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Parkinson's disease

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Introduction

Prior to the 1960s, the clinical features and basic neuropathology of Parkinson's disease had been established, anticholinergic drugs and stereotaxic surgery were in vogue, but the illness progressed relentlessly and was a cause of miserable disability.[1] The only clues to its cause were its increasing incidence with age, the selective pathology involving pigmented brainstem neurones, especially those in substantia nigra pars compacta, and the epidemic of encephalitis lethargica with its disappearing aftermath of post-encephalitic parkinsonism.

Striatal dopamine deficiency

The discovery of selective striatal dopamine deficiency in the parkinsonian brain in the early 1960s[2,3] changed everything. The starting-point for this dramatic finding came from psychiatry. The introduction of antipsychotic drugs to control schizophrenia in the 1950s had led to the appearance of drug-induced parkinsonism. The question was how agents such as reserpine and the phenothiazines produced an akinetic-rigid syndrome indistinguishable from idiopathic Parkinson's disease. Reserpine was found to deplete the brain of monoamines including noradrenaline, serotonin and the newly discovered dopamine. Dopamine was found to be concentrated within the basal ganglia, particularly in the striatum (putamen and caudate nucleus), and the dopaminergic nigrostriatal pathway was identified. The parkinsonism induced by reserpine in animals was shown to be reversed by levodopa, as a result of restoration of striatal dopamine content. The stage was then set for the remarkable demonstration that striatal dopamine content was reduced by 80% or more in the brains of those with Parkinson's disease, due to destruction of the pigmented nigral neurones.[2,4] The logical consequence was to treat Parkinson's disease with levodopa to restore striatal dopamine levels. Initial attempts using small intravenous doses gave inconsistent responses. However, the subsequent use of high dose oral therapy produced spectacular results.

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Dopamine replacement therapy

In the late 1960s and early 1970s high dose oral levodapa therapy was established as the most effective therapy ever found for Parkinson's disease.[5–7] In particular, the most disabling symptoms of the illness, akinesia and bradykinesia, which had shown little improvement with anticholinergics and stereotaxic surgery, responded dramatically to levodopa. Seriously disabled parkinsonians, previously chair- or bed-bound by their illness, became mobile with levodopa. Most patients responded, irrespective of their disability. Parkinson's disease became the first neurodegenerative disease to be treated effectively by neurotransmitter replacement therapy.

There were problems with the introduction of levodopa. A sizeable proportion of patients were plagued by nausea and vomiting, due to dopaminergic stimulation of the vomiting centres via the area postrema, which lies outside the blood–brain barrier. This difficulty was overcome in most by the introduction of selective extracerebral decarboxylase inhibitors, such as carbidopa in Sinemet, and benserazide in Madopar. These drugs prevented the conversion of levodopa into dopamine outside the brain, so preventing nausea and vomiting, and other unwanted extracerebral effects of the drug.

Levodopa, of course, had to be converted into dopamine within the brain to exert its antiparkinsonian actions. The next advance was the design of synthetic directly-acting dopamine agonists, such as bromocriptine, to stimulate cerebral dopamine receptors. Then came agents designed to enhance and prolong the duration of action of dopamine in the brain by inhibition of its major catabolic enzyme monoamine oxidase B, for example, selegiline (Deprenyl).

By the late 1970s neurologists were armed with a range of drugs to treat Parkinson's disease – the old anticholinergics, amantadine (an antiviral agent discovered by chance to enhance dopamine release), combined levodopa and extracerebral decarboxylase inhibitors (Sinemet and Madopar), directly acting dopamine agonists (bromocriptine, lisuride, pergolide), and monoamine oxidase inhibitors (selegiline). The question was how to use them best.

At that stage, it was believed that all these agents were acting as symptomatic therapies, mostly by restoring striatal dopamine levels. None were thought to protect against the basic cause of Parkinson's disease, which was unknown.

It had also become all too obvious that there were problems with long-term levodopa therapy.[6,8,9] Many patients had developed unwanted effects of the drug, the most important of which were abnormal involuntary movements (dyskinesias) and a range of psychiatric complications (including isolated hallucinations, full-blown psychoses, and toxic confusional states). In addition, the therapeutic benefits of the drug began to wane. Fluctuations in response appeared in many long-term treated patients, initially in the form of end-of-dopa deterioration, and then more chaotic variable 'on–off' oscillations. The patient with Parkinson's disease treated for years with levodopa, became brittle, fluctuating between periods of mobility with dyskinesias, and periods of immobility with recurrence of severe parkinsonian symptoms.

In the late 1970s and 1980s, many strategies were devised to try to prevent or overcome these long-term problems. In particular, the early use of directly acting dopamine agonists, restricting and delaying levodopa therapy, was advocated as a means of reducing the risk of emergence of dyskinesias and fluctuations. Recently, longer-acting forms of levodopa have been introduced (Madopar CR and Sinemet CR) in an attempt to both smooth out fluctuations and to prevent them occurring. Those with severe fluctuations have been shown to be stabilized by continuous levodopa administration, either intravenously or by intraduodenal infusion.[10] This has led to the development of a variety of strategies for continuous dopaminergic stimulation by means of subcutaneous delivery of the water-soluble agonists lisuride[11] and apomorphine,[12] and even the transdermal administration of PHNO.[13] Overcoming these severe fluctuations has also been the stimulus to the development of brain-grafting techniques to replace striatal dopaminergic delivery either by autotransplantation of the adrenal medulla,[14] or by transplantation of human fetal nigral tissue into the denervated striatum.[15]

Genetics

In parallel to these increasingly sophisticated attempts at symptomatic therapy, there have been exciting advances in understanding the cause of Parkinson's disease, and devising, for the first time, preventative treatment. The first major step in this saga was the suggestion that inheritance, in its simplest sense, had no major role in the aetiology of Parkinson's disease. It had been assumed that genetic factors were important because at least 10–15% of cases reported relatives similarly affected. However, twin studies showed that monozygotic twins, one of whom had Parkinson's disease, were no more likely to share the illness than dizygotic twins.[16] This data was interpreted to suggest that simple mendelian genetic factors play little role in Parkinson's disease. However, re-analysis of the data has indicated that it does not exclude a genetic contribution, perhaps predisposing susceptible individuals to an environmental agent (see below).

The MPTP story

If Parkinson's disease is not primarily a genetically determined illness, what of the environment? The crucial event was the identification of MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) as a human neurotoxin capable of selectively destroying the substantia nigra, of inducing neuropathological and neurochemical changes remarkably similar to those of Parkinson's disease, and of causing a clinical illness more or less identical to Parkinson's disease itself.[17] The small cluster of young drug addicts in the USA who developed parkinsonism due to MPTP poisoning has provoked a remarkable surge of scientific work in the area. The mechanism of MPTP neurotoxicity has been worked out, and what a fascinating story transpired. MPTP itself is not the active neurotoxin; it must be converted by monoamine oxidase B, perhaps in glia, into N-methyl-4-phenylpyridine (MPP^+) via MPDP.[18] The latter is a substrate for the dopamine neuronal reuptake system, which normally inactivates dopamine released into the synaptic cleft at nigrostriatal terminals.[19] MPP^+ is thus concentrated into dopaminergic neurones. There it binds to neuromelanin.[20] This black pigment, which is a by-product of dopamine synthesis, is found in nigral nerve cells particularly in primates, and in greatest concentration in man. MPTP is most neurotoxic in primates, especially in humans. Indeed, it is now possible to create an accurate model of Parkinson's disease by administration of MPTP to sub-human primates.

How does MPP^+, concentrated in dopaminergic

neurones, and trapped there by neuromelanin, kill those nerve cells? Two complementary mechanisms have been invoked. There is solid evidence that MPP^+ is taken up and concentrated in mitochondria, where it poisons NADH-linked components of complex I of mitochondrial energy metabolism,[21] leading to depletion of ATP and alterations in cellular calcium content. MPP^+ synthesis from MPTP also may induce the formation of free radical species, imposing oxidative stress with consequent lipid membrane peroxidation.[22] Perhaps MPP^+ poisons mitochondrial complex I activity as a result of free radical damage. In this respect, dopamine catabolism by monoamine oxidase B itself generates hydrogen peroxide, thereby imposing oxidative stress upon nigral neurones.[23] The latter are normally protected from such an insult by a variety of enzymes (for example, superoxide dismutase, catalase) and free radical scavengers (for example, reduced glutathione, vitamins E and C). However, with normal ageing these mechanisms may be insufficient, so that dopaminergic nigral neurones may have a tendency to 'self-destruct'.

Environmental toxins as the cause of Parkinson's disease

Discovery of MPTP suggested that Parkinson's disease might be due to exposure to some similar toxic agent in the environment. The simplest explanation was that those who developed the idiopathic disease had inadvertently absorbed, by ingestion, inhalation or some other means, such a toxin. Exposure to such an agent might arise through work, residence, lifestyle or hobbies. On this theory, exposure to such a toxin would be uncommon, for only a minority develop the disease.

MPTP is not a substance normally present in the environment. Exposure has been restricted to a few individuals in laboratories and chemical plants and to some drug addicts. However, the nucleus of MPTP responsible for its toxicity is a common chemical moiety and so may occur in many other substances. Since the discovery of MPTP, over 100 analogues have been described, some of which are substrates for monoamine oxidase-B giving rise to toxic pyridinium species.[24,25] However, none has been identified as possible causes of Parkinson's disease.

The search for such an uncommon environmental toxin has been based upon epidemiological techniques of geographical incidence and prevalence estimates and case control studies.[26] Such surveys have produced a few soft leads. There may be significant factors associated with industrialization or with the use of agrochemicals. The early exposure to a rural environment coupled to the use of well water for drinking appears associated with the early onset of the disease. However, the differences in prevalence rates between countries are relatively small. Also epidemics of Parkinson's disease or clusters of the illness in communities do not seem to occur. This evidence, such as it is, does not suggest that an uncommon toxin in the environment is solely responsible for Parkinson's disease.

An alternative hypothesis is that there exists one or more environmental toxins to which we are all exposed but some of us cannot cope with. Those who develop Parkinson's disease might have an impaired ability to detoxify such potentially damaging agents. Recently, it has been suggested that there is an impairment of sulphur metabolizing enzymes which either renders individuals susceptible to toxins, or depletes the body of sulphydryl groups necessary for the deactivation of toxic oxygen species.[27,28] The inability to handle relevant toxins may be an inherited trait, raising again the role of genetics in Parkinson's disease.

Now it is argued that the original twin studies (see above) were inconclusive, and that a genetic component to Parkinson's disease remains feasible.[29] There are occasional families in which clinical Parkinson's disease, with pathology identical to that seen in the idiopathic disorder, is inherited as an autosomal dominant trait.[30] Other family studies also support inheritance of Parkinson's disease as an autosomal dominant trait with relatively low penetrance.[31] Analysis of familial Parkinson's disease provides no evidence for mitochondrial, X-linked or polygenic inheritance.[31] Thus, a genetically based susceptibility to toxins could contribute to the occurrence of Parkinson's disease. Alternatively, a genetic defect might generate endogenous neurotoxins, or impair the ability to deal with toxic substances produced in the brain through normal metabolism. The normal metabolism of dopamine in the brain results in potentially neurotoxic compounds, not only as a result of its breakdown by monoamine oxidase to its metabolites homovanillic acid and dihydroxyphenyl acetic acid, but also through its auto-oxidation to neuromelanin. Both routes generate free radicals and other reactive oxygen species that might induce oxidative stress.[32–34]

Is oxidative stress responsible for nigral and neuronal death in Parkinson's disease?

Recent studies on the substantia nigra at autopsy in Parkinson's disease have established evidence of increased lipid peroxidation at the time of death. The nigra contains increased levels of malondialdehyde[35] and hydroperoxides,[36] despite lower

concentrations of substrate polyunsaturated fatty acids. These findings point to some continuous toxic process involving oxidative stress occurring in the parkinsonian substantia nigra. Indeed, there is also an increase in nigral mitochondrial superoxide dismutase activity, which can be interpreted to reflect a compensation for increased superoxide production,[37] and depletion of reduced glutathione,[38] which might indicate excessive production of hydrogen peroxide.

Another pertinent observation is that there is accumulation of iron in substantia nigra in Parkinson's disease.[39–41] If this excess nigral iron is in a reactive form, either free or associated with low molecular weight complexes, it would be a potent stimulus to oxidative stress by promoting the formation of the hydroxyl radical.

What is not known at the present time is whether these indices of oxidative stress in the substantia nigra in Parkinson's disease, and of iron accumulation, are primary events related to the cause of the disease, or secondary consequences of another attack on nigral neurones. Such an alternative is the recently discovered abnormality of mitochondrial complex 1 activity in the substantia nigra in Parkinson's disease.

Mitochondrial abnormalities in Parkinson's disease

The discovery that MPTP ultimately destroys the substantia nigra by inhibition of mitochondrial complex 1 activity was the stimulus to examining mitochondrial function in Parkinson's disease. Such studies in post-mortem brain have established a deficiency of complex 1 (NADH CoQ_1 reductase) activity in the substantia nigra in Parkinson's disease.[42,43] Complex 1 deficiency is confined to the substantia nigra, and is not present in caudate nucleus, putamen, globus palidus, cerebral cortex or cerebellum.[44] Activities of the other respiratory chain complexes were not significantly different from age-matched controls in any of the areas of the brain examined, including the substantia nigra. Complex 1 activity in the substantia nigra of patients with multiple system atrophy was normal,[44] suggesting that this defect is not simply the result of neuronal degeneration. Also, the multiple system atrophy patients had been taking L-dopa in amounts comparable to those used to treat the Parkinson's disease patients, so the abnormality in complex 1 activity in Parkinson's disease is unlikely to be due to L-dopa therapy.

Again, the problem arises as to whether the mitochondrial complex 1 deficiency in the substantia nigra in Parkinson's disease is a primary cause of dopaminergic cell death, or merely a secondary phenomenon, perhaps due to oxidative stress. The relationship between complex 1 deficiency and oxidative stress is bilateral. Abnormalities of complex 1 activity can generate oxidative stress through the production of superoxide. Conversely, free radicals can impair respiratory chain activity; complex 1 seems particularly susceptible in this respect. Also, there is argument as to whether abnormalities of mitochondrial complex 1 activity are restricted to the substantia nigra in Parkinson's disease, or whether they occur more widely in other body tissues including platelets and muscle. Further work is required to establish the regional selectivity of mitochondrial changes in Parkinson's disease, and whether they are a primary event or a secondary consequence of the illness.

Implications for new strategies to treat Parkinson's disease

The first attempt to treat the cause of Parkinson's disease, rather than to provide symptomatic relief, came with the suggestion that selegiline might exert a neuroprotective effect in this illness. The theories behind this concept were that: (1) deprenyl prevents the neurotoxicity of MPTP by inhibition of monoamine oxidase-B thereby inhibiting the formation of the active neurotoxin MPP^+; and (2) inhibition of monoamine oxidase-B by deprenyl would reduce toxic oxidative stress resulting from dopamine metabolism in the brain. The large DATATOP study[45] in the United States has established beyond reasonable doubt that the administration of deprenyl at the time of diagnosis of Parkinson's disease significantly delays the need for levodopa therapy. Whether this is due to a true neuroprotective effect against the underlying pathological cause of Parkinson's disease, or to some mild symptomatic benefit from selegiline is argued. This argument is likely to be resolved by further analysis of follow-up studies from the DATATOP investigation. Whatever the outcome, it represents the first attempt to treat the cause of Parkinson's disease.

The biochemical findings found at autopsy in the brains of those with Parkinson's disease provides alternative scenarios for attacking the cause of this illness. Thus, agents that might enhance defence against oxidative stress, or protect against iron toxicity, or induce normal complex 1 activity all might play a role in slowing or stopping progression of the underlying pathology of Parkinson's disease. It does not necessarily matter whether any of these events are primary causes of the illness, or merely secondary consequences. Whatever causes Parkinson's disease might set in train a cascade of events leading to accelerating nigral neuronal cell death. Prevention of such a secondary cascade might slow progression of the illness, even though it would not be attacking the underlying cause of the disease. The next decade is likely to see further attempts to influence the natural history of Parkinson's disease, using drugs designed to reverse the abnormalities discovered in the brain at autopsy.

References

1. Hoehn, M.M. & Yahr, M.D. Parkinsonism: onset, progression and mortality. *Neurology* 1967, **17**: 427–442.
2. Bernheimer, H., Birkmeyer, W., Hornykiewicz, O. *et al.* Brain dopamine and the syndromes of Parkinson and Huntingdon. *J Neurol Sci* 1973, **20**: 415–445.
3. Hornykiewicz, O. Dopamine and brain function. *Pharmacol Rev* 1966, **18**: 925–964.
4. Agid, Y., Javoy-Agid, F. & Ruberg, M. Biochemistry of neurotransmitters in Parkinson's disease. In: Marsden, C.D. & Fahn, S. (eds) *Movement Disorders 2.* Butterworth, London, 1987, pp. 166–230.
5. Cotzias, G.C., Van Woert, M.H. & Schiffer, L.M. Aromatic amino acids and modification of parkinsonism. *N Engl J Med* 1967, **276**: 374–378.
6. Marsden, C.D. & Parkes, J.D. Success and problems of long-term levodopa therapy in Parkinson's disease. *Lancet* 1977, **1**: 345–349.
7. Lees, A.J. L-dopa treatment and Parkinson's disease. *Q J Med* 1986, **59**: 535–547.
8. Marsden, C.D. & Parkes, J.D. On-off effects in patients with Parkinson's disease on chronic levodopa therapy. *Lancet*, 1976, **1**: 292–296.
9. Nutt, J.G., Woodward, W.R., Hammerstad, J.P., Carter, J.P. & Anderson, J.L. The on-off phenomenon in Parkinson's disease. *N Engl J Med* 1984, **310**: 483–488.
10. Quinn, N., Parkes, J.D. & Marsden, C.D. Control of on/off phenomenon by continuous intravenous infusion of levodopa. *Neurology* 1984, **34**: 1131–1136.
11. Obeso, J.A., Luquin, M.R. & Martinez-Lage, J.M. Lisuride infusion pump: a device for the treatment of motor fluctuations in Parkinson's disease. *Lancet* 1986, **1**: 467–470.
12. Frankel, J.P., Lees, A.J., Kempster, P.A. & Stern, G.M. Subcutaneous apomorphine in the treatment of Parkinson's disease. *J Neurol Neurosurg Psychiat* 1990, **53**: 96–101.
13. Coleman, R.J., Lange, K.W., Quinn, N.P. *et al.* The antiparkinsonian actions and pharmacokinetics of transdermal (+)-4-propyl-9-hydroxynapthoxazine (PHNO): preliminary results. *Movement Disord* 1989, **4**: 129–138.
14. Madrazo, I., Drucker-Colin, R., Diaz, V., Martinez-Mata, J., Torres, C. & Becerill, J.J. Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. *N Engl J Med* 1987, **316**: 831–834.
15. Lindvall, O., Brundin, P., Widner, H. *et al.* Grafts of foetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* 1990, **247**: 574–577.
16. Ward, C.D., Duvoisin, R.C., Ince, S.E., Nutt, J.D., Eldridge, R. & Calne, D.B. Parkinson's disease in 65 pairs of twins and in a set of quadruplets. *Neurology* 1983, **33**: 815–824.
17. Ballard, P.A., Tetrud, J.W. & Langston, J.W. Permanent human parkinsonism due to MPTP. *Neurology* 1985, **35**: 949–956.
18. Chiba, K., Trevor, A. & Castagnoli, N. Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem Biophys Res Commun* 1984, **120**: 574–578.
19. Javitch, J.A., D'Amato, R.J., Strittmatter, S.M. & Snyder, S.H. Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc Natl Acad Sci USA* 1985, **82**: 2173–2177.
20. D'Amato, R.J., Benham, D.F. & Snyder, S.H. Characterisation of the binding of N-methyl-4-phenylpyridine, the toxic metabolite of the parkinsonian neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine to neuromelanin. *J Neurochem* 1987, **48**: 653–658.
21. Vyas, I., Heikkila, R.E. & Nicklas, W.J. Studies on the neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Inhibition of NAD-linked substrate oxidation by its metabolite 1-methyl-4-phenylpyridinium. *J Neurochem* 1986, **46**: 1501–1507.
22. Rossetti, Z.L., Sotgiu, A., Sharp, D., Hadjiconstantinou, M. & Neff, N.H. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and free radicals in vitro. *Biochem Pharmacol* 1988, **37**: 4573–4574.
23. Cohen, G. & Spina, M.B. Deprenyl suppresses the oxidant stress associated with increased dopamine turnover. *Ann Neurol* 1989, **26**: 689–690.
24. Ramsay, R.R., Youngster, S.K., Nicklas, W.J. *et al.* Structural dependence of the inhibition of mitochondrial respiration and of NADH oxidase by MPP^+ analogs and their energised accumulation of mitochondria. *Proc Natl Acad Sci USA* 1989, **86**: 9168–9172.
25. Youngster, S.K., Gluck, M.R., Heikkila, R.E. & Nicklas, W.J. 4'-Akylated analogs of MPP^+ are potent inhibitors of mitochondrial respiration. *Biochem Biophys Res Commun* 1990, **169**: 758–764.
26. Tanner, C.M. The role of environmental toxins in the etiology of Parkinson's disease. *Trends Neurosci* 1989, **12**: 49–54.
27. Steventon, G.B., Heafield, H.T.E., Waring, R.H. & Williams, A.C. Xenobiotic metabolism in Parkinson's disease. *Neurology* 1989, **39**: 883–887.
28. Waring, R.H., Sturman, S.G., Smith, M.C.G. *et al.* S-methylation in motor neuron disease and Parkinson's disease. *Lancet* 1989, **i**: 356–357.
29. Johnson, W.G., Hodge, S.E. & Duvoisin, R. Twin studies and the genetics of Parkinson's disease–a reappraisal. *Movement Disord* 1990, **5**: 187–194.
30. Golbe, L.I., Di Iorio, G., Bonavita, V., Miller, D.C. & Duvoisin, R.C. A large kindred with autosomal dominant Parkinson's disease. *Ann Neurol* 1990, **27**: 276–282.
31. Maraganore, D.M., Harding, A.E. & Marsden, C.D. A clinical and genetic study of familial Parkinson's disease. *Movement Disord* 1991, **6**: 205–211.
32. Graham, D.G. Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Mol Pharmacol* 1978, **14**: 633–643.
33. Cohen, G. Oxygen radicals and Parkinson's disease. In: Halliwell, B. (ed) *Oxygen Radicals, and Tissue Injury.* Federation of American Societies for Experimental Biology, Bethesda, 1987.
34. Seiden, L.S. & Vosmer, G. Formation of 6-hydroxydopamine in caudate nucleus of the rat brain after a single large dose of methylamphetamine. *Pharmacol Biochem Behav* 1984, **21**: 29–31.
35. Dexter, D.T., Carter, C.J., Wells, F.R. *et al.* Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J Neurochem* 1989, **52**: 381–389.
36. Dexter, D.T., Holley, A.E., Flitter, W.D. *et al.* Increased levels of lipid hydroperoxides but no evidence for increased free radical formation in the parkinsonian substantia nigra. *J Neurochem* 1992 (in press).
37. Saggu, H., Cooksey, J., Dexter, D. *et al.* A selective increase in particulate superoxide dismutase activity in Parkinsonian-substantia nigra. *J Neurochem* 1989, **53**: 692–697.
38. Perry, T.L., Godin, D.V. & Hansen, S. Parkinson's disease: A disorder due to nigral glutathione deficiency? *Neurosci Lett* 1982, **33**: 305–310.
39. Dexter, D.T., Wells, F.R., Agid, F. *et al.* Increased nigral iron content in post-mortem parkinsonian brain. *Lancet* 1987, **ii**: 219–220.
40. Riederer, P., Sofic, E., Rausch, W.-D. *et al.* Transition metals, ferritin, glutathione, and ascorbic acid in parkinsonian brains. *J Neurochem* 1989, **52**: 515–520.
41. Dexter, D.T., Carayon, A., Javoy-Agid, F. *et al.* Alterations in the levels of iron and ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. *Brain* 1991, **114**: 1953–1975.
42. Schapira, A.H.V., Cooper, J.M., Dexter, D., Jenner, P., Clark, J.B. & Marsden, C.D. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* 1989, **1**: 1269.

43. Schapira, A.H.V., Cooper, J.M., Dexter, D., Clark, J.B., Jenner, P. & Marsden, C.D. Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem* 1990, **54**: 823–827.
44. Schapira, A.H.V., Mann, V.M., Cooper, J.M. *et al.* Anatomic and disease specificity of NADH CoQ reductase (complex 1) deficiency in Parkinson's disease. *J Neurochem* 1990, **55**: 2142–2145.
45. Parkinson Study Group. Effect of deprenyl on the progression of disability in early Parkinson's disease. *N Engl J Med* 1989, **321**: 1364–1371.