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XENOBIOTICS LINKED TO PARKINSON'S DISEASE.

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1. Introduction.

Parkinson's Disease (PD) is manifest as a movement disorder and is one of the most common neurodegenerative conditions in the world, affecting 2% of the population over the age of 65. At present, there is no cure and drug treatment is palliative with many undesirable side effects. The disease was first described in 1817 by William Parkinson, but the final answer as to what initiates the disease process has still not been elucidated. Hundreds of papers in the last decade have been published that investigate some aspect of the pathogenesis of PD, and the mechanisms by which neurodegeneration proceeds seem fairly well defined. However, the initiating factors in PD are still a matter of intense debate.

In this review, the major advances in the field of PD research that have occurred in recent years will be discussed. The evidence behind the claims that certain xenobiotics can directly cause PD will be evaluated in detail. Finally, there will be a discussion about what conclusions can reliably be drawn from the data available, the pros and cons of the techniques currently employed in PD research, where new lines of inquiry can be drawn from and what they may possibly be.

2. Parkinson's Disease.

2.1. Classification of Parkinson's Disease.

Parkinson's disease (PD) in man can be defined histologically as the loss of pigmented neurones in the substantia nigra (see 2.3.). The presentation of 1 or more of the clinical symptoms of PD is known as Parkinsonism.

There are three main types of PD: idiopathic, drug-induced and familial. All three forms present with the clinical symptoms described below. Drug-induced PD can occur after prolonged administration of high doses of phenothiazines, thioxanthenes or butyrophenones (e.g. chlorpromazine, haloperidol). These agents are neuroleptic drugs used in the treatment of schizophrenia and work by antagonising the D₂ class of dopamine receptor in the limbic forebrain¹. The effects are almost totally reversed by the co-administration of anticholinergic agents. The familial form can be traced to genetic malfunctions. It is very rare and no other type of PD has been proven to be heritable. The idiopathic form is diagnosed when no obvious aetiological factor can be identified. A fourth form, postencephalic PD, was more prevalent in the mid 20th century after the epidemics of the disease encephalitis lethargica in the early 1920's².

2.2. Clinical Symptoms of Parkinson's Disease.

There are four cardinal features in all types of PD³:

i. Tremor.

This is defined as rapid contractions of opposing muscle groups, most often observed in the hands when it produces a characteristic 'pill-rolling' action (where the tip of the thumb moves back and forth over the lateral surface of the index finger). The

tremor can also involve facial, jaw and tongue muscles. A tremor usually worsens during periods of anxiety or stress, and may decrease during activity.

ii. Rigidity.

All the muscle groups in the affected limbs are rigid, producing stiffness of movement. This can be detected during a clinical examination by rotating an affected limb through 360°. The rigidity causes an effect rather like trying to turn a sticking cog wheel.

iii. Bradykinesia.

Literally, this means 'slow movement', and is probably the most disabling of the symptoms, mainly affecting the facial and axial muscles. It results in a fixed facial expression, slow monotonous speech, decreased blinking and decreased swallowing which causes dribbling. Other manifestations are reduced arm swinging when walking and trunk rigidity. The most extreme form of bradykinesia is sudden freezing of movement, known as the "on-off phenomena", often precipitated by an attempt to change direction when walking or when standing up from a seated position.

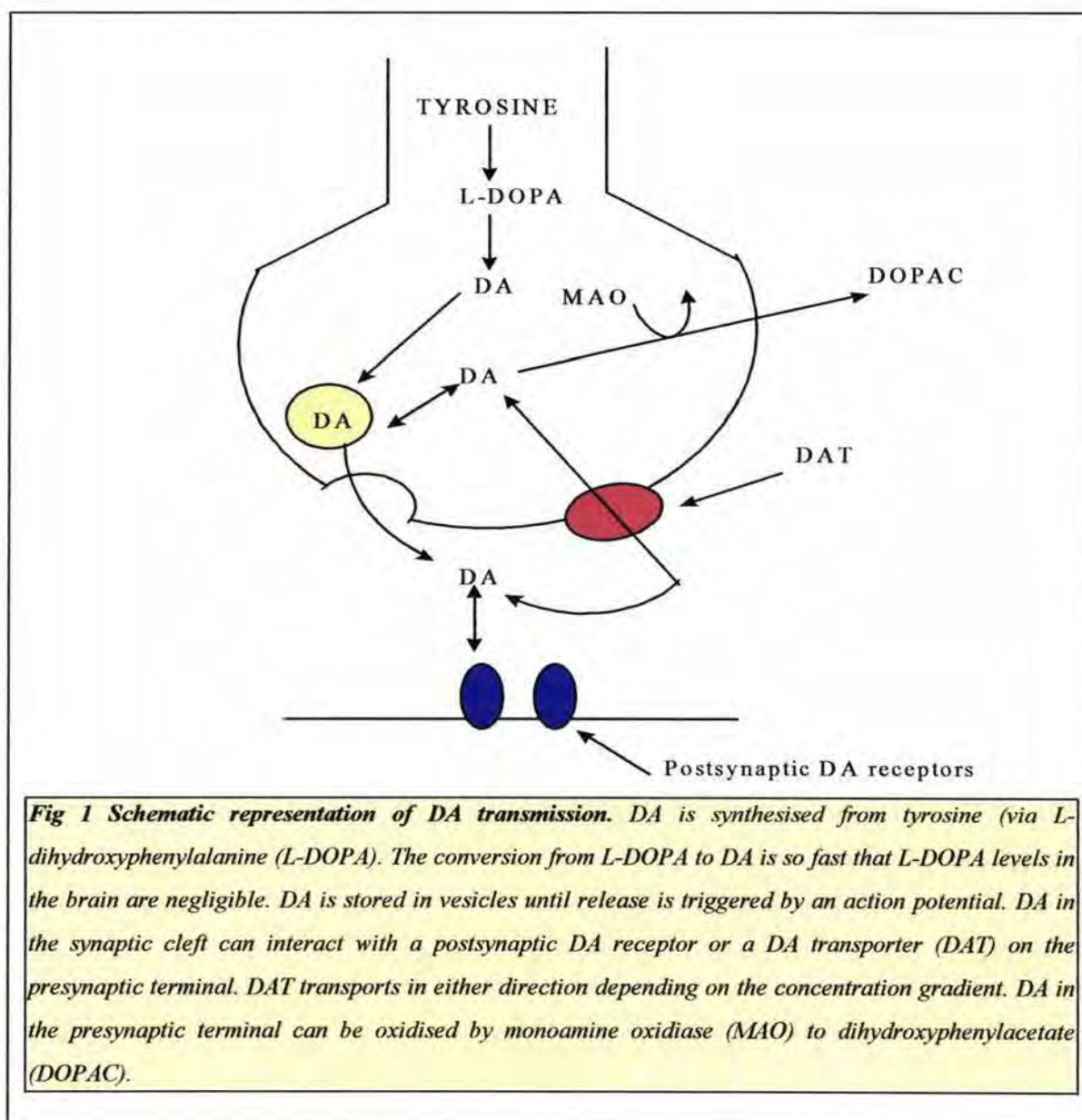
iv. Loss of postural reflexes.

Loss of these innate reflexes makes the patient aware of the constant adjustments required to remain upright, and consequently they feel off balance when standing and moving around. This can often mean that tasks such as turning around or changing direction are made virtually impossible.

Other possible symptoms include depression, personality change, dementia, impaired olfaction, cognitive deficits and pain.⁴

2.3. The PD brain. ^{1,5}

The region of the brain that is most severely affected by Parkinson's disease is the substantia nigra (SN). This is a subcortical nucleus, the fibres of which synapse in the basal ganglia and consequently play a major role in the control of voluntary movements mediated by this region. When stimulated, SN neurones release dopamine (DA) which acts as an inhibitory neurotransmitter. The balance between this inhibitory input and the excitatory input provided by acetylcholinergic neurones is what normal function of the basal ganglia depends upon. Fig 1 shows a DA synapse.



In all forms of PD, there is a loss of pigmented cells in the SN and the remaining neurones contain what are described as Lewy bodies. The pigmented cells are DA-releasing cells, the pigment originating from a by-product of DA synthesis called neuromelanin. Lewy bodies are circular, cytoplasmic lesions, 5-25 μm in diameter, the major component being the protein α -synuclein¹². The neuronal cell loss leads to a substantial decrease in the levels of DA: a 70%-80% decrease is observed in patients with mild PD, and in excess of 90% depletion can be found in those with more severe symptoms.

DA loss in the SN is the major biochemical event that occurs in the PD brain. However, typical PD also involves the degeneration of DA neurones found in other regions such as the hypothalamus and the mesolimbic and mesocortical systems. Other monoamine neurones, such as noradrenergic neurones in the locus ceruleus and serotonin neurones, are also lost. There is a depletion of the neuroactive peptides, substance-P, CCK-8, somatostatin, enkephalin and neurotensin. CCK-8 and neurotensin coexists with DA in the substantia nigra¹, so depletion may cause a direct effect on the function of this area. However this is largely speculative and little attention has been paid to the initiation and consequence of this monoamine and neuroactive peptide loss in the development of PD.

2.4. Current treatments for PD.

Previous to the discovery of the involvement of DA in PD, treatment involved using an anticholinergic agent which, due to the interactions between the DA and non-DA systems, was moderately successful. Current therapies involve replacing DA in the brain by pharmacological means or by grafting dopaminergic cells into the substantia nigra. Theoretically, there are 4 main pharmacological targets for treatment: DA substrate availability, DA agonists, monoamine oxidase inhibition (MAOIs) and re-uptake inhibition. In practice, the most successful treatment is with L-DOPA, a substrate of DA synthesis. Unfortunately, some of the possible side effects of L-DOPA therapy are as debilitating and bizarre as the PD itself. They include involuntary movements, hallucinations, depression and bradykinetic episodes. L-DOPA cannot be administered concomitantly with tricyclic or

MAOI type anti-depressants. There are directly acting and indirectly acting DA agonists which are used but only when L-DOPA therapy is ineffective¹.

2.5. Epidemiology.

PD is found throughout the world and affects all cultures and social classes. The most consistent epidemiological observations when investigating the occurrence of PD in the population are increased risk with ageing, and an inverse relationship between cigarette smoking and the development of PD. The fact that an as yet unidentified component of cigarette smoke seems to confer some protection against developing the disease has not yet been explained or extensively studied. Neither the behavioural characteristics peculiar to smokers, nor the selective increased survival of non-smokers can explain the association⁶.

The discovery in the early '80s that a contaminant of the synthetic street drug "new heroin", 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), could induce symptoms very reminiscent of PD caused a surge in interest in possible environmental influences in the development of PD (see 4.1.). Soon after, epidemiological studies showed there to be a link between an increased incidence of PD and an agricultural lifestyle. Some reports directly link the increase in PD to herbicides and pesticides, and in particular paraquat⁷ (see 4.2.). The association with paraquat is more significant when its structural similarity to MPTP is taken into account. Others confirmed the positive correlation but ruled out the involvement of herbicides and pesticides⁸. These conflicting reports suggest that a gene/environment interaction may well be very important in the aetiology of PD. Purely genetic factors seem to be significant only in juvenile onset PD (see 3.1.).

There are several problems when investigating the environmental links to the aetiology of PD via epidemiological means. Mostly they are methodological difficulties, such as assessment of the level of environmental exposure. Due to the increasing evidence that there is an interaction between genes and environmental exposure to certain toxins, defining populations to study is becoming increasingly more complex⁶.

3. Aetiology of PD.

3.1. Genetic factors.

Current understanding of the aetiology of PD indicates that purely genetic PD is rare, and causes early onset of the disease (before the age of 50)⁹. A small percentage of cases are familial and are inherited in an autosomal-dominant manner¹⁰ but most cases of PD are idiopathic. The study of several kindreds with early onset PD revealed missense mutations in the gene for the protein α -synuclein¹¹, the function of which is unknown. Lewy bodies stain very strongly for it in all cases of PD, the staining being more extensive than for ubiquitin, previously regarded as the most sensitive marker for Lewy bodies¹².

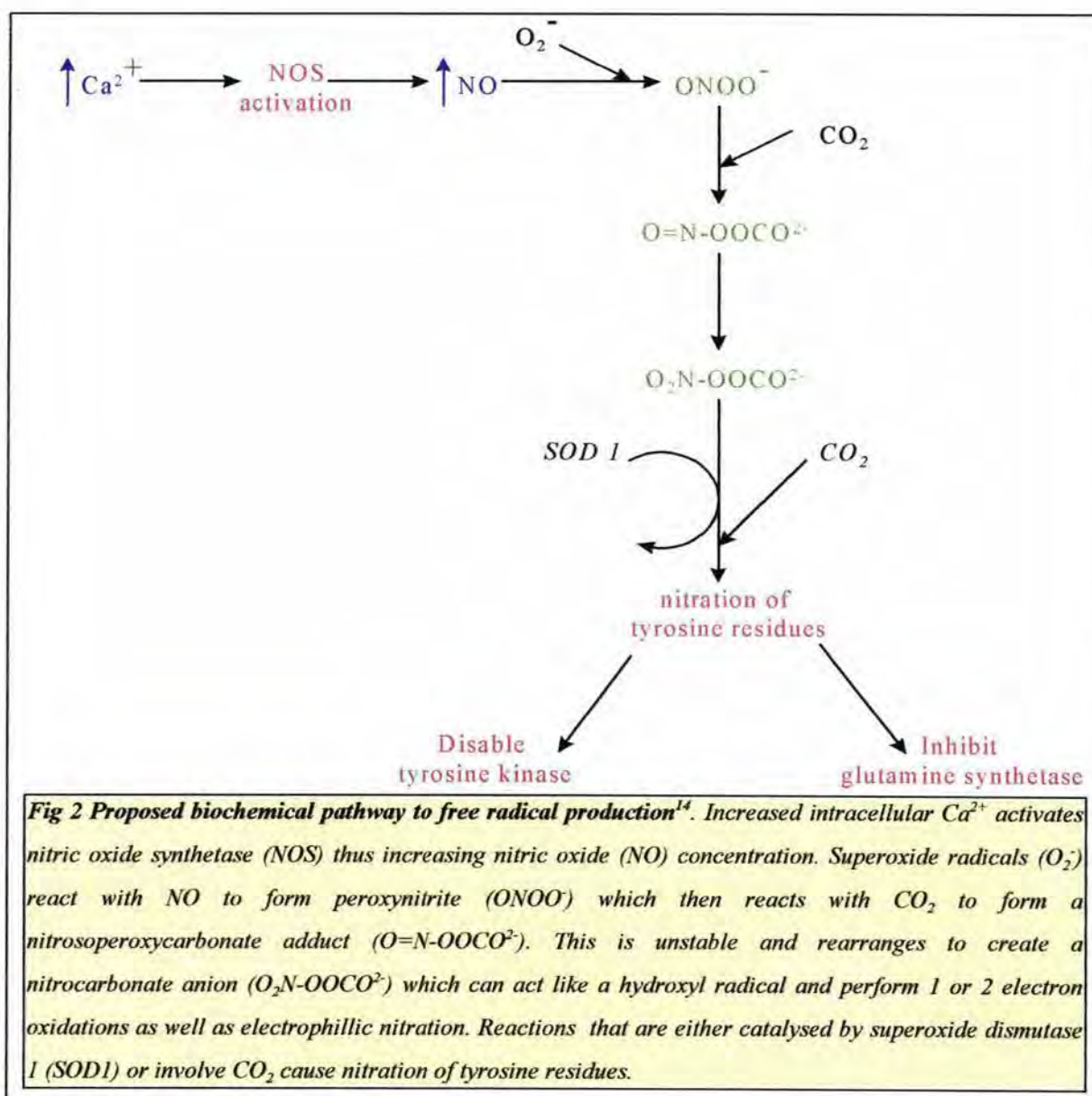
3.2. Non-genetic factors

Oxidative damage caused by free radicals is currently held to be the main pathogenic factor in idiopathic PD. Immunohistochemical studies of PD brains show an increase in 4-hydroxynonenal protein adducts in substantia nigra neurones (4-hydroxynonenal adducts are a biomarker for oxidative stress)¹³. This indicates that free radicals can cause neurodegeneration, but does not reveal where the free radicals originate from. To answer this, the oxidative processes within the cell must be scrutinised, which entails studying the mitochondria of patients with PD.

3.2.1. Mitochondrial defects.

Mitochondrial defects have been identified that would both initiate and exacerbate the production of free radicals. Cybrid cell lines are used when studying potential mutant mtDNA, a technique whereby specimen mitochondria are transferred into mtDNA deficient cells so that it can be determined if defects in the electron transfer chain are attributable to mutant mtDNA¹⁴. Utilising this technique, the reduced complex I (NADH CoQ₁ reductase) activity that was observed in PD nigral neurones¹⁵ has been traced to mtDNA defects, which

compromises electron transport chain function in the mitochondria, leading to a reduction in ATP production. In neurones, a decrease in energy production causes partial depolarisation, as the maintenance of ionic gradients across the cell membrane is an active process. This depolarisation causes activation of excitatory amino acid receptors by local concentrations of the amino acid neurotransmitter glutamate, which in turn causes an increase in intracellular Ca^{2+} ¹⁶. Increased intracellular Ca^{2+} induces free radical generation¹⁷ causing excitotoxic cell death by apoptosis¹⁸.



An increase in intracellular Ca^{2+} activates nitric oxide synthetase (NOS) causing an increase in the intracellular concentrations of NO. Defects in the electron transport chain can cause the production of superoxide radicals (O_2^-), and these react with NO to form peroxynitrite (ONOO^-). Fig 2 summarises what reactions are thought to occur leading up to the observation of free radical damage¹⁶.

The nitration events have two major effects. Glutamine synthetase is crucial in the synthesis of glutamate in glial cells¹. As most glutamate in the brain is synthesised *in situ* and not transported across the blood/brain barrier, inhibition of glutamine synthetase by nitration of tyrosine residues reduces the amount of this neurotransmitter available in the brain. The disabling of tyrosine kinases means that they no longer have the ability to phosphorylate tyrosines which is often a critical process in many cell-cycle and cell-signalling events. This may cause the cell to undergo apoptosis.

Free radicals can also directly cause cell damage by interacting and degrading membrane phospholipids¹⁸.

Much of the evidence supporting the role of oxidative damage in the pathogenesis of PD has come from studying the effects of the mitochondrial toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This compound is discussed in detail later.

4. Chemicals linked to Parkinson's disease.

Ever since the findings that people from some rural communities are at a higher risk of developing PD than other rural and non-rural communities (see 2.4.) and that some synthetic substances can cause some of the symptoms seen in PD (Parkinsonism), the hunt for xenobiotics that could potentially act as pathogenic agents for PD has proceeded in earnest. Many substances have been suggested, the most significant will be discussed here. There will not be a discussion of the Parkinsonian effects of the neuroleptic drugs.

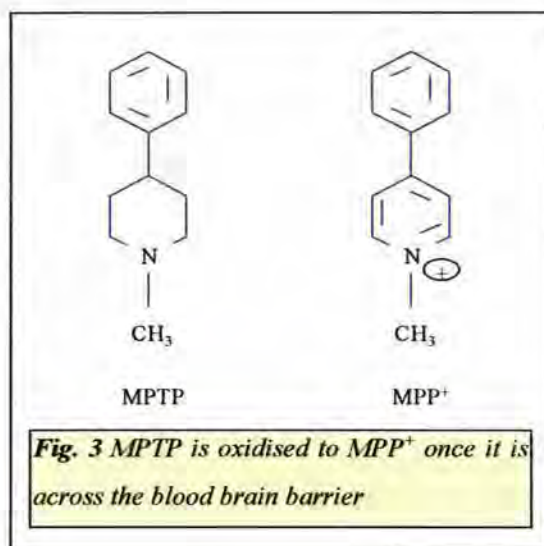
4.1. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

4.1.1. Discovery

In 1982, Parkinsonism suddenly appeared in a group of relatively young people in the San Francisco area. It's appearance was traced to the use of the designer drug "new heroin" which had been self-administered by all the subjects, and this was being manufactured in a clandestine laboratory. Some batches of the drug were defective and were found to contain almost pure MPTP, after the manufacturer (a university student) tried to take a short cut in the manufacturing process. It was soon confirmed that MPTP was toxic to dopaminergic neurones in humans, non-human primates and mice. Soon after that 1-methyl-4-phenylpyridinium (MPP^+), a product of the oxidation of MPTP, was identified as the neurotoxic species¹⁹. Fig 3 shows the structure of MPTP and MPP^+ .

4.1.2. Bioactivation.

MPTP is an uncharged compound and so is lipophilic. This means it is able to penetrate the membrane which comprises the blood/brain barrier. From here it is taken up into astrocytes where it is oxidised to 1-methyl-4-phenyl-1,2,3,6-dihydropyridium ($MPDP^+$) by monoamine-oxidase-B (MAO-B). $MPDP^+$ is then further oxidised to MPP^+ and released by the

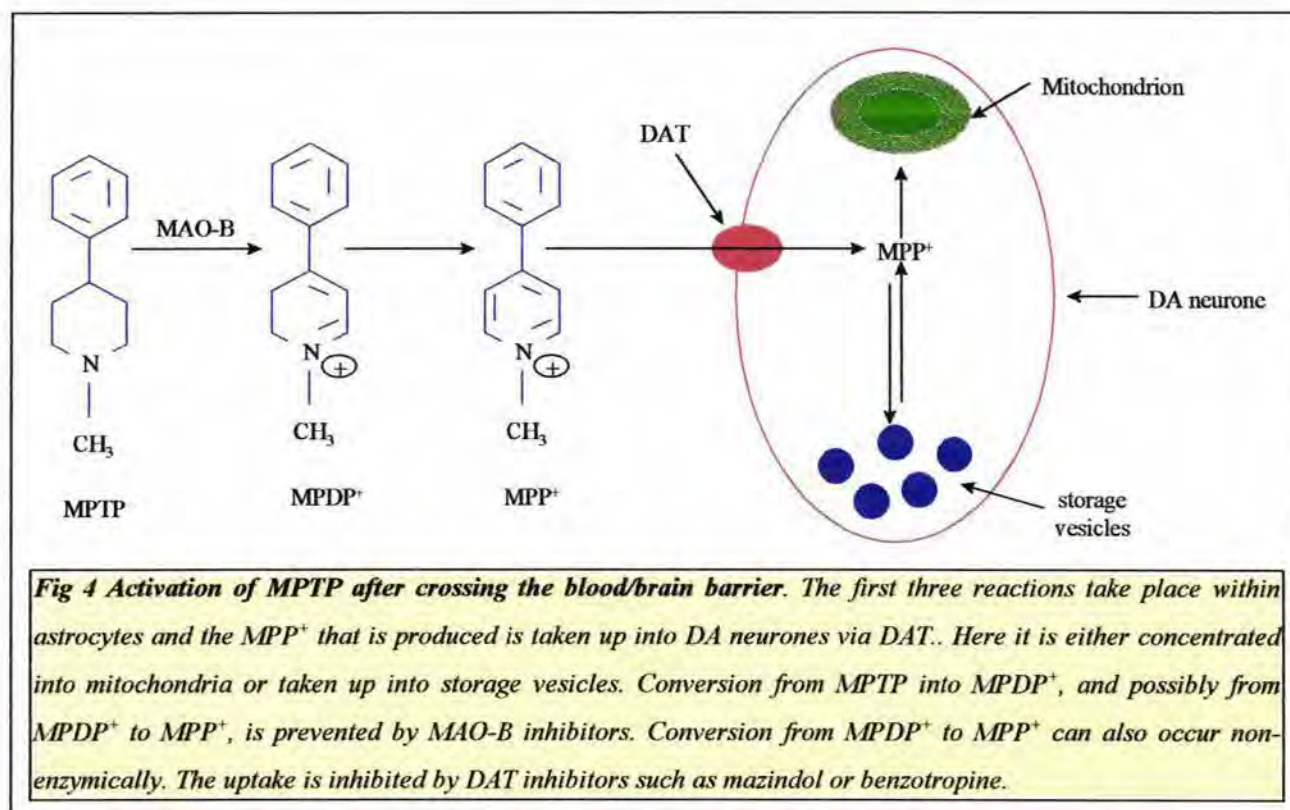


astrocytes²⁰. The role of MAO-B in this process is confirmed by the fact that MPTP toxicity is prevented by MAO-B specific inhibitors such as L-deprenyl²⁰. MPP⁺ is taken up by DA neurones via the presynaptic DA transporter (DAT)¹. This process is halted by DAT inhibitors such as benzotropine and the GBR series, and is not present in mice deficient in DAT²¹. This may answer the question as to why the neurotoxicity seen with MPTP is DA neurone specific: the reactive species is selective for DA neurones because it is taken up specifically by DAT. Another feature of DA neurones which has often been associated with PD and MPTP toxicity is neuromelanin, a by-product of DA synthesis. This component increases with age, an attribute which would indicate that it played a major role in the aetiology of PD. MPP⁺ binds to it and accumulates in pigmented areas (mainly the substantia nigra and the locus ceruleus) and so may act as a MPP⁺ store²². Blocking MPP⁺ binding to neuromelanin causes a slight reduction in the symptoms of MPTP toxicity²³. The role neuromelanin plays in neurodegeneration is yet to be confirmed, especially as only the substantia nigra is affected by MPTP, and other neuromelanated areas (such as the ventral tegmental area) are not affected²³.

Once inside the neurone, MPP⁺ is concentrated into mitochondria where it exerts its toxicity. When stimulated, MPTP-treated cells can release small amounts of MPP⁺ which suggests that some is also taken up into storage vesicles²⁴. Fig 4 summarises these reactions.

4.1.3. Mechanism of MPTP toxicity.

Once inside the neurone, MPP⁺ is concentrated into mitochondria by a transport process. Initial studies with MPP⁺ analogues showed that different analogues were concentrated to



very different levels which was interpreted as indicating that the process was structurally specific and therefore involved a carrier²⁵. However, subsequent studies indicate that the process was non-saturable and so happens passively, powered only by the electrical gradient that exists across the inner mitochondrial membrane²⁶.

Inside the mitochondria, MPP⁺ can have a number of effects. One is to inhibit the replication of mitochondrial DNA (mtDNA), which decreases the total amount of mtDNA in the cell²⁷. Normally there are between 10³ and 10⁴ copies of mtDNA per cell; any reduction in this number compromises the ability of the cell to ensure adequate expression of proteins encoded by mtDNA. Another consequence of MPP⁺ presence in mitochondria is to open the mitochondria transition pore (MTP). This causes release of Ca²⁺ and cytochrome C which can

then initiate apoptosis²⁸. MPP⁺ also causes inhibition of complex I activity, causing a reduction in ATP production and production of free radicals (see 3.2.1.)

4.1.4. Quality of the evidence.

Since the discovery that MPTP causes Parkinsonism, the challenge has really been to see how MPTP toxicity is initiated, and then how similar this mechanism is to the pathology of idiopathic PD. MPTP toxicity is highly species specific and so the choice of test animal is critical. Nonhuman primates and C57 black mice are the most susceptible species (together with humans) and all of these species possess neuromelanin which binds MPP⁺ and may serve as a MPP⁺ store. Rats and other resistant species lack neuromelanin. Rats also have high MAO-B activity in the capillary wall, which would cause the oxidation of MPTP to MPP⁺ before it crossed the blood/brain barrier, thus preventing it from reaching the site of action.

Much of the current evidence points to the fact that the aetiology of MPTP toxicity and idiopathic PD do have many common mechanisms, for example both conditions show evidence of oxidative damage through inhibition of complex I activity in mitochondria. However, there are some questions still to be resolved before MPTP can be hailed as a perfect model for PD. Firstly, what effects do the other consequences of MPTP toxicity have (e.g. inhibition of mtDNA), and how do they cause MPTP-induced Parkinsonism and idiopathic PD to differ? Secondly, MPTP is usually administered in a single acute dose, causing outright Parkinsonism. This is in contrast to the development of idiopathic PD which develops over years, typically with late onset. Both conditions result in the destruction of DA neurones in the substantia nigra, but acute MPTP dosing does not cause the development of Lewy bodies seen in many cases of idiopathic PD³⁰. Chronic dosing of MPTP causes more degeneration in the caudate nucleus than in the putamen, whereas in idiopathic PD the putamen is more affected²⁹. Do these differences in the development of MPTP-induced Parkinsonism and idiopathic PD mean that any remedial therapies that work in an MPTP model will either not work or have limited effect in other cases?

The evidence that MPTP causes Parkinsonism is undeniable, and the pattern of neurodegeneration seen with acute MPTP dosing is almost identical to that seen in idiopathic

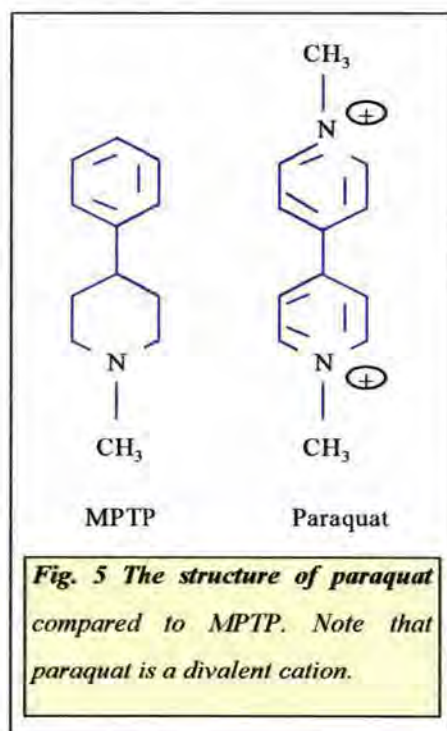
PD. However, any evidence citing a comparison between a MPTP model of PD and the effects of another compound still need to be treated carefully, taking all matters of differing aetiology and disease progression into account, with careful consideration of species suitability.

4.2. Paraquat.

4.2.1. Background.

The structural similarity between MPTP and the herbicide paraquat (1,1'-dimethyl-4,4'-bipyridinium) has often been cited as basis for the belief that paraquat may also be neurotoxic when administered systemically. Fig 5 shows the structure of paraquat and MPTP.

A new impetus was found when several epidemiological studies indicated that there may be a connection between herbicide use and the development of PD in later life (see 2.4.). Many studies have been carried out on whether or not paraquat can cause DA nerve cell death *in*



vivo and the conclusions fall into one of two distinct camps: either paraquat can penetrate the CNS or it cannot. This is the first problem to resolve before any conclusions can be drawn on the safety of paraquat.

4.2.2. Properties and acute toxicity of paraquat ^{31,32}.

Paraquat is a divalent cation that structurally resembles MPTP (see fig 5). It is a non-selective herbicide that was used extensively during the mid 20th century and is still used now as the active ingredient in gramoxone and in dipyridyl phosphate herbicides. It is not reported to be carcinogenic or teratogenic. The effects of acute paraquat poisoning in man are well documented and there have been many cases of accidental, homicidal and suicidal deaths.

Clinical symptoms of acute poisoning progress through three stages: pulmonary oedema, headache and dyspeptic disturbances, and toxic effects due to liver, kidney and lung damage. Pulmonary fibrosis occurs later (about 6 days after initial poisoning) and is associated with the elimination phase. Most deaths occur due to the immediate acute effects mediated by the distribution phase. None of these effects indicate that paraquat activity within the CNS is involved in its acute toxicity. Neuropathological changes have been reported in patients dying from paraquat poisoning, but these can be attributed to either the secondary effects of anoxia due to pulmonary damage, or to the therapeutic regimens employed in the treatment of the poisoning³³.

4.2.3. Mechanisms of paraquat-induced cytotoxicity.

Once within a cell, paraquat is cyclically reduced and reoxidised³¹. This consumes the reserves of NADPH in the cell, causing a reduction in ATP output and leading to free radical production and membrane damage (see 3.2.1.). Paraquat has been shown to induce apoptosis *in vitro*³⁴. There seem to be many contributing factors to the initiation of apoptosis by paraquat, two of which seem contradictory to each other, i.e. paraquat produces reactive oxygen species³⁵ which, in the classical theory of the aetiology of PD, are thought to nitrate tyrosine residues, thus disabling tyrosine kinases and disturbing many cellular processes¹⁶. However it has also been shown that paraquat can increase the tyrosine kinase activity which

leads to apoptosis³⁴. Whether one of these two processes will be found not to occur, or in a different manner, or if there is an interaction between them, remains to be seen.

The binding of AP-1 (a regulatory protein complex in the control of gene expression) to DNA, and protein synthesis are both processes that occur during apoptosis. Blocking these processes when paraquat is present prevents apoptotic cell death. AP-1 binding was also reduced in cells exposed to paraquat and antioxidant enzymes, indicating that the production of reactive oxygen species is a major contributing factor in the cytotoxicity of paraquat. All these effects are potentiated by the co-administration of very low concentrations of chelated iron³⁴.

Paraquat can localise to neurones where neuromelanin is produced (a by-product of DA synthesis). It has been shown that paraquat preferentially locates to cells with this component and so only accumulates in those species that produce it³⁶ (see 4.1.4.). Whether or not the uptake of paraquat is related to DAT, as is the case for MPP⁺, remains to be seen.

4.2.4. The blood/brain barrier and paraquat.

The endothelium of the cerebral vasculature is highly specialised, with tight junctions preventing passage between cells, and a lack of pinocytotic vesicles preventing passage through cells. This limits the kinds of molecules that have access to the brain to those that are lipid soluble, very small, or have a specific carrier system located in the endothelium. This is the blood/brain barrier and it is what prevents many toxins and pathogens from gaining access to neurones within the brain¹.

The blood/brain barrier presents a significant impediment to the passage of paraquat into the brain. The two positive charges that paraquat carries makes it polar and therefore hydrophilic. Its lack of lipid solubility means that it cannot diffuse across the blood/brain barrier and therefore paraquat should not be able to move into the brain from the blood stream. However, several reports have suggested that paraquat is indeed penetrating the blood/brain barrier and gaining access into deeper cerebral structures^{36, 37, 39, 40, 41}. There are four potential mechanisms by which it could penetrate into the brain:

i. Non-barriered brain areas.

There are some areas in the brain that are not protected by the blood/brain barrier because it is either absent or incomplete. These areas include the anterior olfactory bulb, the circumventricular organs and the chemosensitive trigger zone¹.

ii. Variations in permeability.

The neonatal rat has impaired blood/brain barrier integrity (as compared to adult or elderly rats), meaning that certain substances that do not normally accumulate inside the brain can pass through the blood/brain barrier³⁷.

iii. Endogenous transport systems.

Essential substances such as glucose and amino acids have their own transporters located within the endothelium of the blood/brain barrier so that they can penetrate into the brain. Other such transport system exists for a wide variety of substances in many diverse tissues.

iv. The cerebrospinal fluid.

Charged molecules can freely diffuse throughout the extracellular spaces of the brain, if they can enter the CSF.

Limited paraquat entry is observed in brain regions that lack a blood/brain barrier (ventral hypothalamus, area postrema, medulla oblongata, anterior olfactory bulb) and in those areas of the brain that lie outside the blood/brain barrier (pineal gland, lining of the ventricles)⁴². Evidence exists that paraquat can significantly penetrate the blood/brain barrier in neonatal rats, and that the integrity of the lining has also been shown to decrease in elderly animals³⁷. An endogenous paraquat transport system has been identified in rat renal proximal tubule cells in culture³⁸, and in the alveolar epithelium of the lung, uptake of paraquat can occur via the polyamine-sensitive transport system as well as by diffusion⁴³.

Approaches to resolving the question of whether or not paraquat can penetrate the blood/brain barrier have been quite diverse. An approach using HPLC⁴¹ concluded that a significant amount of paraquat entered the brain after systemic administration. However, doubt was cast on this by a study that involved pathological examination of treated rats' brains and quantitative autoradiography³⁷. This concluded that any paraquat found in the brain was associated with the cerebral microvasculature, limited paraquat entry was observed in regions of the brain without a blood/brain barrier and no paraquat was found in the regions of the brain associated with PD. No paraquat-induced cell death was observed either.

4.2.5. Paraquat and the pathogenesis of PD - quality of the evidence.

Paraquat's cytotoxicity, its structural resemblance to the known neurotoxicant MPTP and its epidemiological links to increases in the incidence of PD has singled it out as chief suspect in the search for a pathogenic agent for PD. It has always been known that paraquat is toxic, but it is notable that in a majority of cases of acute paraquat poisoning no neuropathology was recorded. When it has been, the damage was attributable to secondary effects, and there was no evidence of direct neuronal damage³³.

The goal of research in this area is to prove that systemic administration of sub-acute doses of paraquat can cause DA neurone loss in the substantia nigra and behavioural changes resembling those seen with MPTP toxicity. However, evidence to this end is sketchy. A lot of work has been done on establishing the aetiology of paraquat cytotoxicity *in vitro* and, although not conclusive, the data infers that paraquat can induce apoptosis by several different mechanisms, including production of free radicals (see 4.2.3.). One thing that stands out in such studies is that authors often make an assumption that, as some paraquat is able to enter the brain from the systemic circulation, that paraquat is able to penetrate far enough and at an adequate concentration to reach DA neurones and cause the damage *in vivo* that is described *in vitro*. Any conclusions drawn from *in vitro* work must take into account the access paraquat has to DA neurones *in vivo*.

Table 1.

Paper	Method of paraquat administration	Comments
Widdowson (1996) ³⁷	subcutaneous injection (single toxic dose)	Low concentrations of paraquat found in brain, slow entry via blood/brain barrier deficient areas, no evidence of neuronal cell death.
Naylor (1995) ⁴²	subcutaneous injection (single toxic dose)	Paraquat entry to brain limited to blood/brain barrier deficient areas, paraquat present 24hrs post injection associated with cerebrocirculatory system, no evidence of neuronal cell damage.
Bagetta (1992) ³⁹	intracerebral injection and intraperitoneal injection (single toxic dose)	i.c. injection (into hippocampus) - behavioural changes, neuronal cell death observed in several regions. i.p. injection - excitatory behavioural changes, neuronal cell death limited to pyriform cortex.
Brooks (1999) ⁴⁰	repeated intraperitoneal injections	See text.

In vivo studies with paraquat have provided a wealth of contradictory data. Table 1 gives four examples of papers that have utilised *in vivo* methods. Crucially, much of the evidence providing proof that paraquat can induce Parkinsonian changes *in vivo* had, until recently, employed intracerebral injections as the method of administration (the study by Bagetta *et al.* cited in table 1 being an example). Although this gives an insight into the behaviour of paraquat within the brain *in vivo*, it does not take into account the role of biotransformation and transfer across the blood/brain barrier. It also has several methodological problems, for example achieving the correct site of administration, damage caused by the administration process itself. Another consideration is why intracerebral injections were used at all, as this is not the way in which paraquat exposure would ever occur naturally.

The most recent study (Brooks *et al.* [40]) did establish links between non-intracerebral paraquat administration and pathological and behavioural changes consistent with Parkinsonism. An MPTP-sensitive species was used (C57 black mice) and ambulatory locomotor activity was assessed with an automated device. DA neurone density was measured using tyrosine hydroxylase immunocytochemistry and Fluoro-gold visualisation. Paraquat, MPTP and saline were administered i.p. at doses derived empirically from several previous studies. A behavioural baseline was measured after Fluoro-gold surgery but before treatment commenced. Saline treated mice acted as a control group. The results show a reduction in ambulatory activity and DA neurone density in the paraquat and MPTP treated animals.

The data seem quite compelling, however, a few questions remain unanswered. There is still no explanation as to how the paraquat is crossing the blood/brain barrier. Also the serum levels of paraquat were not measured, no other pathology data was submitted, only one area of the brain was examined and one cell type was selectively visualised, so it could be possible that neurological damage was secondary to paraquat's primary toxic effects. If there is an effect on less discrete regions of the brain (as has been reported in some acute paraquat poisoning cases in humans³³), then if only one cell type in one region of the brain is visualised then the effect will only be observed in that region.

Another possible flaw is that the Fluoro-gold labelling technique requires stereotactic delivery of Fluoro-gold, which violates the blood/brain barrier thus reducing its integrity. This matter is addressed by the authors, who cite unpublished evidence that a breached blood/brain barrier is impenetrable to serum-derived immunoglobulins after 5 days healing. The first injection of paraquat was 7 days post Fluoro-gold delivery. Paraquat could have gained entry to the brain though a malfunctioning blood/brain barrier if it took more than the stated 5 days to heal. Also, immunoglobulins are considerably larger with a higher molecular weight and therefore are more likely to be excluded by an incompletely healed blood/brain barrier.

The tyrosine hydroxylase immunocytochemistry could have been used to great effect if it had been used in a control group that had not undergone the Fluoro-gold surgery.

4.2.6. The future for paraquat research.

All the evidence that is currently available on the neurotoxicity of paraquat demonstrates that there are no clear and concise answers. Biological convention says that paraquat cannot penetrate the brain due to its polar nature and the defences provided by the blood/brain barrier. However, doubt must be cast on these assumptions if data comes to light suggesting that paraquat can penetrate the blood/brain barrier and cause selective degeneration of DA neurones at sub-acute dose levels. Further studies must be done in different species to

investigate the permeability of the blood/brain barrier to paraquat, and the role of genetics in determining susceptibility.

4.3. Organochlorines.

4.3.1. Background.

Polychlorinated biphenyls (PCBs) have been associated with a decrease in DA levels in primate brains⁴⁶, and PCBs, together with organochlorine (OC) pesticide residues, have been found by various studies in human adipose tissue for over 20 years⁴⁷. Consequently, these observations have justified investigation into possible links with the pathogenesis of PD in humans. PCB congeners have been detected in some brain regions, both within the basal ganglia and elsewhere, but not in the frontal or occipital cortices. However, they were detected at similar concentrations in both PD and non-PD brains^{48, 49}. It has been observed in primates that decreased DA concentrations are still observed after PCB concentrations have declined⁴⁶. There are conflicting reports regarding the presence of the OC dieldrin in PD brain tissue^{48, 49}.

A metabolite of DDT (pp'-DDE) has not been detected in greater concentrations in PD brain tissues compared to non-PD brain tissue⁴⁹. Heptachlor, an OC insecticide, has been found to increase the expression of DAT (the gateway into the cell for MPP⁺, see 4.1.2) and the vesicular monoamine transporter (VMAT2) in C57 black mice, whilst also inhibiting the function of VMAT2 (thought to sequester toxins into vesicles and thus provide some neuroprotection)⁵⁰.

4.3.3. The evidence.

It has been necessary to investigate agricultural chemicals other than paraquat for possible links with PD, not only because they show neurotoxic properties, but also to provide further evidence that can be taken into account when evaluating the epidemiological studies on PD. The research in this area has gone down a different path to that of paraquat research. It has

started off by looking for traces of these chemicals in human brain tissue, comparing PD brains to non-PD brains, and checking the effects of the chemicals once they are in the brain. Paraquat research has been more mechanism-based, concentrating on how it may produce neurodegeneration. Only recently has OC research begun to look into possible mechanisms⁵⁰. To date, the data from OC research has been inconclusive and there have not been enough well controlled studies to provide fair comparisons. However it is important to keep this line of enquiry open, as if there is an interaction between agricultural chemicals and the development of PD, it is more likely that it is not one chemical (e.g. paraquat) acting alone, but a complex interaction between a number of environmental contaminants and genetic anomalies.

4.4. Manganese.

It has long been known that occupational exposure to manganese causes atypical Parkinsonism; in 1907 “manganese crusher’s disease” was described in workers in manganese ore crushing plants. The symptoms were muscle weakness, limb tremor, bent posture, whispering speech and salivation. Miners seemed to be the most susceptible group, however, manganese toxicity has also been reported in other industrial and agricultural workers⁴⁴.

Although manganese toxicity manifests itself in a very similar way to idiopathic PD, it does have a different pathology, which causes slight differences in presentation between the two diseases and allows them to be differentiated. Manganese toxicity is indicated by the presence of atypical Parkinsonism with dystonia and infrequent tremor. Morphologically, the most severe neurodegeneration is seen in the globus pallidus, particularly in the medial segment. This is in contrast to the pattern of neurodegeneration seen in idiopathic PD where the most severe neuronal loss is seen in the substantia nigra. Neurodegeneration is seen in the substantia nigra with manganese toxicity, as well as in the putamen and caudate nucleus, but to a lesser extent. Histologically, manganese toxicity involves a decrease in the number of myelinated fibres and a proliferation of astrocytes, which is also significantly different to the changes seen in PD⁴⁴.

Some studies have tried to find a link between manganese toxicity and oxidative stress (as implicated in PD)⁴⁵, but these have proved negative. Consequently, it is not wise to compare the development and treatment of PD to that of manganese toxicity, because although the clinical symptoms are similar, the pathological processes are very different.

5. Concluding remarks.

The pathogenesis and aetiology of idiopathic PD has long been an enigma to science. Over the past twenty years, the disease process has been elucidated by directly studying PD brains and MPTP models of PD. This process is not yet complete, but a clearer picture is emerging with each paper published.

However, an initiating factor is still proving to be elusive. When the effects of MPTP were discovered, the herbicide paraquat moved to the fore of research as it was a structural analogue of MPTP and it had been implicated in epidemiological studies of PD. Confusion still surrounds the question of whether or not paraquat can induce neurodegeneration akin to that seen in PD. There is no doubt that it is cytotoxic, and can localise to neuromelanin producing neurones, but evidence from *in vivo* studies does not provide conclusive evidence that paraquat can cause significant damage. The fact that paraquat should not be able to diffuse across the blood/brain barrier has still not been satisfactorily addressed in those studies claiming that non-intracerebral administration of paraquat can cause Parkinsonism. There is enough evidence to the contrary to cast doubt on these claims.

Other substances have been linked to PD, including organochlorine pesticides and manganese. Currently there is little hard evidence to indict the organochlorine pesticides, although research needs to start mimicking what is being done with MPTP so as to provide a good comparison. Long term occupational exposure to manganese does produce a motor disorder which is very similar to PD, however, different brain regions are affected and it seems that oxidative damage is not involved.

In conclusion, idiopathic PD must still be classified as idiopathic, that is of unknown or obscure origin. The desire to postulate a cause to explain this unknown is tempting, especially with the abundance of possible mechanisms currently circulating. The exact role of genetics must be uncovered, and it may transpire that genetic testing and discovery of substances acting to exacerbate these aberrations may be the best way of discovering who needs to avoid what to reduce the chances of developing the disease.

6. References.

1. Cooper, J.R. *et al.* (1996) The Biochemical Basis of Neuropharmacology. Oxford University Press Inc. 7th ed.
2. Duvoisin, R.C. History of Parkinsonism. *Pharmacol Ther* 1987; **14**: 414-418.
3. Patten, J. (1996) Neurological differential diagnosis. London Springer. 2nd ed.
4. Quinn, N.P. Parkinson's Disease: clinical features. *Baillieres Clin Neurol* 1997; **6**: 1-13.
5. Vander, A.J. *et al.* (1998) Human Physiology: The Mechanisms of Body Function. WCB McGraw-Hill Inc. 7th ed.
6. Checkoway, H. and Nelson, L.M. Epidemiologic approaches to the study of Parkinson's disease etiology. *Epidemiology* 1999; **10**: 327-336.
7. Barbeau, A. *et al.* Ecogenetics of Parkinson's disease: prevalence and environmental aspects in rural areas. *Can J Neurol Sci* 1987; **14**: 36-41.
8. Rajput, A.H. *et al.* Geography, drinking water chemistry, pesticides and herbicides and the etiology of Parkinson's disease. *Can J Neurol Sci* 1987; **14**: 414-418.
9. Tanner, C.M. *et al.* Parkinson's Disease in twins: an etiologic study. *JAMA* 1999; **281**: 341-346.
10. Duvoisin, R.C. Familial Parkinson's Disease. *Neurosci News* 1998; **1**: 43-46.
11. Polymeropoulos, M.H. *et al.* Mutation in the α -synuclein gene identified in families with Parkinson's Disease. *Science* 1997; **276**: 2045-2047.
12. Spillantini, M.G. *et al.* α -Synuclein in filamentous inclusions of Lewy bodies from Parkinson's Disease and dementia with Lewy bodies. *Proc Natl Acad Sci USA* 1998; **95**: 6469-6473.
13. Yoritaka, A. *et al.* Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson's Disease. *Proc Natl Acad Sci USA* 1996; **93**: 2696-2701.

14. King, M.P. and Attardi, G. as cited by [16].
15. Swerdlow, R.H. *et al.* Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann Neurol* 1996; **40**: 663-671.
16. M. Flint Beal. Mitochondria, free radicals and neurodegeneration. *Curr Op Neuro* 1996; **6**: 661-666.
17. Reynolds, I.J. and Hastings, T.G. as cited by [16].
18. Plaa, G.L. and Witschi, H. (1976) as cited by [39]
19. Singer, T.P. and Ramsay, R.R. Mechanism of the neurotoxicity of MPTP. *FEBS Letters* 1990; **274**: 1-8.
20. Tipton, K.F. and Singer, T.P. Advances in our understanding of the mechanisms of the neurotoxicity of MPTP and related compounds. *J Neurochem* 1993; **61**: 1191-1203.
21. Gainetdinov, R.R. *et al.* Dopamine transporter is required for in vivo MPTP neurotoxicity: evidence from mice lacking the transporter. *J Neurochem* 1997; **69**: 1322-1325.
22. Calne, D.B. (ed.). Drugs for the treatment of Parkinson's Disease. *Handbook Ex Pharm* 1989; **88**. Springer-Verlag.
23. D'Amato *et al* (1987) as cited by [22].
24. Keller and De Prada (1985) as cited by [19].
25. Ramsay and Singer (1986) as cited by [19]
26. Aiuchi *et al* (1992) as cited by [19]
27. Miyako, K. *et al.* 1-Methyl-4-phenylpyridinium ion (MPP⁺) selectively inhibits the replication of mitochondrial DNA. *Eur J Biochem* 1999; **259**: 412-418.

28. Cassarino, D.S, *et al.* The Parkinsonian neurotoxin MPP⁺ opens the mitochondrial permeability transition pore and releases cytochrome C in isolated mitochondria via an oxidative mechanism. *Biochem Biophys Acta* 1999; **1453**: 49-62.
29. Riederer and Youdim (1987) as cited by [30].
30. McCrodden, J.M. *et al.* The neurotoxicity of MPTP and the relevance to Parkinson's Disease. *Pharmacol Toxicol* 1990; **67**: 8-13.
31. Riddell, R.H. (ed.) (1982). Pathology of Drug-induced and Toxic Diseases. Churchill Livingstone, 1st ed.
32. Richardson, M.L. (ed.) (1994). Dictionary of Substances and their Effects. The Royal Society of Chemistry.
33. Hughes, J.T. Brain damage due to paraquat poisoning: a fatal case with neuropathological examination of the brain. *Neurotoxicology* 1988; **9**: 243-248.
34. Li, X. and Sun, A.Y. Paraquat induced activation of transcription factor AP-1 and apoptosis in PC12 cells. *J Neural Trans* 1999; **106**: 1-21.
35. Yang, W. And Sun, A.Y. (1998b) as cited by [34].
36. Lindquist, N.G. *et al.* Autoradiography of [¹⁴C] paraquat or [¹⁴C] diquat in frogs and mice: accumulation in neuromelanin. *Neurosci Lett* 1988; **93**: 1-6.
37. Widdowson, M.J. *et al.* Influence of age on the passage of paraquat through the blood/brain barrier in rats: a distribution and pathological examination. *Human Exp Toxicol* 1996; **15**: 231-236.
38. Chan, B.S. *et al.* Characterisation and uptake of paraquat by rat renal proximal tubular cells in primary culture. *Hum Exp Toxicol* 1996; **15**: 949-956.
39. Bagetta, G. *et al.* Production of limbic motor seizures and brain damage by systemic and intracerebral injections of paraquat in mice. *Pharmacol Toxicol* 1992; **71**: 443-448.

40. Brooks, A.I. *et al.* Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. *Brain Res* 1999; **823**: 1-10.
41. Corasaniti, M.T. *et al.* Determination of paraquat in rat brain by high performance liquid chromatography. *J Chromatog* 1993; **643**: 419-425.
42. Naylor, J.L. *et al.* Further evidence that the blood/brain barrier impedes paraquat entry into the brain. *Hum Exp Toxicol* 1995; **14**: 587-594.
43. Wyatt, I. *et al.* The accumulation and localisation of putrescine, spermidine, spermine and paraquat in the rat lung. *Biochem Pharmacol* 1988; **37**: 1909-1918.
44. Pramod Kr. Pal *et al.* Manganese neurotoxicity: a review of clinical features, imaging and pathology. *Neurotoxicology* 1999; **20**: 227-238.
45. Brenneman, K.A. *et al.* Manganese-induced developmental neurotoxicity in the CD rat: is oxidative damage a mechanism of action? *Neurotoxicology* 1999; **20**: 477-488.
46. Seegal, R.F. *et al* (1994) as cited by [49]
47. Abbott, D.C. *et al* (1981) as cited by [49]
48. Fleming, L. *et al.* Parkinson's Disease and brain levels of organochlorine pesticides. *Ann Neurol* 1994; **36**: 100-103.
49. Corrigan, F.M. *et al.* Organochlorine compounds in human brain. *Hum Exp Toxicol* 1996; **15**: 262-264.
50. Miller, G.W. *et al.* Heptachlor alters expression and function of dopamine transporters. *Neurotoxicology* 1999; **20**: 621-637.