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THE EFFECT OF MANGANESE ON MAMMALIAN MITOCHONDRIA

A thesis presented in partial fulfilment of the requirements for the degree of
Master of Science in Biochemistry
at Massey University, New Zealand

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ABSTRACT

Manganese (Mn) is an essential trace element, but excessive inhalation can cause serious disorders of the central nervous system, lungs and liver, and results in the condition known as manganism. The general population is exposed to Mn through its use in the fungicide Maneb and MMT, which is used as an anti-knock agent to replaced lead in petrol. Also there have been a number of reports of Mn contaminated drinking water. Victims of Mn poisoning suffer from serious neurological disorders, such as an intermittent tremor of small amplitude, speech impairments and disruption of postural reflexes, which are caused by damage to certain regions of the brain. After prolonged exposure severe symptoms develop that generally resemble those associated with Parkinson's disease.

The action of Mn on the brain is not well understood, although three possible mechanisms have been proposed:

1. Inhibition of the mitochondrial electron transfer chain following Mn accumulation by mitochondria.
2. Neuronal degradation by free radicals such as O$_2^-$ and ·OH causing lipid peroxidation and damage to DNA and protein.
3. Induction of mutation of the mitochondrial genome, as has previously been shown in both eukaryotes and prokaryotes.

It has been shown in this study that Mn inhibits the mitochondrial electron transfer chain. An overall ionic strength inhibition of the entire electron transfer chain was observed, probably mediated by an interference of the electrostatic interactions between cytochrome $c$ and the cytochrome $bc_1$ complex or cytochrome oxidase. Also a direct inhibition of succinate dehydrogenase, NADH dehydrogenase and cytochrome oxidase was observed. This inhibition would be associated with a decrease the production of ATP and could be sufficient to cause the degradation of brain tissue seen in victims of Mn poisoning.

It seems likely that if Mn can inhibit the mitochondrial electron transfer chain, this inhibition would lead to an increase in the generation of free radical species by the mitochondria. However, this was not shown in this work, due to difficulties with
detector molecules. It was observed that sheep liver mitochondria can oxidise and reduce acetylated cytochrome c, which may not have been previously reported.

The effect of Mn on isolated mtDNA showed a decrease in the intensity of PCR products after exposure to Mn, which may have been cause by an interference of the activity of Taq polymerase. It has previously been shown that Mn interferes with the activity of both Taq polymerase and chicken liver mitochondrial polymerase-γ and, if it could interfere with the activity of mitochondrial DNA polymerase, this would also decrease further both the number of functional mitochondria and the production of ATP.

A decrease in the production of ATP by mitochondria, or a decrease in the production of functional mitochondria, would lead to cellular death of affected cells and could provide an explanation of the symptoms observed in victims of Mn poisoning.
ACKNOWLEDGEMENTS

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To my friends, thank you for the support, especially those who have proof read some of my essays and listened to my speeches, I know they didn’t make any sense to you but it helped me a lot.

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<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>adenine</td>
<td></td>
</tr>
<tr>
<td>$A_{XXX}$</td>
<td>absorbance (XXX-wavelength of measurement)</td>
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<tr>
<td>ADP</td>
<td>adenosine 5’-diphosphate</td>
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<td>AMProp</td>
<td>2-amino-2-methyl-1 propanol</td>
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<td>ATP</td>
<td>adenine triphosphate</td>
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<td>bp</td>
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<td>cytochrome $b_{c_1}$ complex</td>
<td>ubiquinol : ferricytochrome-c oxidoreductase (EC 1.10.2.2)</td>
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<td>BSA</td>
<td>bovine serum albumin (fraction V powder)</td>
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<tr>
<td>C</td>
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<tr>
<td>CCCP</td>
<td>carbonyl cyanide m-chlorophenylhydrazone</td>
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<td>CO1</td>
<td>cytochrome c oxidase subunit 1 gene</td>
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<td>CR</td>
<td>control ratio (= rate after DNP addition/rate before DNP addition)</td>
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<tr>
<td>cytochrome oxidase</td>
<td>ferrocytochrome c : oxygen oxidoreductase (EC 1.9.3.1)</td>
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<td>DCPIP</td>
<td>2,6-dichlorophenol-indo-phenol</td>
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<td>DMSO</td>
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<td>5,5-dimethyl-1-pyrrole N-oxide</td>
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<td>2,4-dinitrophenol</td>
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<td>EDTA</td>
<td>ethylene diamine tetra-acetic acid</td>
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<td>EGTA</td>
<td>ethylene glycol-bis(β-aminoethyl)ether-N,N,N’,N’-tetra-acetic acid</td>
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<tr>
<td>EPR</td>
<td>electron paramagnetic resonance</td>
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<td>FECN</td>
<td>potassium ferricyanide</td>
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<tr>
<td>G</td>
<td>guanine</td>
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<tr>
<td>GSH</td>
<td>glutathione</td>
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<tr>
<td>$H_2O_2$</td>
<td>hydrogen peroxide</td>
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<td>HEPES</td>
<td>N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid]</td>
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<tr>
<td>$\cdot\text{OH}$</td>
<td>hydroxyl radical</td>
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<td>kb</td>
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KO$_2$  potassium superoxide
L-DOPA  l-3,4-dihydroxyphenylalanine
Maneb  [ethylenebis(dithiocarbamato)]manganese
$\Delta \psi$  membrane potential
Mg  magnesium
MMT  methylcyclopentadienyl manganese tricarbonyl
Mn  manganese
MPTP  1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mtDNA  mitochondrial DNA
NAD$^+$  nicotinamide adenine dinucleotide (oxidised form)
NADH  nicotinamide adenine dinucleotide (reduced form)
NADH dehydrogenase  NADH : ubiquinone oxidoreductase (EC 1.5.5.3)
NBT  2,2'-di-p-nitrophenyl-5-5'-diphenyl-3,3'-[3,3'-dimethoxy-4-4'-diphenylene]-ditetrazolium chloride
RAPD  random amplified polymorphic DNA
RCR  respiratory control ratio
RFLP  restriction fragment length polymorphism
SOD  superoxide dismutase (EC 1.15.1.1)
$O_2^-$  superoxide radical
PCR  polymerase chain reaction
PD  Parkinson’s disease
succinate dehydrogenase  succinate : ubiquinone oxidoreductase (EC 1.3.5.1)
T  thymine
TE buffer  Tris-HCl (10 mM) EDTA (1 mM) pH 8.0
TAE buffer  Tris (40 mM) acetate (20 mM) EDTA (1 mM) pH 8.0
$Taq$ polymerase  *Thermus aquaticus* DNA polymerase
Tris  2-amino-2-(hydroxymethyl)propane-1,3-diol
$\Delta \mu_H^+$  transmembrane proton electrochemical potential
tRNA  transfer RNA
U  unit
UQ  ubiquinone
UQH  ubiquinol
UV  ultraviolet
V  volts