TABLE I

Distribution of the polarographically determined biochemical defects in patients with mitochondrial myopathy

These groups have been subclassified according to the activity of NADH CoQ_1 reductase (D, decreased; N, normal; NA, not assayed). The coding regions deleted were; ND, complex I; COX, complex IV; ATP, complex V. Data obtained from; [a] [23, 25]; [b] [52].

	Number of patients			
Polarographic defect:	I	I–III	I–IV	normal
NQ_1R activity:	D/N/NA	D/N/NA	D/N/NA	D/N/NA
mtDNA Deletion involving				
NDs only [a]	4/–/1	–	–	–
ND/COX/ATP [a]	1/–/–	2/–/3	–/–/3	–/5/3
mtDNA Point mutation				
$tRNA^{leu}$ (MELAS) [b]	4/–/2	1/–/2	–	–/2/1
$tRNA^{lys}$ (MERRF) [b]	–	–/–/1	–	–
None detected	5/–/5	1/1/4	1/–/8	–/4/–
	14/–/8	4/1/10	1/–/11	–/11/4
Total	22	15	12	15

observed in a variety of clinical conditions, including mitochondrial myopathies [3,17], Parkinson's disease [18–20], Lebers hereditary optic neuropathy (LHON) [21], and ageing [22]. This predominance of complex I dysfunction suggests that its structure and/or function is relatively easily perturbed, although the underlying causes of the complex I dysfunction are not always apparent.

Mitochondrial myopathies and encephalomyopathies

Dysfunction of the mitochondrial respiratory chain is classically recognised in patients with a variety of neurological disorders, typically presenting some of the following features: lactic acidosis, muscular weakness, ataxia, dementia, chronic progressive external opthalmoplegia (CPEO), myoclonus and stroke-like episodes. The morphological hallmark of the mitochondrial myopathies is the 'ragged red' muscle fibre, seen with the modified Gomori trichrome stain, which represents subsarcolemmal mitochondrial proliferation.

Patients with mitochondrial myopathy can be classified using molecular genetic data according to whether the disease is associated with defects of mtDNA, either deletions or point mutations. Polarographic analysis of isolated mitochondria can be used to localise the position of the abnormality [23] (Table I).

mtDNA point mutations

The biochemical expression of these mtDNA defects is quite diverse, even between patients with apparently the same molecular defect. This can be most easily observed in 12 patients with the same point mutation of the $tRNA^{leu}$ gene (MELAS), who present with different functional defects of the respiratory chain. In 6 of these patients the defect is localised to complex I, 3 have a defect involving several complexes, and 3 have normal respiratory chain function (Table I). This particular point mutation of the $tRNA^{leu}$ gene (A to G conversion at position 3243) could have two possible effects: either alteration of the function of the $tRNA^{leu}$ or interference with the binding of the mt-TERM [24] transcription termination protein and therefore with the levels of rRNA. However, these two mechanisms do not explain the variability in the biochemical defect, nor why complex I deficiency should predominate.

mtDNA deletions

Patients with a deletion in mtDNA fell into two broad categories: those in which the deletion involved only ND products (+ intervening tRNAs) and those which involved subunits from complexes I, IV and V, as well as various tRNAs [25]. The former group has a complex I defect in good agreement with the subunits involved in the deletion. However, in the latter group the biochemical abnormality was variable, affecting either only complex I, several of the complexes or, in approximately half the group, no detectable functional defect. Consequently, there was no correlation between the site of the deletion and the biochemical defect.

One possible explanation for the lack of correlation between the molecular and functional defects may lie in the fact that, in all cases studied with abnormal mtDNA, the mtDNA populations are heteroplasmic with a variable ratio of mutant vs. wild-type mtDNA. Consequently, the variability in functional expression of these mtDNA abnormalities may reflect (a) the differences in the percentage of wild-type and mutant mtDNA, or (b) the distribution of the mutant mtDNA. These two variables could lead to different levels of

TABLE II

Analysis of the complex I polypeptide composition of patients with mitochondrial myopathy

The specific polypeptide deficiency invariably involved the 24 kDa and/or the 13 kDa subunits of complex I. Data obtained from; [a] [23, 25]; [b] [52].

mtDNA mutation	Pattern of complex I polypeptides			
	normal	general decrease	gen + specific decrease	not analysed
Deletion [a]	8	4	–	10
$tRNA^{leu}$ [b]	2	5	3	2
$tRNA^{lys}$ [b]	–	–	1	–
None defined	5	5	2	17

normal mtDNA products and, through varying degrees of complementation between mutant and wild-type DNA, varying levels of mutant products [26]. Unfortunately, the lack of any correlation between the proportion of deleted DNA and either the severity of the clinical or biochemical abnormalities [25] does not support this suggestion. An alternative hypothesis is that these mutations are only partially responsible, and there is a second factor which could involve either mtDNA or nuclear DNA, as suggested for LHON [27].

The relatively high occurrence of complex I dysfunction in patients with defects of mtDNA may reflect a greater vulnerability of the function of this complex (at least as measured polarographically) to the absence or partial deficiency of some, or all, of its mtDNA products. The importance of the ND products in complex I function is supported by the involvement of ND1 in the binding site for both rotenone [28] and MPP^+ [29].

Normal mtDNA

A mtDNA abnormality has not yet been observed in approximately half the cases studied (Table I). While it is possible that all these patients have an as yet undefined mtDNA mutation, some are likely to be due to defects of nuclear genes. The localisation of the biochemical defect in this group is similar to that seen in patients with a mtDNA abnormality, with a predominance of cases having a defect of complex I activity.

Complex I polypeptide analysis

Analysis of a number of nuclear encoded complex I subunits by Western blotting has revealed three main patterns of cross-reactive complex I polypeptides in the mitochondria from patients with mitochondrial respiratory chain dysfunction: (a) normal patterns, (b) general decrease in all cross-reactive proteins and (c) a general decrease in conjunction with the absence of one or two specific nuclear encoded proteins (24 kDa and/or 13 kDa subunits) [3]. There was a broad correlation between the severity of the biochemical defect and the severity of the polypeptide deficiency.

In patients with a mtDNA deletion the complex I polypeptide patterns fell into categories (a) or (b). However in patients with the $tRNA^{leu}$ mutation all three patterns (a, b and c) have been observed. This implies that if the $tRNA^{leu}$ mutation is acting by decreasing the levels of mtDNA protein products, then decreased levels of ND products must also result in the failure of the complex to assemble correctly resulting in a deficiency of nuclear encoded subunits. The specific absence of several nuclear encoded subunits, in three cases (and also the $tRNA^{lys}$ case), may reflect a greater reliance on the presence of the ND products for the assembly of these subunits into the complex.

The patients with an as yet undefined genetic abnormality show the same immunoreactive pattern of complex I subunits as those observed for the $tRNA^{leu}$ patients (a, b and c). The interpretation of this could be that either these patients have a similar underlying primary abnormality (i.e., a mutation of a tRNA gene), or an abnormality that disrupts the assembly/stability of the complex in a similar manner, which could be due to either a nuclear or mtDNA abnormality.

Respiratory chain dysfunction in other clinical phenotypes

There are indications that mitochondrial respiratory chain dysfunction may play a role in other disorders which do not possess the classical ragged red muscle fibres and therefore fall outside the strict definition of the mitochondrial myopathies. These include ageing, Parkinson's disease (PD) and Leber's hereditary optic neuropathy (LHON).

Ageing

There are numerous theories to explain the ageing phenomenon. However, the pivotal role mitochondria play in cellular metabolism has focused attention on the involvement of the mitochondrial respiratory chain in ageing.

Analyses of respiratory chain function in purified human muscle mitochondria revealed declining rates of oxygen utilisation using pyruvate ($r = -0.767$, $P = 0.016$) and glutamate ($r = -0.767$, $P = 0.026$) with age. This was matched by a similar decline in NADH CoQ_1

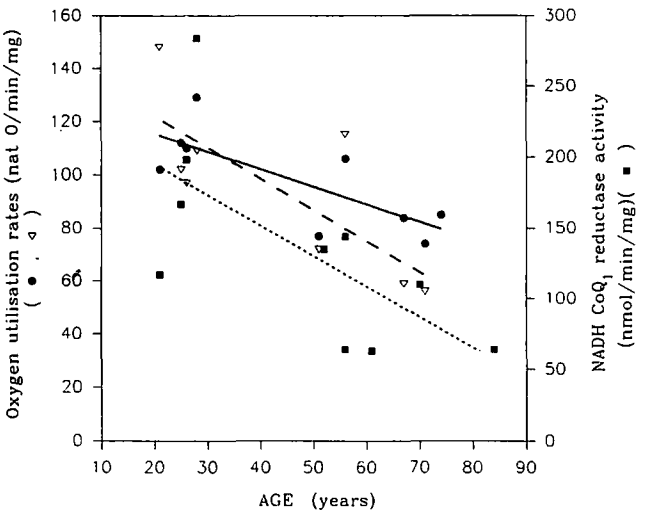

Fig. 1. Correlation of complex I dependent parameters in control human muscle mitochondria with age. Oxygen utilisation rates using pyruvate (closed circle), or glutamate (open triangle) and rotenone sensitive NADH-CoQ_1 reductase activities (closed square). Lines of correlation are; pyruvate, solid line; glutamate, dashed line; NADH-CoQ_1 reductase dotted line.

reductase activity ($r = -0.677$, $P = 0.032$) (Fig. 1). There was also a statistically significant decline in cytochrome *c* oxidase activity with age ($r = -0.803$, $P = 0.005$). However, the decline in the rates of succinate oxidation ($r = -0.288$, $P > 0.6$, data not shown) and succinate cytochrome *c* reductase activity ($r = -0.344$, $P = 0.35$) with age were not statistically significant and the activity of a non-respiratory-chain enzyme, citrate synthase, did not change with age ($r = 0.201$, $P > 0.35$, data not shown).

The somatic cell mutation theory of ageing proposes that the ageing process is due to the accumulation of DNA mutations with age. With subunits encoded by nuclear DNA and mtDNA the function of the respiratory chain is vulnerable to the mutation of either genome. MtDNA is particularly susceptible to mutation because of its location in the mitochondrial matrix in the vicinity of the respiratory chain, a known generator of potentially mutagenic free radicals [30]. This, in addition to a lack of histone proteins [31], a lack of some of the repair mechanisms associated with nuclear DNA [32], a high turnover rate [33], and a high error rate of the DNA polymerase-gamma [32], makes mtDNA a candidate for the accumulation of mutations with age. There are indications that there is an accumulation of mutant mtDNA with age [34]; however, the levels of deleted mtDNA are small (less than 5%) relative to those seen in patients with mitochondrial myopathy (20–80%), some of which do not show any functional abnormality (Table I). However, mtDNA dysfunction may still participate in the ageing process as both transcription and translation of mtDNA have been reported to decline significantly with age [35,36] and could be responsible for the decline in the activities of both complexes I and IV.

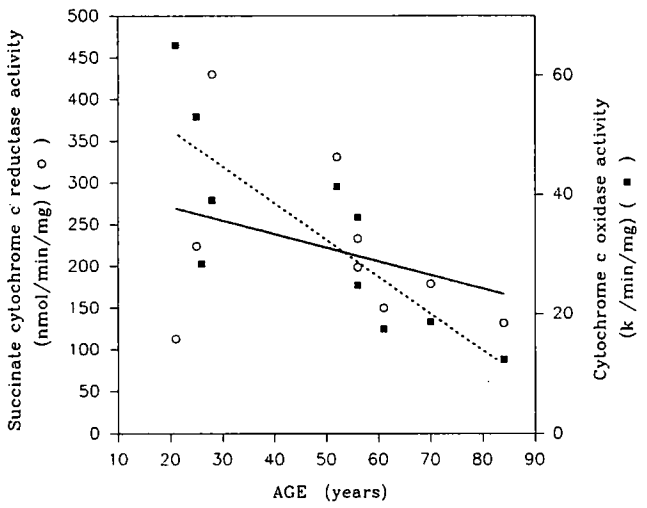

Fig. 2. Correlation of complex II/III and complex IV activities in control human muscle mitochondria with age. Spectrophotometric analyses of succinate cytochrome *c* reductase (open circle) and cytochrome *c* oxidase (closed square) activities. Lines of correlation are; succinate cytochrome *c* reductase, solid line; cytochrome *c* oxidase, dashed line.

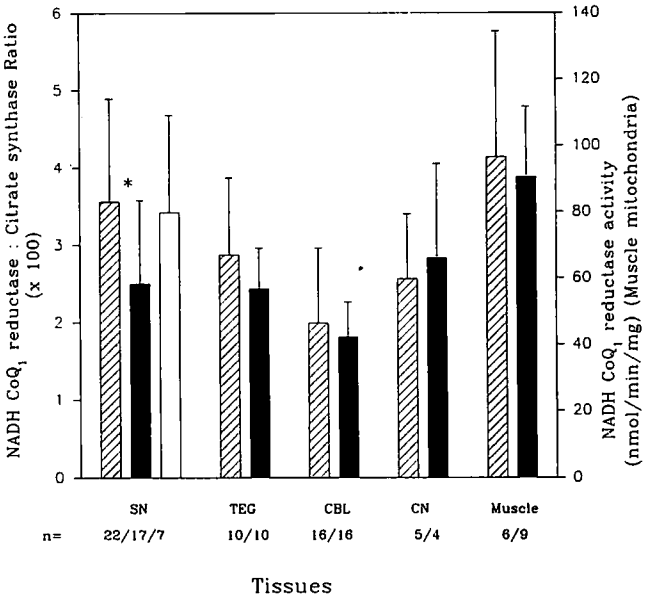

Fig. 3. Rotenone sensitive NADH-CoQ_1 reductase activities in brain regions and isolated muscle mitochondria from control (hatched bars), Parkinson's disease (solid bars) and MSA (open bar) patients. Mann Whitney U-test, * $p < 0.002$.

Parkinson's disease

Parkinson's disease (PD) results from the degeneration of the dopaminergic neurones in the substantia nigra. The mechanism underlying this neurodegeneration is unknown. However, analysis of respiratory chain function in the substantia nigra of patients dying with PD revealed a 37% decrease in NADH CoQ_1 reductase activity in comparison to age matched control samples (Fig. 3), suggesting that complex I may be involved in the underlying causes of PD. This finding correlates with an earlier finding that a Parkinson-like disorder was caused by MPTP, a byproduct of an illicit merperidine analogue, which was taken by drug addicts. The cause of the Parkinson-like symptoms was due to the active MPTP metabolite MPP^+, whose mode of action has been shown to be via inhibition of complex I activity [37].

The underlying cause of complex I dysfunction in PD substantia nigra is not known. The specificity of the defect for both the substantia nigra and PD is supported by normal complex I activities in other PD brain regions, and in multiple system atrophy (MSA), another neurodegenerative disease that involves the substantia nigra (Fig. 3).

Mitochondrial respiratory chain function in freshly isolated muscle mitochondria failed to reveal any abnormality of complex I activity in comparison to age matched control values (Fig. 3). This is in contrast to other reports which found defects of several complexes in muscle mitochondria [38,39]. However, the severity of these reported abnormalities in muscle function are surprising given the absence of muscle weakness and exercise induced lactic acidosis in PD expected from such abnormalities.

A severe defect of complex I activity was reported in platelet mitochondria prepared from patients with Parkinson's disease [19]. Using platelet homogenates we failed to confirm this finding [40]. However, in recent studies using mitochondrial fractions prepared from platelets, we have observed a small but statistically significant decrease (16%) in complex I activity in Parkinson patients (Krige et al., unpublished data).

The vulnerability of complex I function to abnormalities of mtDNA has led to speculation that this genome may be abnormal in PD. Southern blot analyses of mtDNA in PD failed to detect any abnormality [41]. Using the polymerase chain reaction (PCR), Ikebe et al. [42] reported an increase in the levels of a 5kb deletion of mtDNA in PD striatum, commonly observed in patients with a mitochondrial myopathy [25] and observed to increase with age [43]. This phenomenon was not confirmed when compared to age-matched controls [44].

It is becoming increasingly clear that the causes of PD may be multi-factorial, possibly involving both environmental and genetic factors.

Relevant observations in PD include increased incidence of abnormal xenobiotic metabolism [45], increased iron in PD brain [46], markers of increased oxidative stress in the substantia nigra [47], and evidence for genetic factors [48]. At present there is no direct link between the deficiency of complex I activity and any of these other observations, although complex I inhibition has been shown to generate oxygen radicals [49], which could contribute to the oxidative stress observed in the substantia nigra. Conversely experimentally induced oxidative stress has been demonstrated to inhibit all the respiratory chain complexes [50], unless the generation of free radicals is localised as in the case of MPP^+ inhibition, which specifically results in complex I inhibition [51].

Abnormal xenobiotic metabolism in some cases of PD could result in abnormally high levels of some environmental toxins. If any of the toxins have uptake, conversion and inhibition characteristics similar to MPTP, this could result in inhibition of complex I activity.

Genetic factors could play a role in conferring susceptibility to PD. This could relate to abnormal xenobiotic metabolism, abnormal iron metabolism or to the susceptibility of complex I to ageing or free radical generation.

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References

1 Petty, R.K.H., Harding, A.E. and Morgan-Hughes, J.A. (186) Brain 109, 915–938.
2 Lombes, A., Bonilla, E. and DiMauro, S. (1989) Rev. Neurol. (Paris) 10, 671–689.
3 Morgan-Hughes, J.A., Schapira, A.H.V., Cooper, J.M. and Clark, J.B. (1988) J.Bioenerg. Biomembr. 20, 365–382.
4 Rivner, M.H., Shamsmia, M., Shift, T.R., Tressz, J., Roesel, R.A., Carter, A.L., Yanamura, W. and Hommes, F.A. (1989) Neurology 39, 693–696.
5 Kennaway, N.G. (1988) J. Bioenerg. Biomembr. 20, 325–352.
6 DiMauro, S., Zeviani, M., Rizzuto, R., Lombes, A., Nakase, H., Bonilla, E., Miranda, A. and Schon, E.A. (1988) J. Bioenerg. Biomembr. 20, 353–364.
7 Mizusawa, H., Watanabe, M., Kanazawa, I., Nakanishi, T., Kobayashi, M., Tanaka, M., Suzuki, H., Nishikimi, M. and Ozawa, T. (1988) J. Neurol. Sci. 86, 171–184.
8 Fearnley, I.M., Runswick, M.J. and Walker, J.E. (1989) EMBO J. 8, 665–672.
9 Toda, H., Hosokawa, Y., Nishikimi, M., Suzuki, H., Kato, K. and Ozawa, T. (1989) Int. J. Biochem. 21, 1161–1168.
10 Runswick, M.J. Gennis, R.B., Fearnley, I.M. and Walker, J.E. (1991) Biochemistry 28, 9452–9459.
11 Patel, S.D., Aebersold, R. and Attardi, G. (1991) Proc. Natl. Acad. Sci. USA. 88, 4225–4229.
12 Pilkington, S.J., Skehel, J.M. and Walker, J.E. (1991) Biochemistry 30, 1901–1908.
13 Dupuis, A., Skehel, J.M. and Walker, J.E. (1991) 30, 2954–2960.
14 Skehel, J.M., Pilkington, S.J., Runswick, M.J., Fearnley, I.M. and Walker, J.E. (1991) FEBS. Lett. 282, 135–138.
15 Dupuis, A, Skehel, J.M., and Walker, J.E. (1991) Biochem. J.277, 11–15.
16 Fearnley, I.M., Finel, M., Skehel, J.M. and Walker, J.E. (1991) Biochem. J. 278, 821–829.
17 Koga, Y., Nonaka, I., Kobayashi, M., Tojyo, M. and Nihei, K. (1988) Ann. Neurol. 24, 749–756.
18 Schapira A.H.V., Cooper, J.M., Dexter, D., Jenner, P., Clark, J.B. and Marsden, C.D. (1989) Lancet i, 1269.
19 Parker, W.D., Boyson, S.J. and Parks, J.K. (1989) Ann. Neurol. 26, 719–723.
20 Schapira, A.H.V., Cooper, J.M., Dexter, D., Clark, J.B., Jenner, P. and Marsden, C.D. (1990) J. Neurochem. 54, 823–827.
21 Larsson, N.G., Andersen, O., Holme, E., Oldfors, A. and Wahlstrom, J. (191) Ann.Neurol. 30, 701–708.
22 Trounce, I., Byrne, E. and Marzuki, S. (1989) Lancet 637–639.
23 Morgan-Hughes, J.A., Schapira, A.H.V., Cooper, J.M., Holt, I.J., Harding, A.E. and Clark, J.B. (1990) Biochim. Biophys. Acta 1018, 217–222.
24 Hess, J.F., Parisi, M.A., Bennett, J.L. and Clayton, D.A. (1991) Nature 351, 236–239.

25 Holt, I.J., Harding, A.E., Cooper, J.M., Schapira, A.H.V., Toscano, A., Clark, J.B. and Morgan-Hughes, J.A. (1989) Ann. Neurol. 26, 699–708.
26 Hayashi, J.-I., Ohta, S., Kikuchi, A., Takemitsu, M., Goto, Y-I and Nonaka, I. (1991) Proc. Natl. Acad. Sci. USA 88, 10614–10618.
27 Vilkki, J., Ott, J., Savontaus, M-L., Aula, P. and Nikoskelainen, E.K. (1991) Am. J. Hum. Genet. 48, 486–491.
28 Earley, F.G.P., Patel, S.D., Ragan, C.I. and Attardi, G. (187) FEBS. Lett. 219, 108–113.
29 Ramsay, R.R., Kreuger, M.J., Youngster, S.K., Gluck, M.R., Casida, J.E. and Singer, T.P. (1991) J. Neurochem. 56, 1184–1190.
30 Boveris, A., Oshino, N. and Chance, B. (1972) Biochem. J. 128, 617–630.
31 Caron, F., Jacq, C. and Rouviere-Yaniv, J. (1979) Proc. Natl. Acad. Sci. USA. 76, 4265–4269.
32 Clayton, D.A. (1982) Cell 28, 693–705.
33 Gross, N.J., Getz, G.S. and Rubinowitz, M. (1969) J. Biol. Chem. 244, 1552–1562.
34 Piko, L., Hougham, A.J. and Bulpitt, K.J. (1988) Mechanisms of Ageing and Development 43, 279–293.
35 Fernandez-Silva, P., Petruzzella, V., Fracasso, F., Gadaleta, M.N. and Cantatore, P. (1991) Biochem. Biophys. Res. Commun. 176, 645–653.
36 Attardi, G., Chomyn, A., King, M.P., Kruse, B., Loguercio-Polosa, P. and Narasimhan-Murdter, N. (1990) Biochem. Soc. Trans. 18, 509–513.
37 Nicklas, W.J., Vyas, I. and Heikkila, R.E. (1985) Life Sci. 36, 2503–2508.
38 Bindoff, L.A., Birch-Machin, M., Cartlidge, N.E.F., Parker, W.D. and Turnbull, D.M. (1989) Lancet ii, 49.
39 Shoffner, J.M., Watts, R.L. Juncos, J.L., Torroni, A. and Wallace, D.C. (1991) 30, 332–339.
40 Schapira, A.H.V., Mann, V.M., Cooper, J.M., Dexter, D., Daniel, S.E., Jenner, P., Clark, J.B. and Marsden, C.D. (1990) J. Neurochem. 55, 2142–2145.
41 Schapira, A.H.V., Holt, I.J., Sweeney, M., Harding, A.E., Jenner, P. and Marsden, C.D. (1990) Movement Disorders 5, 294–297.
42 Ikebe, S-I., Tanaka, M., Ohno, K., Sato, W., Hattori, K., Kondo, T., Mizuno, Y. and Ozawa, T. (1990) Biochem. Biophys. Res. Commun. 170, 1044–1048.
43 Cortopassi, G.A. and Arnheim, N. (1990) Nucleic Acids Res. 18, 6927–6933.
44 Mann, V.M., Cooper, J.M. and Schapira, A.H.V. (1992) FEBS. Lett. 299, 218–222.
45 Green, S., Buttrum, S., Mollay, H., Steventon, G., Sturman, S., Waring, R., Pall, H. and Williams A. (1991) Lancet 338, 120–121.
46 Dexter, D., Carter, C., Javoy-Agid, F., Agid, Y., Lees, A.J., Jenner, P. and Marsden, C.D. (1987) Lancet ii, 1219–1220.
47 Dexter, D., Carter, C., Wells, F.R., Javoy-Agid, F., Agid, Y., Lees, A.J., Jenner, P. and Marsden, C.D. (1989) J. Neurochem. 52, 381–389.
48 Golbe, L.I., Di Iorio, G., Bonavita, V., Miller, D.C. and Duvoisin, R.C. (1990) Ann. Neurol. 27, 276–282.
49 Turrens, J.F. and Boveris, A. (1980) Biochem. J. 191, 421–427.
50 Zhang, Y., Marcillat, O., Giulivi, C., Ernster, L. and Davies, K.J.A. (1990) J. Biol. Chem. 265, 16330–16336.
51 Cleeter, M.W.J., Cooper, J.M. and Schapira, A.H.V. (1992) J. Neurochem. 58, 786–789.
52 Hammans, S.R., Sweeney, M.G., Brockington, M., Morgan-Hughes, J.A. and Harding, A.E. (1991) Lancet 337 1311–1313.