and the possibility of secondary deficiencies complicates the picture even more, as discussed below.

Combined Defect of Cytochrome Oxidase and the bc_1 Complex

Three patients have been described with infantile mitochondrial myopathy or encephalomyopathy, DeToni–Fanconi–Debré syndrome, and lactic acidosis in whom cytochrome aa_3 was absent and cytochrome *b* reduced in

skeletal muscle. Cytochrome oxidase activity was 5 and 6% of normal in two patients (Stansbie *et al.*, 1982; DiMauro *et al.*, 1980) but was not measured in the third (Van Biervliet *et al.*, 1977). Cytochromes aa_3 and *b* were normal in other tissues examined (heart of two patients; liver, kidney, and brain of one patient) except that low levels were found in kidney of one patient in whom cytochrome oxidase activity was 38% of normal (DiMauro *et al.*, 1980).

Morgan-Hughes *et al.* (1984b) reported a 14-year-old female with mitochondrial myopathy and peripheral neuropathy dating from infancy. State 3 respiration was low with all substrates, and cytochromes aa_3 and *b* were deficient.

Aprille (1985) described two siblings with infantile mitochondrial encephalomyopathy in whom cytochrome oxidase activity was severely reduced in muscle but normal in liver; cytochromes aa_3 and *b* were undetectable in muscle but normal in liver, heart, brain, and kidney of both except for a total absence of cytochrome *b* in liver from case 2. Clearly the major abnormality was confined to muscle in these two patients and the isolated deficiency of cytochrome *b* in liver from one sibling only could be a secondary phenomenon. However, a second cousin, who had died at 5 months of age with hepatic encephalopathy, hypoglycemia, and generalized aminoaciduria, had marked deficiency of cytochrome oxidase and cytochromes aa_3 and *b* in liver. Although muscle was not available, the distinct clinical presentation and absence of lactic acidosis suggested that the disorder was confined to liver in this patient. Another similar but unrelated patient was reported by the same author. The basis of the tissue specificity of these disorders has been discussed already. Of interest is the fact that the biochemical defect was absent in kidney of three out of four patients with DeToni–Fanconi–Debré syndrome and deficient cytochromes aa_3 and *b* in muscle or liver, suggesting that the renal dysfunction may be a secondary phenomenon in some patients. Possible causes of a combined defect of cytochrome oxidase and the bc_1 complex are discussed later along with additional data on another case of combined complex III and complex IV deficiency (Kennaway *et al.*, 1987).

Multiple Respiratory Chain Defects and/or Secondary Defects of the bc_1 Complex

Several patients have been described with multiple respiratory chain deficiencies, the underlying cause of which is obscure. A young woman with Kearns–Sayre syndrome had low levels of cytochrome oxidase, succinate: cytochrome *c* reductase, citrate synthetase, and carnitine palmityltransferase in muscle mitochondria (Allen *et al.*, 1983). Free carnitine was also markedly reduced. Tanaka *et al.* (1986) described an infant with mitochondrial

encephalomyopathy and DeToni–Fanconi–Debré syndrome in whom the activities of NADH dehydrogenase, succinate dehydrogenase, ubiquinol: cytochrome *c* reductase, and cytochrome oxidase were all markedly reduced in muscle mitochondria. Cytochromes aa_3 and *b* were undetectable and $c + c_1$ was 14% of control. The family described by Barth *et al.* (1983) is unusual in that the disease affected cardiac muscle, skeletal muscle, and neutrophil leukocytes and was inherited as an X-linked recessive condition. Finally, the infant described by Fischer *et al.* (1986), with mitochondrial encephalomyopathy, had relatively minor reductions of succinate: ubiquinone reductase and succinate: cytochrome *c* reductase activities which were probably secondary to a profound abnormality of complex I, leading to a disturbance in the environment of coenzyme Q in that patient. Another infant with mitochondrial encephalopathy and combined deficiencies of complexes I and III was reported by Morgan-Hughes *et al.* (1984b).

Many patients with respiratory chain defects have had additional abnormalities and it is not always clear what is primary and what is secondary. One of the more common findings has been a reduced level of free carnitine in muscle. This may contribute to the increased lipid in muscle, carnitine being required for the uptake of fatty acids into mitochondria where they undergo β-oxidation. Low muscle carnitine may be a consequence of a respiratory chain defect since ATP is required for transport of carnitine into muscle. Alternatively, low tissue carnitine may result from a disturbance in oxidation of long-chain acyl-CoA due to formation of acylcarnitines which are subsequently exported from the cell. Ultimately the carnitine deficiency may cause further accumulation of these acyl-CoA esters which are known to inhibit a variety of enzymes and translocases, particularly the adenine nucleotide translocase (Lauquin *et al.*, 1977). Other secondary effects may be mediated by the activation of proteases or phospholipases by increased intracellular Ca^{+2}, or the generation of free radicals as a consequence of a block in the respiratory chain. Supplementation with oral carnitine and/or vitamin E may be beneficial in such patients.

Stadhouders and Sengers (1987) have presented an alternative view of the etiology of respiratory chain deficiencies, especially in some patients with CPEO. They suggest that the increased oxygen supply resulting from proliferation of capillaries around ragged red fibers (seen in only some patients) leads to increased free radical attack on membrane phospholipids, with disruption of membrane micro-architecture. This may lead to decreased assembly of respiratory chain complexes, decreased electron transport chain activity, and crystallization of the unincorporated proteins. This view is consistent with the variability of biochemical findings seen in patients with, for example, the Kearns–Sayre syndrome.

Finally, the diminished oxidation of succinate and NADH-linked substrates and the low succinate : cytochrome *c* reductase activity in muscle and/or liver in Zellweger's syndrome should be mentioned (Goldfisher *et al.*, 1973; Trijbels *et al.*, 1983). This syndrome is one of a group of disorders with multiple peroxisomal dysfunctions, possibly due to a disorder of peroxisomal biogenesis or assembly (Schutgens *et al.*, 1986). How the peroxisomal abnormality impinges on the bc_1 complex of the mitochondrial respiratory chain is not clear.

Molecular and Genetic Considerations

Attempts to identify the primary molecular defect in patients with disorders of the bc_1 complex have been successful in only two cases with a deletion of muscle mtDNA (see note added in proof). The subunit composition of complexes III and IV has been examined in muscle from only two patients, one with an isolated defect of complex III and the other with a combined defect of complexes III and IV. In the first (patient 6, Tables I and III) mitochondrial proteins showed severe deficiency of cytochrome *b* (Table III) and of core proteins 1 and 2, the nonheme iron-sulfur protein, and peptide VI of complex III (Fig. 2) (Darley-Usmar *et al.*, 1983). Cytochrome c_1, although present in normal amount, was more sensitive to proteolysis than normal, suggesting loose attachment to the membrane.

Deficiency of multiple subunits of complex III, including cytochrome *b*, a product of mtDNA, was at first surprising. Although some yeast mutants with deletions in the cytochrome *b* gene have defective assembly of complex III and a similar pattern of subunit deficiencies (Sen and Beattie, 1986), a genetic defect of mtDNA in this patient is unlikely for several reasons. First, the onset of symptoms in late childhood is inconsistent with a defect of mtDNA which would presumably have been present at conception. Second, the patient's mother was clinically normal and her resting levels of phosphocreatine and recovery following exercise were normal by ^{31}P-NMR spectroscopy. Third, a defect of mtDNA should affect all tissues and there was no evidence to suggest that any tissue other than muscle was affected in the patient (Darley-Usmar *et al.*, 1986). Since a defect of mtDNA is unlikely in this patient the primary abnormality could be in a nuclear coded subunit of complex III that is unique to muscle, possibly not normally expressed until adolescence, and in the absence of which complex III cannot be assembled properly. A model for this is provided by a yeast mutant in which the gene for subunit II has been disrupted, resulting in severe reduction in levels of apocytochrome *b*, the nonheme iron-sulfur protein, and two other subunits, whereas cytochrome c_1 was present (Oudshoorn *et al.*, 1987). A primary deficiency of one subunit may be sufficient to prevent the proper assembly of other subunits into the membrane, resulting in multiple deficiencies.

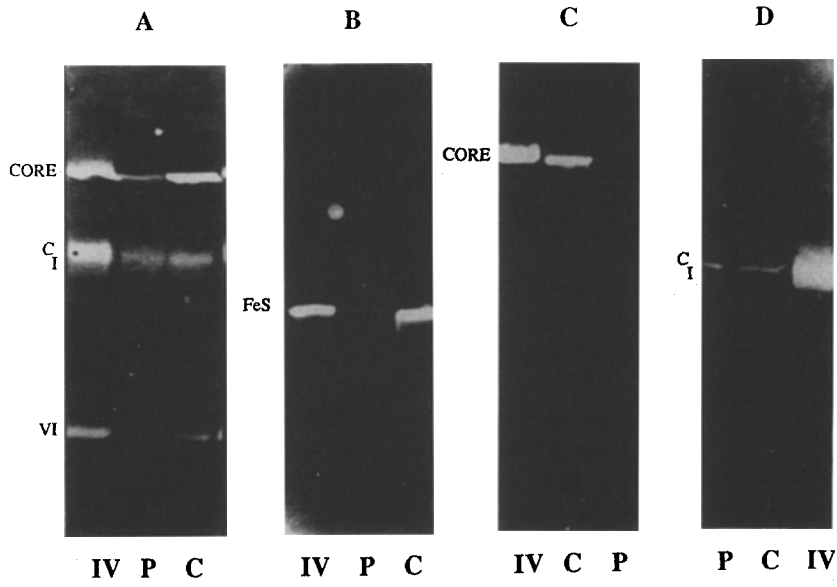

Fig. 2. Antibody binding to subunits of complex III in patient 6. Experimental details are described in Darley-Usmar *et al.*, 1983. (A) Binding of anti-holocomplex III antibody; (B) Binding of anti-FeS protein antibody; (C) Binding of anti-core protein (I and II) antibody; (D) Binding of anti-cytochrome c_1 antibody. III, 2 μg of purified complex III from beef heart; C, 80 μg of protein isolated from control human muscle mitochondria; P, 80 μg of protein isolated from patient muscle mitochondria. Modified from Darley-Usmar *et al.*, 1983, with permission.

Alternatively there could be a defect of a nuclear coded gene required for the synthesis of cytochrome *b* and/or other subunits of the bc_1 complex. Again, there are models for this in yeast (Dieckmann *et al.*, 1982; Dieckmann and Tzagoloff, 1985).

The subunit composition of the defective respiratory chain has also been examined in a patient with a combined defect of complex III and complex IV (Sengers *et al.*, 1984; Kennaway *et al.*, 1987). This infant, with mitochondrial myopathy and cardiomyopathy, died at 5 months of age of cardiorespiratory insufficiency and lactic acidosis. Cytochrome oxidase and succinate : cytochrome *c* reductase in muscle mitochondria were less than 10% of controls. Cytochromes aa_3 and *b* were 7.0 and 15.7% of the normal mean respectively. Free carnitine in muscle was also low. Antibody binding to subunits of the bc_1 complex documented deficiency of core proteins 1 and 2 and the nonheme iron-sulfur protein but normal cytochrome c_1. Interestingly, this is the same

pattern of subunit deficiencies as seen in patient 6. Additional experiments documented marked deficiency of at least 7 of the 13 subunits of cytochrome oxidase (MtII, MtIII, CIV, CV, ASA, AED, and STA) (Takamiya *et al.*, 1986; Kennaway *et al.*, 1987) using the nomenclature of Capaldi (see Takamiya *et al.*, 1987).

Deficiency of multiple subunits has also been described in two patients with isolated cytochrome oxidase deficiency (Bresolin *et al.*, 1985; Tanaka *et al.*, 1987) and one patient with combined cytochrome oxidase and complex I deficiency (Tanaka *et al.*, 1987). Thus, it appears that multiple subunit deficiencies may be the rule rather than the exception in disorders of the respiratory chain. Mechanisms to account for this have been discussed above. However, involvement of more than one complex, as in this patient, requires further explanation.

The biosynthesis of the respiratory chain is unique in that it requires the coordinated expression of both the nuclear and mitochondrial genomes

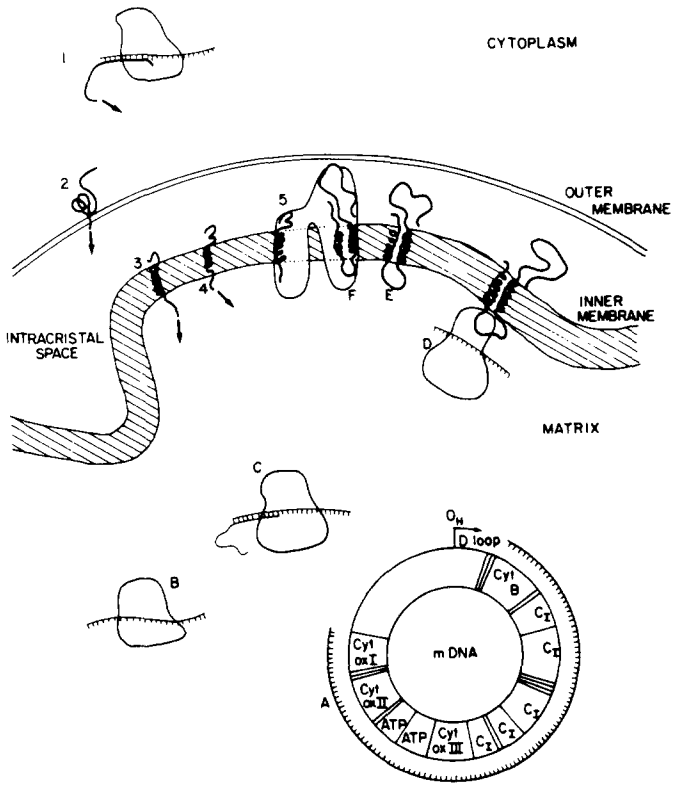

Fig. 3. Schematic of the synthesis and assembly of the repiratory chain complexes. Reproduced from Takamiya *et al.*, 1986, with permission.

(Poyton, 1980; Clayton, 1984). Figure 3 outlines schematically the steps involved in synthesis and assembly of cytochrome oxidase, as an example. It could apply equally to complexes I, III, or V. Mitochondrial DNA, which codes for three subunits of cytochrome oxidase, is thought to be transcribed as a polycistronic message which is then processed to give mRNA corresponding to the individual reading frames. These are translated on mitochondrial ribosomes close to or associated with the inner membrane. The remaining polypeptides are synthesized on cytoplasmic ribosomes as precursors with an N-terminal extension which directs their incorporation into mitochondria and, after cleavage of the leader sequence, into the inner membrane (Schatz and Butow, 1983). The presence of cytochrome c_1 and the two components of succinic dehydrogenase as mature polypeptides indicated that those mechanisms were intact in the patient described above. An error of mtDNA seems unlikely since the mother was healthy, and consanguinity of the parents suggested autosomal recessive inheritance. As far as we know, the synthesis of the bc_1 complex and cytochrome oxidase are linked only at the level of transcription of the mtDNA and processing of the polycistronic mRNA, suggesting that the defect in this patient and others with multiple deficiencies could be a defect of a nuclear coded gene required for these events. Such genes have been described in yeast (Malczewski and Whitfield, 1984).

Expression of Respiratory Chain Defects *in vivo* and Response to Treatment

It is important to consider whether the abnormalities of the respiratory chain, measured *in vitro*, represent a functional defect *in vivo*. Two techniques are relevant in this regard. The first is an incremental exercise test with measurement of O_2 consumption and CO_2 production, and determination of the respiratory exchange ratio (CO_2 production/O_2 consumption). Under conditions of increasing exercise, normal controls maintain a straight-line relationship between minute ventilation (volume of air passing through the lungs/min) and oxygen consumption, up to the ventilatory threshold (Fig. 4A). At this point, increasing work results in a change of slope, resulting from increased lactate production from anaerobic glycolysis. From Fig. 4A it can also be seen that O_2 consumption increased approximately 10-fold during strenuous exercise in a normal control. In contrast (Fig. 4B) O_2 consumption in patient 6 increased only by a factor of about 2 and she was anaerobic almost from the onset of the test. The patient's respiratory exchange ratio rose to 2.28, a highly abnormal value, in spite of the fact that the maximum work performed (5 watts) was much less than that of the control (147 watts) whose respiratory exchange ratio rose only to 1.12. Similar data were reported

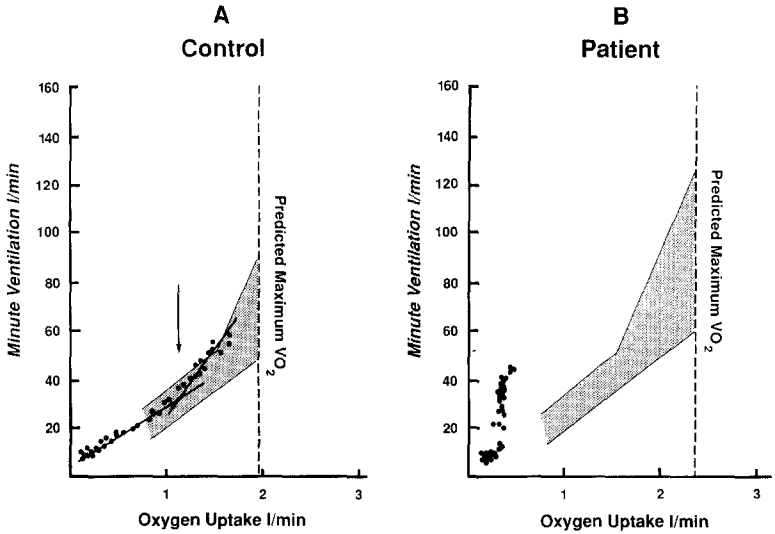

Fig. 4. Relationship between minute ventilation and oxygen uptake during incremental exercise testing in a control female (A) and patient 6 (B). Maximum work was 147 watts for the control and 5 watts for the patient. Arrow indicates ventilatory threshold (see text for definition).

for patient 2 (Morgan-Hughes *et al.*, 1977). This test provides persuasive evidence of a defect of skeletal muscle aerobic metabolism in these patients.

The second *in vivo* technique which is valuable is ^{31}P-NMR spectroscopy (Chance *et al.*, 1986). In patient 6, it enabled us to document markedly reduced levels of phosphocreatine (PCr), especially in relation to the levels of inorganic phosphate (P_i) (Eleff *et al.*, 1984). During exercise, PCr/P_i falls to around 1, below which discomfort due to lactic acidosis soon occurs. Recovery from exercise, with return of PCr/P_i to resting levels, is usually complete in 3–4 min (Fig. 5). In the patient, recovery took over 20 minutes, illustrating the severe deficiency of energy generation in muscle and suggesting a method of assessing the efficacy of different therapeutic trials. Figure 5 (lower panel) shows a spectrum from patient 6, on treatment with menadione and ascorbate which may bypass a block at the level of the bc_1 complex. Resting levels of PCr/P_i increased dramatically as did recovery from exercise. This improvement, as well as considerable clinical improvement, has been maintained over several years of treatment (Argov *et al.*, 1986).

Similar therapy has been tried in at least two other patients with defects of the bc_1 complex (cases 5 and 12), with no obvious clinical success (Reichmann *et al.*, 1986; Przyrembel, 1987). In patient 5 (nonresponsive), cytochrome *b* was present in normal amounts whereas in patient 6 (responsive) it was low, indicating that the molecular defects in these two patients were distinct. In

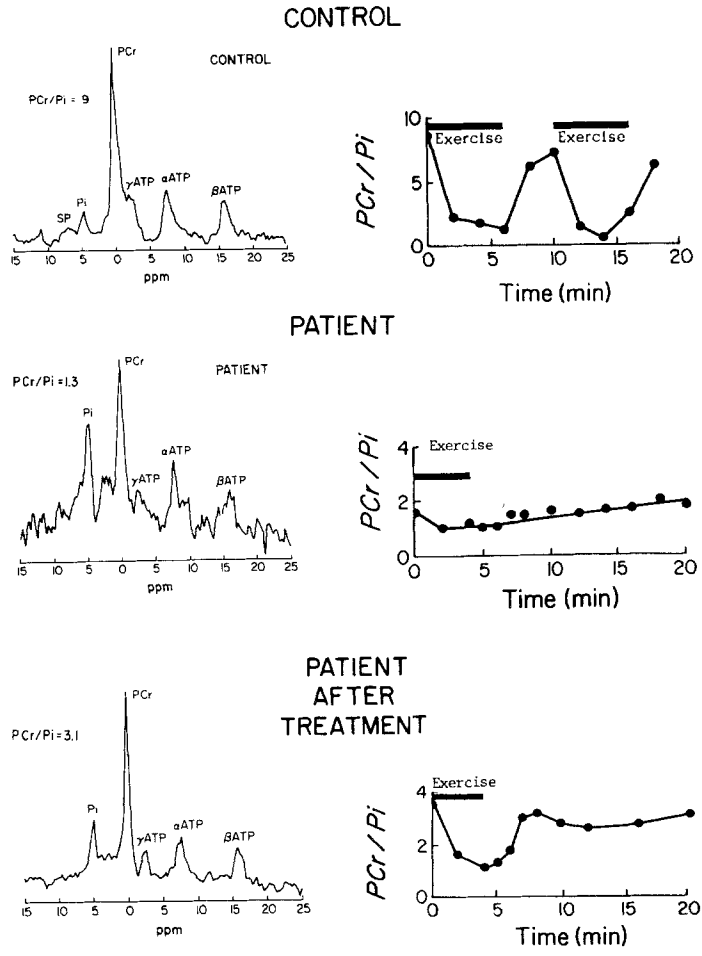

Fig. 5. ^{31}P-NMR spectra of the arm of a control female (top panel) and patient 6 (middle and lower panels). Experimental details are given in Eleff *et al.*, 1984. The left panel shows resting spectra with peaks corresponding to the α, β, and γ phosphorus of ATP, phosphocreatine (PCr), inorganic phosphate (P_i) and sugar phosphates (SP) identified. The right panel shows results following isokinetic ergometer exercise indicating intervals of rest and exercise. The lower panel shows results following therapy with menadione and ascorbate in the patient. Reproduced from Eleff *et al.*, 1984, with permission.

patient 5, the defect may involve the region of coenzyme Q or the nonheme iron-sulfur protein which in some way precludes interaction with menadione. It has been suggested that therapy with coenzyme Q_{10} might be beneficial in some patients and, indeed, was reported to decrease exercise-stimulated lactate production in another patient with a defect of complex III (Morgan-Hughes

et al., 1984b). The application of ^{31}P-NMR in evaluating such patients is obvious.

Conclusions

This review has attempted to summarize the available data on 14 patients with a well-defined defect of the bc_1 complex. The variability of clinical features and biochemical findings defies logical classification at present, and presumably reflects the complexity of this multisubunit protein with components that are coded on both nuclear and mitochondrial DNA. Variability of expression of the disease in different tissues suggests that tissue-specific forms of some of the polypeptides of complex III may exist, or that the expression of these polypeptides is under tissue-specific control. The occurrence of multiple subunit deficiencies in one patient with a defect of complex III, and one patient with a combined defect of complex III and cytochrome oxidase, suggests interference with the synthesis and/or assembly of these complex proteins in the mitochondrial inner membrane. Finally, measurement of O_2 consumption and CO_2 production during exercise or ^{31}P-NMR spectroscopy documents the extent of the deficiency *in vivo* and illustrates that knowledge of the site of the defect in certain patients with disorders of the bc_1 complex can be used to design specific therapy to circumvent that defect, and thus provide therapeutic benefit to the patient.

Note Added in Proof

In a recent report, Holt *et al.* (1988) identified deletions of mtDNA in 9 out of 25 cases of mitochondrial myopathy. Two of these patients had complex III deficiency. The deletions were identified by the restriction enzyme digest pattern, using probes to HeLa cell mtDNA, and were present in 18–79% of total muscle mtDNA but absent in leukocytes.

In one patient with complex III deficiency, the deletion of muscle mtDNA was about 5.9 kb in length and extended from at least position 7441 to 12,871 with upper limits of position 7204 to 13,259. This includes coding regions for subunits of complex I, cytochrome oxidase, and the ATPase as well as several tRNAs, but does not include cytochrome *b*. Thus, the relationship of the biochemical defect to the deletion of mtDNA is not at all clear. As the authors point out, a defect of complex I cannot be identified polarographically in the presence of a defect further along the respiratory chain. Likewise, a defect of complexes I and/or III cannot be identified polarographically in the presence of a defect of cytochrome oxidase. This illustrates

the necessity of activity measurements and antibody binding experiments in addition to polarographic studies in documenting specific and/or multiple defects of the respiratory chain.

These data demonstrate conclusively that heteroplasmy of mtDNA occurs in man, and that defects of mtDNA can be associated with human disease. The presence of the deletion in muscle but not leukocyte mtDNA in the patients described above suggests that selection against mutant mitochondria in rapidly dividing tissues may account in part for the variable expression of these diseases in different tissues.

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References

Allen, R. J., DiMauro, S., Coulter, D. L., Papadimitriou, A., and Rothenberg, S. P. (1983). *Anal. Neurol.* **13**, 679–682

Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R., and Young, I. G. (1981). *Nature* (*London*) **290**, 457–465.

Angelini, C. (1984). In *Neuromuscular Diseases* (Serratrice, G. *et al.*, eds.), Raven Press, New York, pp. 87–94.

Aprille, J. R. (1985). In *Achievements and Perspectives of Mitochondrial Research*, Vol. II: *Biogenesis* (Quagliariello, E. *et al.*, eds.), Elsevier, Amsterdam, pp. 465–476.

Argov, Z., Bank, W. J., Maris, J., Eleff, S., Kennaway, N. G., Olson, R. E., and Chance, B. (1986). *Ann. Neurol.* **19**, 598–602.

Barth, P. G., Scholte, H. R., Berden, J. A., Van der Klei-Van Moorsel, J. M., Luyt-Houwen, I. E. M., Van'T Veer-Korthof, E. Th., Van Der Harten, J. J., and Sobotka-Plojhar, M. A. (1983). *J. Neurol. Sci.* **62**, 327–355.

Behbehani, A. W., Goebel, H., Osse, G., Gabriel, M., Langenbeck, U., Berden, J., Berger, R., and Schutgens, R. B. H. (1984). *Eur. J. Pediatr.* **143**, 67–71.

Berenberg, R. A., Pellock, J. M., DiMauro, S., Schotland, D. L., Bonilla, E., Eastwood, A., Hays, A., Vicale, C. T., Behrens, M., Chutorian, A., and Rowland, L. P. (1977). *Anal. Neurol* **1**, 37–54.

Bookelman, H., Trijbels, J. M. F., Sengers, R. C. A., and Janssen, A. J. M. (1978a). *Biochem. Med.* **19**, 366–373.

Bookelman, J., Trijbels, J. M. F., Sengers, R. C. A., Janssen, A. J. M., Veerkamp, J. H., and Stadhouders, A. M. (1978b). *Biochem. Med.* **20**, 395–403.

Bresolin, N., Zeviani, M., Bonilla, E., Miller, R. H., Leech, R. W., Shanske, S., Nakagawa, M., and DiMauro, S. (1985). *Neurology* **35**, 802–812.

Chance, B., Leigh, J. S., Smith, D. S., Nioka, S., and Clark, B. J. (1986). *Ann N.Y. Acad. Sci.* **488**, 140–153.

Chomyn, A., Mariottini, P., Cleeter, M. W. J., Ragan, C. I., Doolittle, R. F., Yagi, A. M., Hatefi, Y., and Attardi, G. (1985). In *Achievements and Perspectives of Mitochondrial Research*, Vol. II: *Biogenesis* (Quagliariello, E. *et al.*, eds.), Elsevier, Amsterdam, pp. 259–275.

Clark, J. B., Hayes, D. J., Byrne, E., and Morgan-Hughes, J. A. (1983). *Biochem. Soc. Trans.* **11**, 626–627.

Clayton, D. A. (1984). *Annu. Rev. Biochem.* **53**, 573–594.

Darley-Usmar, V. M., Kennaway, N. G., Buist, N. R. M., and Capaldi, R. A. (1983). *Proc. Natl. Acad. Sci. USA* **80**, 5103–5106.

Darley-Usmar, V. M., Watanabe, M., Uchiyama, Y., Kondo, I., Kennaway, N. G., Gronke, L., and Hamaguchi, H. (1986). *Clin. Chim. Acta* **158**, 253–261.

Dieckmann, C. L., and Tzagoloff, A. (1985). *J. Biol. Chem.* **260**, 1513–1520.

Dieckmann, C. L., Pape, L. K., and Tzagoloff, A. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 1805–1809.

DiMauro, S., Mendell, J. R., Sahenk, Z., Bachman, D., Scarpa, A., Scofield, R. M., and Reiner, C. (1980). *Neurology* **30**, 795–804.

DiMauro, S., Bonilla, E., Zeviani, M., Nakagawa, M., and DeVivo, D. C. (1985). *Ann. Neurol.* **17**, 521–538.

Eleff, S., Kennaway, N. G., Buist, N. R. M., Darley-Usmar, V. M., Capaldi, R. A., Bank, W. J., and Chance, B. (1984). *Proc. Natl. Acad. Sci. USA* **81**, 3529–3533.

Fischer, J. C., Ruitenbeek, W., Berden, J. A., Trijbels, J. M. F., Veerkamp, J. H., Stadhouders, A. M., Sengers, R. C. A., and Janssen, A. J. M. (1985). *Clin. Chim. Acta* **153**, 23–36.

Fischer, J. C., Ruitenbeek, W., Gabreëls, F. J. M., Janssen, A. J. M., Renier, W. O., Sengers, R. C. A., Stadhouders, A. M., ter Laak, H. J., Trijbels, J. M. F., and Veerkamp, J. H. (1986). *Eur. J. Pediatr.* **144**, 441–444.

Fukuhara, N., Tokiguchi, S., Shirakawa, K., and Tsubaki, T. (1980). *J. Neurol. Sci.* **47**, 117–133.

Gabreëls, F. J. M., Prick, M. J. J., Trijbels, J. M. F., Renier, W. O., Jaspar, H. H. J., Janssen, A. J. M., and Slooff, J. L. (1984). *Acta Neurol. Scand.* **70**, 145–154.

Giles, R. E., Blanc, H., Cann, H. M., and Wallace, D. C. (1980). *Proc. Natl. Acad. Sci. USA* **77**, 6715–6719.

Goldfischer, S., Moore, C. L., Johnson, A. B., Spiro, A. J., Valsamis, M. P., Wisniewski, H. K., Ritch, R. H., Norton, W. T., Rapin, I., and Gartner, L. M. (1973). *Science* **182**, 62–64.

Hayes, D. J., Lecky, B. R. F., Landon, D. N., Morgan-Hughes, J. A., and Clark, J. B. (1984). *Brain* **107**, 1165–1177.

Holt, I. J., Harding, A. E., and Morgan-Hughes, J. A. (1988). *Nature (London)* **331**, 717–719.

Kadenbach, B., Hartmann, R., Glanville, R., and Buse, G. (1982). *FEBS Lett.* **138**, 236–238.

Kennaway, N. G., Buist, N. R. M., Darley-Usmar, V. M., Papadimitriou, A., DiMauro, S., Kelley, R. I., Capaldi, R. A., Blank, N. K., and D'Agostino, A. (1984). *Pediat. Res.* **18**, 991–999.

Kennaway, N. G., Wagner, M. L., Capaldi, R. A., Takamiya, S., Yanamura, W., Ruitenbeek, W., Sengers, R. C. A., and Trijbels, J. M. F. (1987). *J. Inher. Metab. Dis.* **10**, Suppl. 2, 247–251.

Kim, S. J., Lee, K. O., Takamiya, S., and Capaldi, R. A. (1987). *Biochim. Biophys. Acta* **894**, 270–276.

Kuhn-Nentwig, L., and Kadenbach, B. (1985). *Eur. J. Biochem.* **149**, 147–158.

Lauquin, G. J. M., Villiers, C., Michejda, J. W., Hryniewiecka, L. V., and Vignais, P. V. (1977). *Biochim. Biophys. Acta* **460**, 331–345.

Lee, C-P., Sottocasa, G. L., and Ernster, L. (1967). *Methods Enzymol.* **10**, 33–37.

Malczewski, R. M., and Whitfield, C. D. (1984). *J. Biol. Chem.* **259**, 11103–11113.

Mitsumoto, H., Aprille, J. R., Wray, S. H., Nemni, R., and Bradley, W. G. (1983). *Neurology* **33**, 452–461.

Morgan-Hughes, J. A., Darveniza, P., Kahn, S. N., Landon, D. N., Sherratt, R. M., Land, J. M., and Clark, J. B. (1977). *Brain* **100**, 617–640.

Morgan-Hughes, J. A., Hayes, D. J., Clark, J. B., Landon, D. N., Swash, M., Stark, R. J., and Rudge, P. (1982). *Brain* **105**, 533–582.

Morgan-Hughes, J. A., Hayes, D. J., and Clark, J. B. (1984a). In *Neuromuscular Diseases* (Serratrice, G. *et al.*, eds.), Raven Press, New York, pp. 79–85.

Morgan-Hughes, J. A., Hayes, D. J., Clark, J. B., and Cooper, J. M. (1984b). In *Biomedical and Clinical Aspects of Coenzyme Q* (Folkers, K., and Yamamura, Y., eds.), Vol. 4, Elsevier, Amsterdam, pp. 417–424.

Morgan-Hughes, J. A., Hayes, D. J., Cooper, M., and Clark, J. B. (1985). *Biochem. Soc. Trans.* **13**, 648–650.

Morgan-Hughes, J. A., Cooper, J. M., Schapira, A. H. V., Hayes, D. J., and Clark, J. B. (1987). In *The London Symposium* (EEG Suppl. 39) (Ellingson, R. J. *et al.*, eds.), Elsevier, Amsterdam, pp. 103–114.

Oudshoorn, P., Van Steeg, H., Swinkels, B. W., Schoppink, P., and Grivell, L. A. (1987). *Eur. J. Biochem.* **163**, 97–103.

Papadimitriou, A., Neustein, H. B., DiMauro, S., Stanton, R., and Bresolin, N. (1984). *Pediat. Res.* **18**, 1023–1028.

Pavlakis, S. G., Phillips, P. C., DiMauro, S., DeVivo, D. C., and Rowland, L. P. (1984). *Ann. Neurol.* **16**, 481–488.

Poyton, R. O. (1980). *Curr. Top. Cell. Regul.* **17**, 231–295.

Przyrembel, H. (1987). *J. Inher. Metab. Dis.* **10**, Suppl. 1, 129–146.

Reichmann, H., Rohkamm, R., Zeviani, M., Servidei, S., Ricker, K., and DiMauro, S. (1986). *Arch. Neurol.* **43**, 957–961.

Riggs, J. E., Schochet, S. S. Jr., Fakadej, A. V., Papadimitriou, A., DiMauro, S., Crosby, T. W., Gutmann, L., and Moxley, R. T. (1984). *Neurology* **34**, 48–53.

Rosing, H. S., Hopkins, L. C., Wallace, D. C., Epstein, C. M., and Weidenheim, K. (1985). *Ann. Neurol.* **17**, 228–237.

Schatz, G., and Butow, R. A. (1983). *Cell* **32**, 316–318.

Scholte, H. R., Luyt-Houwen, I. E. M., and Busch, H. F. M. (1985). *J. Inher. Metab. Dis.* **8**, Suppl. 2, 149–150.

Scholte, H. R., Busch, H. F. M., Luyt-Houwen, I. E. M., Vaandrager-Verduin, M. H. M., Przyrembel, H., and Arts, W. F. M. (1987). *J. Inher. Metab. Dis.* **10** Suppl. 1, 81–97.

Schutgens, R. B. H., Heymans, H. S. A., Wanders, R. J. A., Bosch, H. V. D., and Tager, J. M. (1986). *Eur. J. Pediatr.* **144**, 430–440.

Sen, K., and Beattie, D. S. (1986). *Arch. Biochem. Biophys.* **251**, 239–249.

Sengers, R. C. A., Fischer, J. C., Trijbels, J. M. F., Ruitenbeek, W.,, Stadhouders, A. M., ter Laak, H. J., and Jaspar, H. H. J. (1983). *Eur. J. Pediatr.* **140**, 332–337.

Sengers, R. C. A., Trijbels, J. M. F., Bakkeren, J. A. J. M., Ruitenbeek, W., Fischer, J. C., Janssen, A. J. M., Stadhouders, A. M., and ter Laak, H. J. (1984). *Eur. J. Pediatr.* **141**, 178–180.

Sottocasa, G. L., Kuylenstierna, B., Ernster, L., and Bergstrand, A. (1967). *J. Cell Biol.* **32**, 415–438.

Sperl, W., Ruitenbeek, W., Trijbels, J. M. F., Sengers, R. C. A., Stadhouders, A. M., and Guggenbichler, J. P. (1988). *Eur. J. Pediat.*, in Press.

Spiro, A. J., Moore, C. L., Prineas, J. W., Strasberg, P. M., and Rapin, I. (1970). *Arch. Neurol.* **23**, 103–112.

Stadhouders, A. M., and Sengers, R. C. A. (1987). *J. Inher. Metab. Dis.* **10**, Suppl. 1, 62–80.

Stansbie, D., Dormer, R. L., Hughes, I. A., Minchom, P. E., Hendry, G. A. F., Jones, O. T. G., Cross, A. R., Sherratt, H. S. A., Turnbull, D. M., and Johnson, M. A. (1982). *J. Inher. Metab. Dis.* **5**, Suppl. 1, 27–28.

Takamiya, S., Yanamura, W., Capaldi, R. A., Kennaway, N. G., Bart, R., Sengers, R. C. A., Trijbels, J. M. F., and Ruitenbeek, W. (1986). *Ann. N.Y. Acad. Sci.* **448**, 33–43.

Takamiya, S., Lindorfer, M. A., and Capaldi, R. A. (1987). *FEBS Lett.* **218**, 277–282.

Tanaka, M., Nishikimi, M., Suzuki, H., Ozawa, T., Okino, E., and Takahashi, H. (1986). *Biochem. Biophys. Res. Commun.* **137**, 911–916.

Tanaka, M., Nishikimi, M., Suzuki, H., Ozawa, T., Koga, Y., and Nonaka, I. (1987). *Biochem. Int.* **14**, 525–530.

Trijbels, J. M. F., Berden, J. A., Monnens, L. A. H., Willems, J. L., Janssen, A. J. M., Schutgens, R. B. H., and Van Den Broek-Van Essen, M. (1983). *Pediatr. Res.* **17**, 514–517.

Van Biervliet, J. P. G. M., Bruinvis, L., Ketting, D., DeBree, P. K., Van Der Heiden, C., Wadman, S. K., Willems, J. L., Bookelman, H., Van Haelst, U., and Monnens, L. A. H. (1977). *Pediat. Res.* **11**, 1088–1093.

Vianey-Liaud, C., Divry, P., Gregersen, N., and Mathieu, M. (1987). *J. Inher. Metab. Dis.* **10**, Suppl. 1, 159–198.

Wallace, D. C. (1986). *Hospital Practice* **21**, 77–92.