TETRAHYDROBIOPTERIN METABOLISM IN DEMENTIA

by

Julia Margaret Anderson

A thesis submitted for the Degree of Doctor of Philosophy

The University of Aston in Birmingham

May 1987

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SUMMARY

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The aim of this study was to establish normal levels of the enzymes involved in tetrahydrobiopterin (BH_4) metabolism in human and rat brain preparations; to determine whether BH_4 metabolism is altered in dementia, particularly in relation to senile dementia of the Alzheimer type (SDAT); and to examine the effect of aluminium on BH_4 metabolism.

Overall BH_4 synthesis and dihydropteridine reductase (DHPR) activity were greater in the locus coeruleus than in the neocortex of elderly subjects. Sepiapterin reductase and DHPR activity showed a linear correlation with age in the temporal cortex. DHPR activity in the frontal cortex was relatively constant until the mid 60s and then fell with age.

Overall BH_4 synthesis showed a non-significant decline in temporal cortex and was significantly reduced in locus coeruleus preparations from SDAT subjects compared to control subjects. As DHPR, sepiapterin reductase and GTP cyclohydrolase activity were unaltered in SDAT we suggest that there is a lesion on the biosynthetic pathway between dihydroneopterin triphosphate and BH_4 in SDAT, possibly at the level of 6-pyruvoyl tetrahydropterin synthase. DHPR activity and BH_4 synthesis capacity were unaltered in temporal cortex preparations from Huntington's disease subjects indicating that the defect in BH_4 metabolism in SDAT is specific to the disease process and not a secondary consequence of dementia. The implications of altered BH_4 metabolism in aging and dementia are discussed.

BH_4 metabolism was examined in temporal and frontal cortex preparations from 4 subjects who had received peritoneal dialysis treatment. All patients had elevated serum aluminium levels. The data suggests that aluminium may inhibit DHPR activity in the frontal cortex resulting in diminished BH_4 levels in the cells which leads to a compensatory increase in the activity of the biosynthetic pathway.

Aluminium reversibly inhibited sepiapterin reductase activity in rat brain preparations but did not alter sepiapterin reductase activity *in vivo* Overall BH_4 synthesis and GTP cyclohydrolase activity were not affected by aluminium *in vitro* The biosynthetic pathway was unaltered in rat brain preparations from animals receiving aluminium orally compared to control animals. DHPR activity was unaltered or increased in rat brain preparations from aluminium treated rats compared to the control group.

Key words: Tetrahydrobiopterin, Senile dementia of the Alzheimer type, Huntington's disease, Aging, Aluminium

To

My Parents

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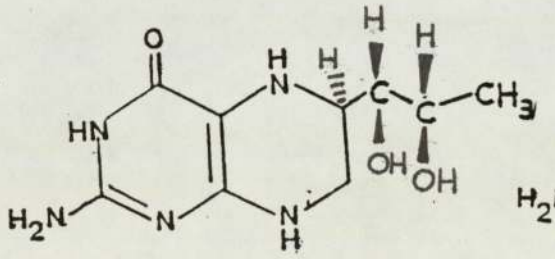
ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
AD	Alzheimer's disease
BH_4	tetrahydrobiopterin
Br	Brodmann area
CNS	central nervous system
CSF	cerebrospinal fluid
DHFR	dihydrofolate reductase
DHPR	dihydropteridine reductase
DOPA	3,4-dihydroxyphenylacetic acid
$FPydP_3$	2-amino-6-(triphosphoribosyl)-amino-5 or 6-formamido-4-hydroxy pyrimidine triphosphate
GABA	gamma-aminobutyric acid
GTP	guanosine triphosphate
HD	Huntington's disease
HPLC	high performance liquid chromatography
HVA	homovanillic acid
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
MHPG	3-methoxy-4-hydroxyphenylglycol
NH_2TP	dihydroneopterin triphosphate
N:B	ratio of neopterin:biopterin
PEE	phosphate eliminating enzyme (6-pyruvoyltetrahydropterin synthase)
PH_4	tetrahydropterin
PM	postmortem
qBH_2	quinonoid dihydrobiopterin
SDAT	senile dementia of the Alzheimer type

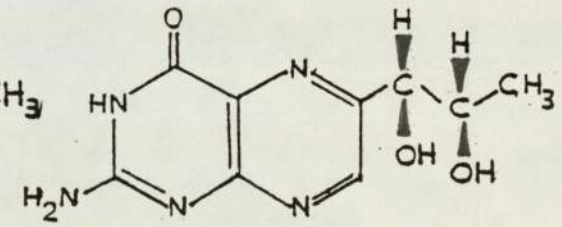
CHAPTER 1

INTRODUCTION

L-erythro-5,6,7,8-tetrahydrobiopterin (I), BH_4, is widely distributed in nature. It belongs to the class of compounds designated pteridines. The basic structure of most naturally occuring pteridines is 2-amino-4-hydroxypteridine (pterin). In 1956 Patterson and co-workers isolated biopterin (II) from human urine. Subsequently biopterin was identified in a wide variety of mammalian tissues and body fluids (Leeming *et al* 1976, Fukushima and Nixon 1980). Biopterin in body tissues and fluids exists primarily in the 5,6,7,8-tetrahydro form (Fukushima and Nixon 1980, Abou-Donia and Viveros 1981, Katoh and Sueoka 1986). BH_4 is slowly absorbed from the small intestine (Leeming *et al* 1983) and rats fed a diet deficient in BH_4 show normal development and continue to excrete biopterin in urine (Pabst and Rembold 1966) indicating that the major source of BH_4 is *de novo* synthesis within the cell.



I. 5,6,7,8-tetrahydrobiopterin



II. Biopterin

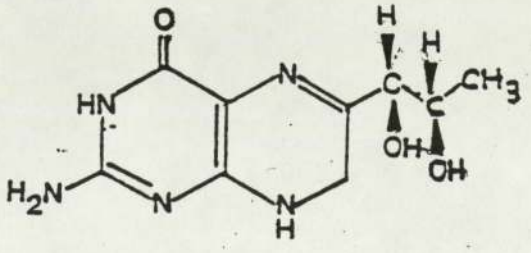
1.1.1 BH_4 biosynthesis

Initial work on the biosynthetic pathway of pteridines in bacteria indicated that a phosphorylated purine was the precursor of pterins *in vivo* (Vieira *et al* 1961, Krumdieck *et al* 1966, Goto and Forrest 1961). In 1968 Burg and Brown isolated an enzyme from *E. coli* which converted guanosine triphosphate (GTP) to dihydroneopterin triphosphate (NH_2TP) and proposed that GTP is enzymically cleaved at the C-8 of the imidazole ring to form 2-amino-6-(triphosphoribosyl)- amino-5 or 6-formamido -4- hydroxypyramidine ($FPydP_3$). Formate is released.

the product undergoes an Amadori rearrangement and ring closure to form NH_2TP (Figure 1.1). As no intermediates were isolated they suggested that the conversion of GTP to NH_2TP requires only one enzyme to which all intermediates remain bound. The enzyme was designated GTP cyclohydrolase 1.

Buff and Dairman (1975) and Fukushima and Shiota (1974) demonstrated that mouse neuroblastoma cells and Chinese hamster ovary cells in culture incorporate radioactivity from 2-^{14}C guanosine but not 8-^{14}C guanosine into pterins suggesting that the biosynthetic pathway in higher animals is similar to that in bacteria. GTP cyclohydrolase has since been isolated and partially purified from several species (Fukushima *et al* 1977, Bellahsene *et al* 1984, Blau and Niederwieser 1983). GTP cyclohydrolase from mammalian tissues has similar properties to the bacterial enzyme: one enzyme is required to convert GTP to NH_2TP, the enzyme is relatively heat stable and shows no cofactor requirements.

The mechanism by which NH_2TP is converted to BH_4 has been difficult to determine due to the lability of the intermediates. Initial studies suggested that NH_2TP is converted to 7,8-dihydrobiopterin (III) via dihydro intermediates (Krivi and Brown 1979, Tanaka *et al* 1981). It was proposed that 7,8-dihydrobiopterin is subsequently reduced to BH_4 by dihydrofolate reductase (Kaufman 1967). However studies using DukX-B11 cell line cultures which lack DHFR or using the DHFR inhibitor methotrexate showed that this was a minor pathway of BH_4 biosynthesis (Smith and Nichol 1983, Culvenor *et al* 1984).



III. 7,8-dihydrobiopterin

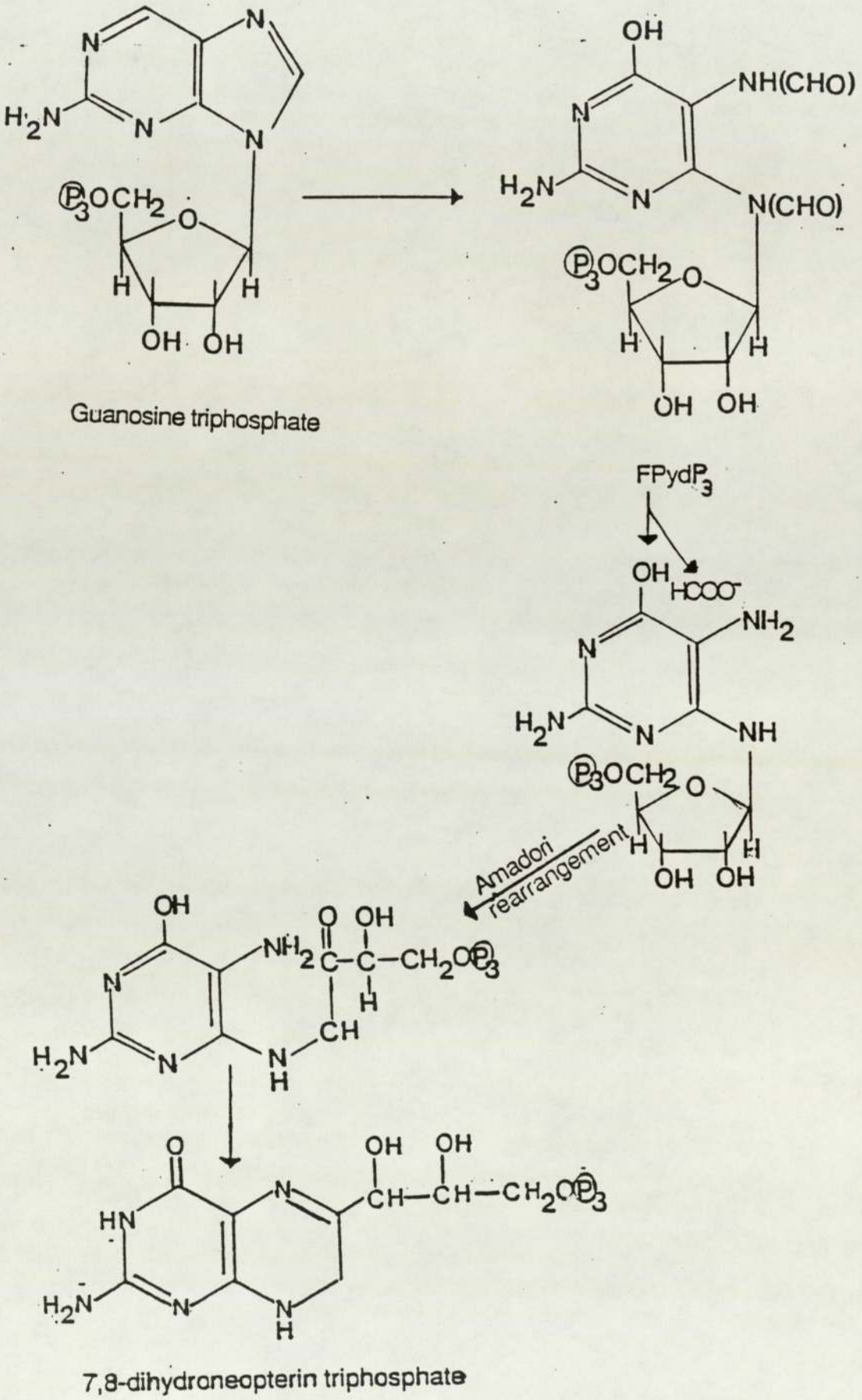
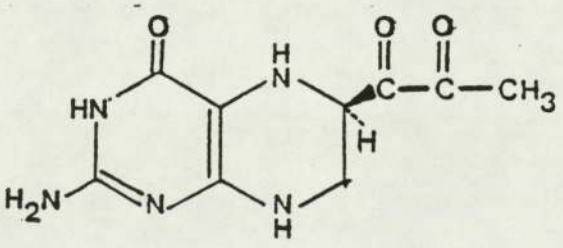
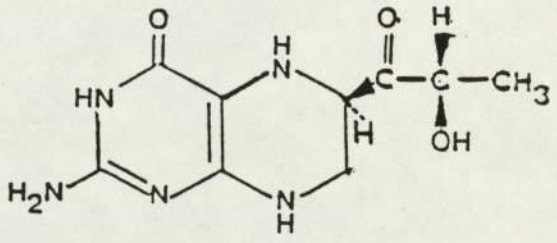


Figure 1.1 GTP cyclohydrolase catalyses the conversion of GTP to NH_2TP

Several groups reported that the intermediates on the biosynthetic pathway are tetrahydropterins but were unable to conclusively identify the nature of the sidechain (Milstein and Kaufman 1983, Milstein and Kaufman 1985, Switchenko and Brown 1985, Smith and Nichol 1984). A recent report by Smith and Nichol (1986) on BH_4 synthesis in the adrenal medulla provides strong evidence that the intermediates are 6R-(1',2'-dioxopropyl)-tetrahydropterin (IV) and 6R-(L-1'-hydroxy-2'-oxopropyl)-tetrahydropterin (V) (Figure 1.2). The first step is catalysed by a magnesium dependent enzyme which they designated pyruvoyl-H_4pterin synthase. Electrons are transferred from the side-chain (C-1') moiety of NH_2TP to the pterin ring; tautomerization of the enol formed and elimination of the phosphate group leads to the formation of 6-pyruvoyl-H_4pterin. Switchenko and co-workers (1985) using *Drosophila melanogaster* extracts and Heintel and co-workers (1985) using partially purified enzyme from human liver also proposed that the first step in the conversion of NH_2TP to BH_4 is the formation of 6-pyruvoyl-H_4-pterin. Heintel *et al* (1985) designated this enzyme phosphate eliminating enzyme (PEE). It is suggested that one enzyme is required to convert 6-pyruvoyl-H_4-pterin to 6R-(L-1'-hydroxy- 2'-oxopropyl)tetrahydropterin and finally to BH_4. This enzyme is very similar to sepiapterin reductase: NADPH dependent and inhibited by N-acetyl serotonin. Smith and Nichol (1986) suggested that this enzyme is more correctly designated H_4-biopterin synthase.



IV. 6-pyruvoyl-tetrahydropterin



V. 6-lactoyl-tetrahydropterin

In summary, BH_4 is synthesized in the mammal and insect from GTP. This requires three enzymes: GTP cyclohydrolase, 6-pyruvoyl-tetrahydropterin synthase (PEE) and BH_4 synthase (sepiapterin reductase).

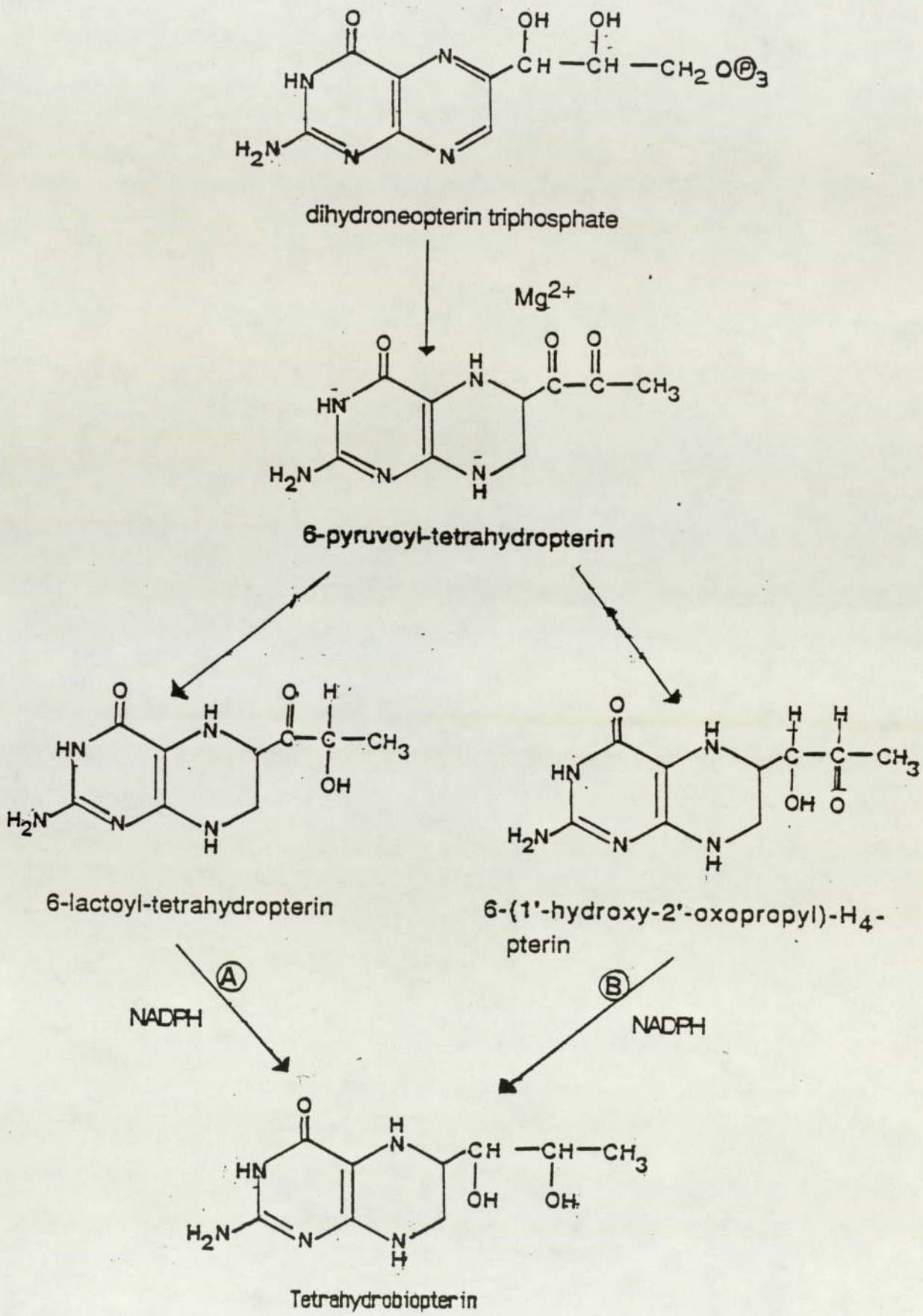


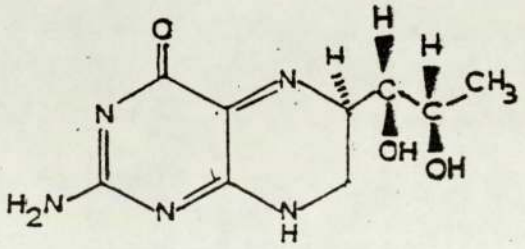
Figure 1.2. Biosynthesis of BH_4 from NH_2TP

Pathways proposed by (A) Switchenko *et al* (1984), Milstein and Kaufman (1985) (B) Smith and Nichol (1986).

The conversion of NH_2TP to BH_4 proceeds via tetrahydropterin intermediates and requires magnesium and NADPH.

1.1.2 Salvage pathway

Tetrahydrobiopterin acts as the electron donor during reactions catalysed by the pterin-dependent monooxygenases. The mechanism has been studied in most detail for phenylalanine hydroxylase but it is suggested that other monooxygenases act in a similar manner. Kaufman (1976) proposed that a hydroperoxide of BH_4 is formed (4a-hydroperoxy-tetrahydrobiopterin) which acts as the hydroxylating agent in the conversion of phenylalanine to tyrosine. The 4a-carbinolamine derivative subsequently formed loses H_2O to form quinonoid dihydrobiopterin (qBH_2, VI). qBH_2 may spontaneously tautomerize to 7,8-dihydrobiopterin which is subsequently lost from the cell. A salvage pathway operates in the cell to prevent this occurring: qBH_2 is reduced to BH_4 by the enzyme dihydropteridine reductase (DHPR) in the presence of NADH (Craine *et al* 1972. Figure 1.3). DHPR is of major importance in the maintenance of cellular BH_4 levels.



VI. Quinonoid dihydrobiopterin

1.1.3 Cofactor function of tetrahydrobiopterin

BH_4 is the cofactor for phenylalanine hydroxylase (Kaufman 1964), tyrosine hydroxylase (Nagatsu 1964), and tryptophan hydroxylase (Hosoda and Glick 1966). The reactions catalysed by these enzymes are shown in figure 1.4. As this work concerns BH_4 metabolism in the CNS the role of BH_4 in tyrosine hydroxylation and tryptophan hydroxylation

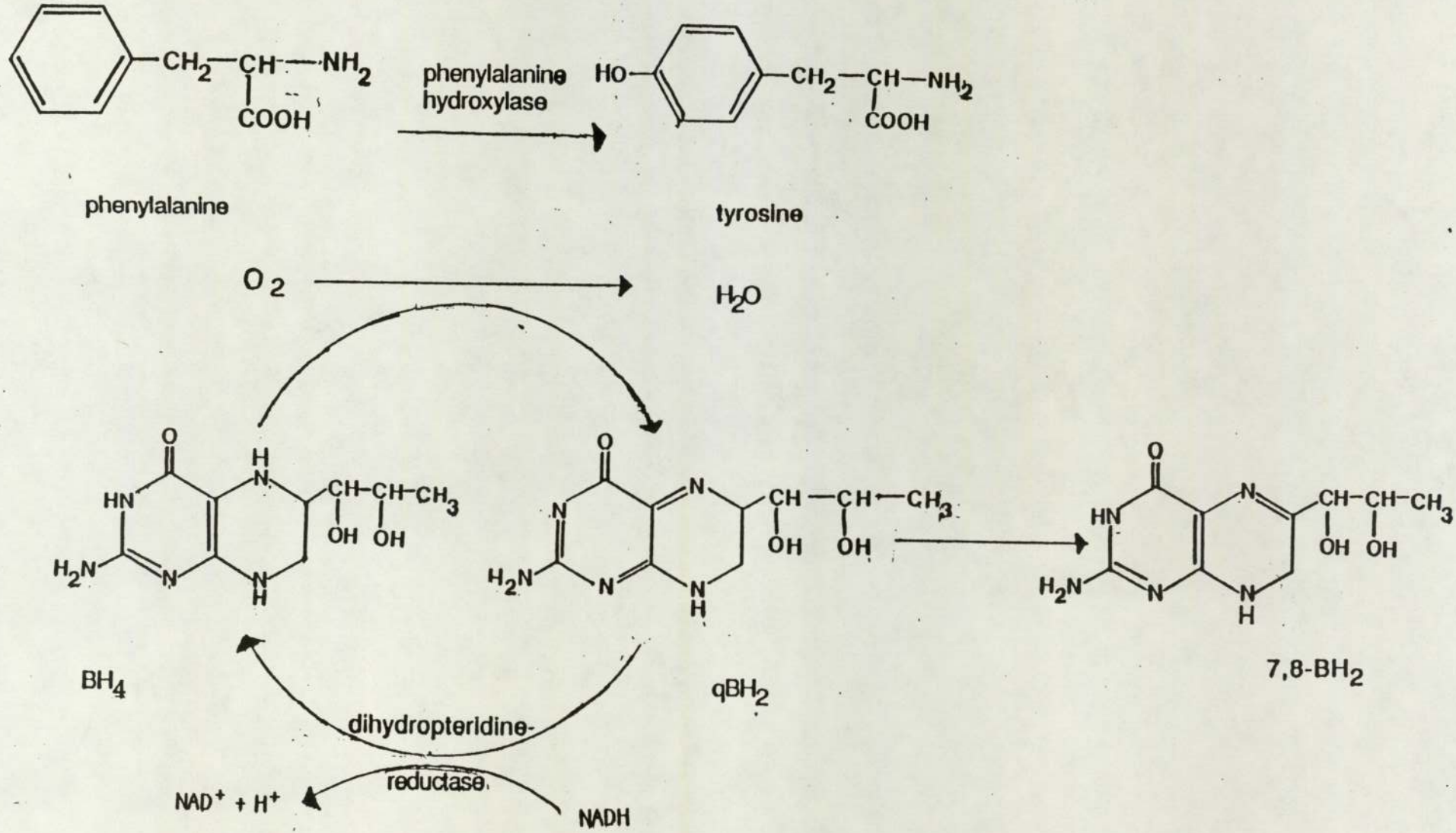


Figure 1.3. Cofactor activity of BH_4

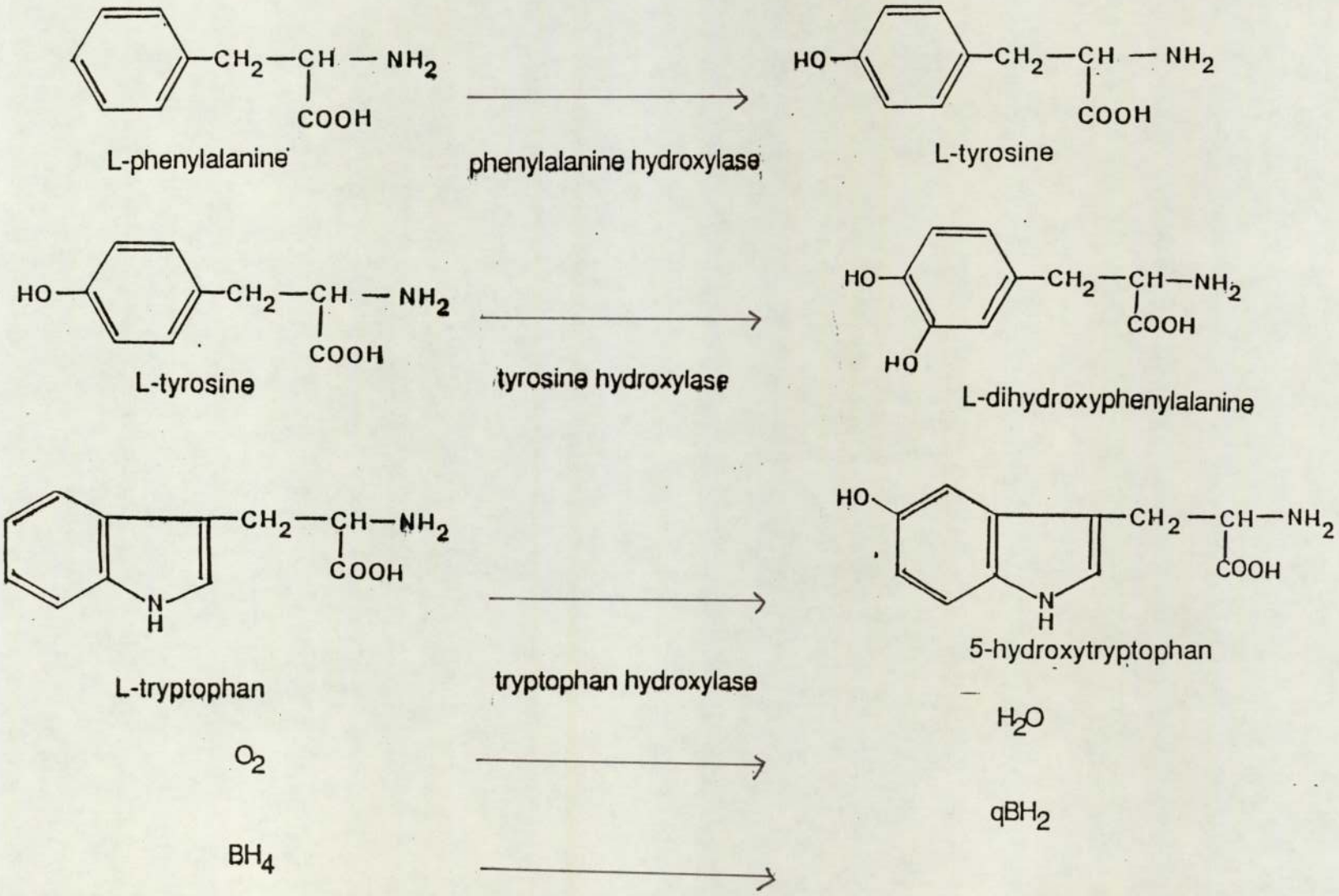
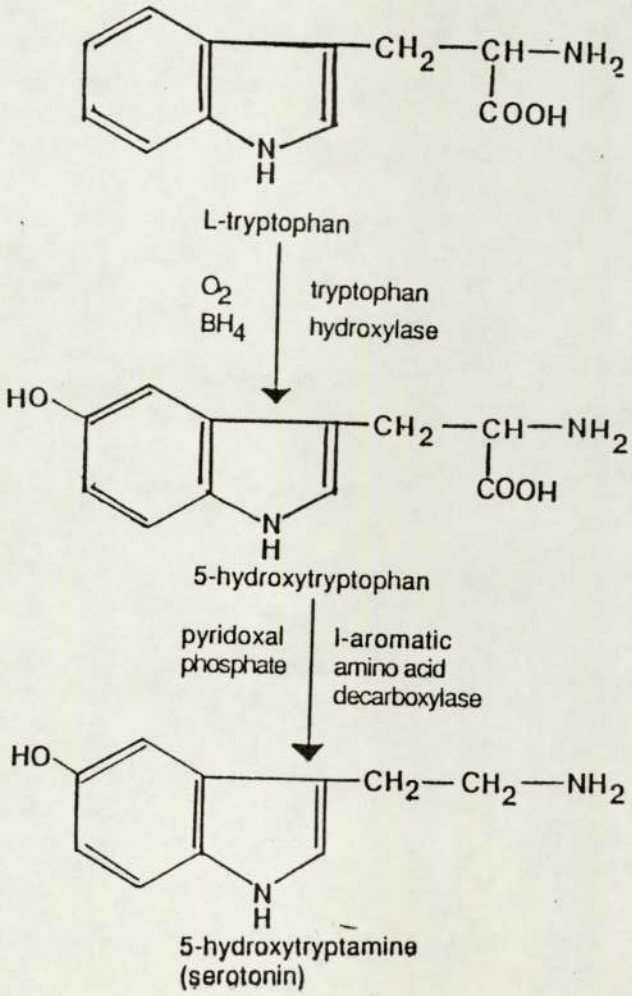


Figure 1.4 BH_4 dependent hydroxylase reactions

will be considered in more detail.

Tyrosine hydroxylase and tryptophan hydroxylase catalyse the initial and rate-limiting steps in the biosynthesis of the neurotransmitters dopamine, noradrenaline and serotonin (Figure 1.5 Nagatsu *et al* 1964, Levitt *et al* 1965, Mandell and Knapp 1974). BH_4 levels show regional variation within the brain (Bullard *et al* 1978, Leeming *et al* 1976, Fukushima and Nixon 1980) where it is primarily located within the catecholaminergic and serotonergic systems (Bullard *et al* 1978, Hennings and Rembold 1982, Levine *et al* 1981). Tyrosine hydroxylase exists in two forms: a high affinity form (Km 10-30μM) and a low affinity form (Km 100-600μM) with respect to BH_4 (Levine *et al* 1981, Vulliet *et al* 1980). Several lines of evidence suggest that BH_4 levels in catecholaminergic neurons are subsaturating for the low affinity form and possibly for the high affinity form of the enzyme. Thus it has been shown that intracellular BH_4 levels in adrenal medulla (10-20μM), cultured chromaffin cells (4μM) and rat striatum (100μM) are below the apparent Km of the low affinity form of the enzyme (Abou-Donia and Viveros 1981, Abou-Donia *et al* 1986, Levine *et al* 1981). The administration of exogenous BH_4 to rat striatum *in vivo*, rat striatum synaptosomes and adrenal medullary chromaffin cells in culture and cultured sympathetic neurones enhances tyrosine hydroxylation (and thus neurotransmitter biosynthesis) by the activation of the low affinity form of tyrosine hydroxylase. Further, it implies that factors which increase the availability of the cofactor will also influence tyrosine hydroxylase activity.

It has been shown that a variety of physiological and pharmacological stimuli convert the low affinity enzyme to the high affinity form: Ca^{2+} activates tyrosine hydroxylase *in vitro* at concentrations estimated to occur in the cell during membrane depolarization (Iuvone 1984); cAMP-dependent phosphorylation of tyrosine hydroxylase activates the enzyme and it is suggested that an analogous situation may occur on nerve stimulation (Lovenberg *et al* 1975); nerve growth factor activates tyrosine hydroxylase in PC12 cells in culture (Greene *et al* 1984).



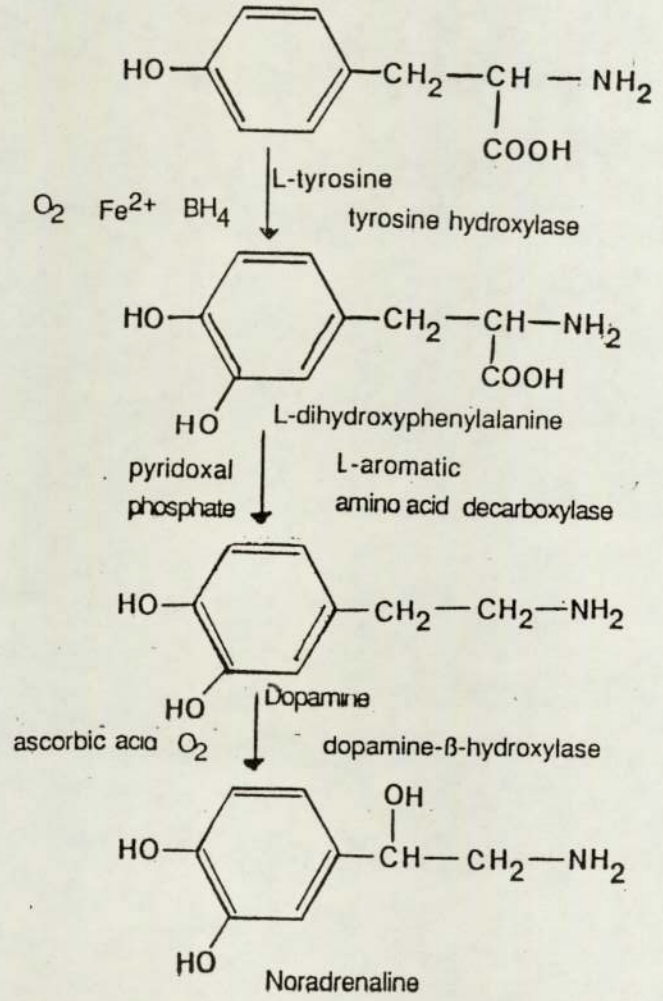
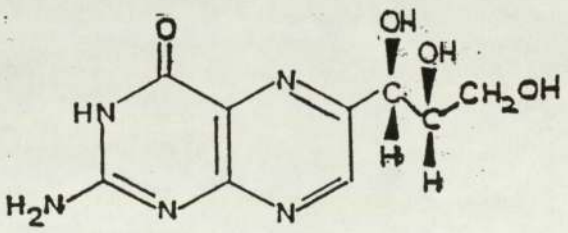


Figure 1.5 Biosynthesis of serotonin, dopamine and noradrenaline

Thus enzyme activation provides a sensitive mechanism for the rapid modification of catecholaminergic activity on appropriate stimulation.

1.1.4 Regulation of BH_4 biosynthesis

Although much attention has been focussed on the regulation of neurotransmitter biosynthesis little work has been carried out on the regulation of BH_4 synthesis. Indirect evidence suggests that species variation occurs in the control of BH_4 synthesis. Neopterin (VII) is undetectable in body fluids and tissues of lower mammals including the rat using high performance liquid chromatography, whereas neopterin is present in primate tissues and body fluids in similar quantities to biopterin (Fukushima and Nixon 1980, Duch *et al* 1984A). Nagatsu and co-workers (1984) were able to detect very low levels of neopterin in rat body tissues and fluids by using a sensitive radioimmunoassay procedure. However, GTP cyclohydrolase activity in human brain tissue is much lower than that in rat brain (Sawada *et al* 1986). It is suggested that pyruvoyl-H_4pterin synthase may be an important regulatory enzyme in human and monkey tissue (Blau and Niederwieser 1983, Duch *et al* 1984).



VII. Neopterin

GTP cyclohydrolase catalyses the initial step in BH_4 synthesis. Feedback control of GTP cyclohydrolase by BH_4 occurs *in vitro* (Bellahsene *et al* 1984, Fukushima *et al* 1977) and in mouse neuroblastoma cells in culture (Kapatos and Kaufman 1983). Abou-Donia and co-workers examined the regulation of GTP cyclohydrolase in adrenal medulla and cortex *in vivo* and in cultures of adrenomedullary chromaffin cells (Abou-Donia *et al* 1981, 1986, Viveros *et al* 1981). They showed that

depletion of cellular catecholamines by reserpine or insulin-induced hypoglycaemia enhances GTP cyclohydrolase activity by enzyme induction and increases BH_4 levels. There is differential control of GTP cyclohydrolase in the adrenal gland: increased splanchnic nerve discharge stimulates GTP cyclohydrolase synthesis in the adrenal medulla whereas release of ACTH by the pituitary gland increases GTP cyclohydrolase synthesis in the adrenal cortex. They further suggest that there is a cAMP-dependent mechanism by which GTP cyclohydrolase activity and BH_4 levels are increased in response to functional demand.

The regulation of BH_4 metabolism in the brain has not been as intensively studied as that in the adrenal gland. BH_4 synthesis does not appear to be under hormonal control in the brain (Duch *et al* 1986). Stimulation of dopamine autoreceptors enhances tyrosine hydroxylase and dopamine synthesis in dopamine nerve terminals in the brain but does not alter biopterin levels (Galloway and Levine 1986). cAMP-dependent activation of GTP cyclohydrolase does not operate in neuroblastoma cells in culture (Woolf *et al* 1986). Noradrenaline and serotonin inhibit brain sepiapterin reductase *in vitro* suggesting co-regulation of BH_4 synthesis and neurotransmitter synthesis (Katoh *et al* 1982). By analogy to the situation in the adrenal gland several factors are probably involved in the regulation of BH_4 metabolism in the brain and factors which stimulate tyrosine hydroxylase activity may also enhance BH_4 synthesis but the identity of such factors is unknown at the present time.

1.1.5 Malignant hyperphenylalaninaemia

In the mid 1970s a variant form of phenylketonuria was described in which patients developed neurological impairment despite good dietary control of phenylalanine levels and which was further characterised by abnormal pterin profile in serum and urine (Smith *et al* 1975, Leeming *et al* 1976). Subsequently metabolic defects at the level of DHPR or BH_4 synthesis were identified (Kaufman 1975, Firgaira *et al* 1981, Leeming *et al* 1976, Kaufman *et al* 1978, review by Dhondt 1984). Defects on the biosynthetic pathway have been identified at the level of

GTP cyclohydrolase (Niederwieser *et al* 1984) and pyruvoyl-H_4pterin synthase (Niederwieser *et al* 1985). Patients with BH_4 deficiency show progressive neurological deterioration indicating the importance of BH_4 in normal development. Further support for the key role of BH_4 in neurotransmitter biosynthesis is provided by the demonstration that CSF and urinary levels of biogenic amines and their metabolites are greatly reduced in BH_4 deficiency (Brewster *et al* 1979, Niederwieser *et al* 1984, Koslow and Butler 1977). These may be increased by the use of 6-methyltetrahydropterin replacement therapy with a concomitant improvement of neurological symptoms in some but not all patients (Kaufman *et al* 1983, Niederwieser *et al* 1984, McInnes *et al* 1984). The neurological consequences of BH_4 deficiency imply that alterations in BH_4 metabolism may contribute to the pathogenesis of other neurological disorders.

1.1.6 BH_4 metabolism and neurological disorders

Biopterin levels in body fluids and tissues have been measured for several neurological disorders (Tables 1.1, 1.2). The primary aim of this project is to examine BH_4 metabolism in the CNS particularly in relation to ageing and senile dementia of the Alzheimer type (SDAT). SDAT is an age-related disorder in which there is gradual and progressive loss of cognitive and intellectual function accompanied by characteristic neuropathological changes, principally neurofibrillary degeneration and senile plaques (Reisberg 1983, Review: British Medical Bulletin 1986). While this work was in progress several groups also published reports on altered BH_4 metabolism in dementia. It has been shown that serum and CSF biopterin levels are reduced in SDAT and there is an increase in the neopterin:biopterin (N:B) ratio in the CSF (Table 1.2). Measurement of pterins in body fluids is an indirect index of levels in the brain. Biopterin (B) levels show regional variation in brain tissue (Bullard *et al* 1978, Leeming *et al* 1976, Fukushima and Nixon 1980, Nagatsu *et al* 1986). Neuropathological alterations in SDAT show regional selectivity with the hippocampus and temporal cortex being most affected (Brun

1983). Thus it is important when trying to establish the role of BH_4 in SDAT to examine several regions of the brain. Barford and co-workers (1983) reported that the N:B ratio is increased and the capacity to synthesize BH_4 is diminished in the temporal lobe of SDAT patients. Nagatsu and co-workers (1986) reported that biopterin levels are reduced in the locus coeruleus and substantia nigra of SDAT patients. This report examines BH_4 metabolism in SDAT brain in order to further clarify the defect in BH_4 metabolism which occurs in this disorder.

Aluminium is a neurotoxin. People on long-term renal dialysis who develop encephalopathy have extremely high levels of aluminium in body fluids and aluminium is implicated as the causative agent in this disorder (Sideman and Manor 1982). Aluminium levels are raised in SDAT brain and are localised in the central region of the senile plaque core (Candy *et al* 1986). There is evidence that aluminium may disrupt BH_4 metabolism. Aluminium inhibits DHPR *in vitro* and *in vivo* (Leeming and Blair 1979, Brown 1981, Dhondt and Bellahsene 1983). Dhondt and co-workers (1982) found an increase in the N:B ratio in the serum of patients on maintenance dialysis. This investigation assesses the role of aluminium in the alteration of BH_4 metabolism *in vitro* and *in vivo*

Table 1.1 BH_4 metabolism in neurological disorders

Neurological Disorder	Tissue	Observation[#]	Reference
Down's Syndrome	serum	↑BH_2	Aziz *et al* 1982
	blood	normal DHPR	Barford *et al* 1983
Huntington's Disease	CSF	↓BH_4	Williams *et al* 1980
Parkinson's Disease	CSF	↓BH_4	Lovenberg *et al* 1979
	CSF	↓BH_4	Lovenberg *et al* 1979
	CSF	↓BH_4	Williams *et al* 1980
	caudate nucleus	↓GTP cyclohydrolase	Sawada *et al* 1986
	caudate nucleus	↓GTP cyclohydrolase	Nagatsu *et al* 1986
Unipolar depression	urine	↑biopterin	Duch *et al* 1984
	urine	↑neopterin	Duch *et al* 1984
	urine	normal biopterin	Blair *et al* 1984
Bipolar depression	urine	↓biopterin	Blair *et al* 1984
	urine	normal biopterin	Duch *et al* 1984
	urine	↑neopterin	Duch *et al* 1984
Depression	urine	↑biopterin	Garbutt *et al* 1985
	temporal lobe	↓BH_4 synthesis	Blair *et al* 1984
Schizophrenia	urine	normal biopterin	Duch *et al* 1984
	urine	normal neopterin	Duch *et al* 1984
	CSF	normal BH_4	Garbutt *et al* 1985
Shy-Drager Syndrome	CSF	↓BH_4	Williams *et al* 1980
Steele-Richardson Syndrome	CSF	↓BH_4	Williams *et al* 1980
Adult onset dystonia	CSF	normal BH_4	Williams *et al* 1980
Dystonia	CSF	↓N↓B	LeWitt *et al* 1986
Essential tremor	CSF	↓BH_4	Williams *et al* 1980

[#] biopterin and neopterin refers to the total oxidized pterin.

Table 1.2. BH_4 metabolism in dementia

Diagnosis	Tissue	Observation[#]	Reference
Presenile dementia	CSF	↓BH_4	Williams *et al* 1980
SDAT	serum	↓biopterin	Leeming and Blair 1980
	CSF	normal neopterin	Morar *et al* 1983
	CSF	↓biopterin	Morar *et al* 1983
	CSF	↓biopterin	LeWitt *et al* 1985
	CSF	normal neopterin	LeWitt *et al* 1985
	serum	↓biopterin	Aziz *et al* 1983
	plasma	↓biopterin	Young *et al* 1982
	plasma	normal neopterin	Young *et al* 1982
	lymphocyte	↓DHPR	Young *et al* 1982
	erythrocyte	normal DHPR	Jeeps *et al* 1986
	temporal lobe	↓BH_4 synthesis	Barford *et al* 1984
	temporal lobe	↕N:B ratio	Barford *et al* 1984
	temporal lobe	normal DHPR	Barford *et al* 1984
	locus coeruleus	↓biopterin	Nagatsu *et al* 1986
	substantia nigra	↓biopterin	Nagatsu *et al* 1986

[#]biopterin and neopterin refers to total oxidized pterin.

CHAPTER 2

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Animals

Male Wistar rats, grade 4, (Bantin and Kingman Ltd.) weighing approximately 200g (age 50-60 days) were used. The animals were fed rat and mouse breeding diet *ad libitum*.

2.1.2 Human tissue samples

Human brain samples were stored at -80°C. Tissue samples from the locus coeruleus, temporal cortex (Brodmann areas 20/21, Br20/21) and frontal cortex (Brodmann area 9, Br9) of senile dementia of the Alzheimer type (SDAT) patients and age-matched controls were provided by Dr. Reynolds of the MRC Brain Bank, Cambridge (Table 2.1). SDAT was confirmed postmortem (PM) by the presence of numerous senile plaques and neurofibrillary tangles. Postmortem delay in the control group was greater than in the SDAT group. Tissue samples from the temporal cortex (Br 21) of Huntington's disease (HD) and age-matched control patients were provided by Dr. Reynolds of the MRC Brain Bank, Cambridge (Table 2.2). Postmortem delay in the control group was greater than in the HD group. Statistical analysis using the least squares method showed no correlation between PM delay and DHPR, GTP cyclohydrolase, sepiapterin reductase or BH_4 synthesis.

Tissue samples from the inferior temporal cortex and frontal cortex of neurologically normal subjects including four patients on renal dialysis were provided by Dr. Altmann at the London Hospital, Whitechapel (Table 2.3). Subject A.H. showed early signs of encephalopathy. Subject D.T. showed evidence of neurofibrillary degeneration on histopathological examination.

2.1.3 Chemicals

Tris base, NADPH, coenzyme B12, ascorbate (sodium salt), EDTA (disodium salt), GTP, sodium azide, horseradish peroxidase, pterin, 6-methyl-5,6,7,8-tetrahydropterin and alkaline phosphatase, type III, from *E.coli* were obtained from Sigma Chemical Company. Alkaline phosphatase (Grade 1) from calf intestine, lyophilized alkaline phosphatase (Grade 1) from calf intestine and alkaline phosphatase (Grade 3) suspension from calf intestine were obtained from Boehringer Mannheim GmBH, W. Germany. Sephadex G-25M columns (PD-10) were obtained from Pharmacia, Uppsala, Sweden. L-erythrobiopterin, D-threoneopterin and sepiapterin were obtained from Dr. Schirks, Wettswill A.A., Switzerland. $MgCl_2$, KCl, methanol (HPLC grade) and glycerol were obtained from Fisons PLC (UK). Calcium-5-methyl-tetrahydrofolate.$4H_2O$ was obtained from Eprova, Switzerland.

2.1.4 High performance liquid chromatography (HPLC)

Automated sample injection was carried out by a Waters Intelligent Sample Processor 710B (Waters Associate Inc. USA). Samples were chromatographed on Spherisorb S5 ODS1 analytical column (Phase Sep Ltd.). Pterins were detected by measuring the relative fluorescence at 360nm excitation wavelength and 450nm emission wavelength using a spectrofluorometer, model SFM23 (Kontron, USA) and a W+W chart recorder, model 302 (Scientific Instruments, Switzerland). A flow rate of 1ml/minute of 5% methanol through the system was achieved using a Constametric model III pump (Data Control, LDC, UK). To obtain a standard curve neopterin or biopterin was dissolved in water and the concentration determined by measuring the absorbance at 360nm in 0.1 M NaOH using the molar extinction coefficient 8.3 x 10^3 M^{-1} cm^{-1} (Fukushima and Nixon 1980). The concentrated standard solution was serially diluted and analysed by HPLC. A typical standard curve for biopterin and neopterin is shown in figure 2.1.

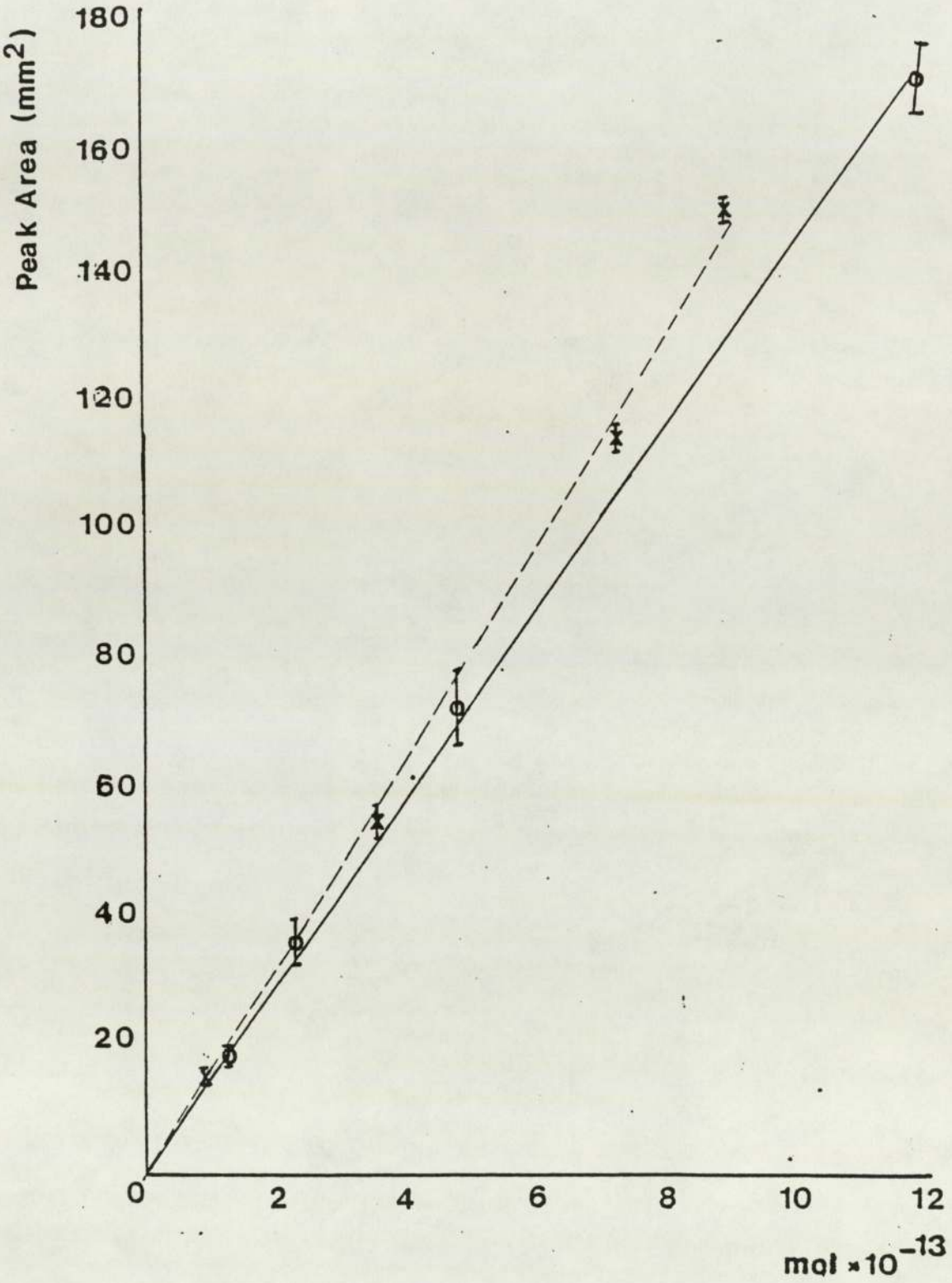


Figure 2.1 Standard curve for neopterin and biopterin using HPLC with spectrofluorometric detection (Mean. n=2)

neopterin x---x biopterin o——o

2.2 METHODS

2.2.1 Preparation of enzyme source

Human or rat tissue was homogenised in 0.1 M Tris buffer, pH 7.6 and centrifuged at 40000 rpm, 4 °C for 45 minutes in a MSE Superspeed centrifuge (Measuring and Scientific Equipment Ltd.). The supernatant was assayed for dihydropteridine reductase, tetrahydrobiopterin synthesis, GTP cyclohydrolase and sepiapterin reductase activity. Protein was measured by the Biuret method.

2.2.2 Dihydropteridine reductase assay

The method used was based on that of Craine and co-workers (1972). The reaction mixture contained 0.05 M Tris buffer, pH 6.8, 2.5×10^{-4} M sodium azide, 8µg horseradish peroxidase, 1×10^{-4} M NADH, 1×10^{-3} M hydrogen peroxide, 20µl supernatant and 1×10^{-4} M 6,7-dimethyl-5,6,7,8-tetrahydropterin ($DMPH_4$) in a total volume of $1cm^3$. The mixture was preincubated at 37°C in the absence of $DMPH_4$ for 90 seconds. $DMPH_4$ was added and the decrease in absorbance at 340nm measured 30 seconds later. Blanks contained no supernatant. A second blank in which $DMPH_4$ was omitted was also assayed. The extinction coefficient of NADH at 340nm is 6.17×10^3 M^{-1} cm^{-1}. DHPR activity is expressed as nmol NADH consumed/mg protein/minute.

2.2.3 Tetrahydrobiopterin synthesis assay

Tetrahydrobiopterin synthesis was determined by the method of Brown (1981) as described by Barford *et al* (1984). Preliminary experiments were carried out to determine the optimal conditions for the measurement of BH_4 synthesis in brain preparations. The optimum pH was 8.0 for rat brain and 7.6 for human brain preparations. The optimum substrate concentrations were 6×10^{-3} M GTP and 6×10^{-3} M $MgCl_2$ for rat brain and 4×10^{-3} M GTP and 4×10^{-3} M $MgCl_2$ for human brain preparations. 100µl of brain preparation was incubated for

3 hours at 37°C in the dark in a medium containing GTP, $MgCl_2$, 1.07 x 10^{-2} M Tris buffer and 3 x 10^{-3}M NADPH in a total volume of $1cm^3$. Blanks containing no $MgCl_2$ and no GTP were used to determine endogenous BH_4. The reaction was terminated with $2cm^3$ of 0.1M HCl and reduced biopterins were oxidized with iodine. Oxidation was stopped by the addition of ascorbate. The sample was concentrated by freeze-drying. Biopterin produced was measured by HPLC.

2.2.4 GTP cyclohydrolase assay

The method used was based on that of Duch and co-workers (1984A). The supernatant was first passed through a G-25 minicolumn equilibrated with 0.1 M Tris buffer pH 7.8 containing 2.5 x 10^{-3} M EDTA, 0.3 M KCL and 10% glycerol. The desalted preparation was assayed for GTP cyclohydrolase activity. It was found that near saturating conditions for both the rat and the human enzyme preparation were achieved with 0.06 M GTP (Figure 2.2 and 2.3 respectively). 50µl of the eluate was incubated in the presence of 0.06 M GTP in a total volume of 62.5 µl for 90 minutes at 37°C. Blanks in which GTP or enzyme source were omitted were also assayed. 6.5 µl of 1 M HCl containing 1%I / 2%KI was added and the incubation continued for a further 30 minutes. Excess iodine was reduced with 6.5 µl of 2% ascorbate and the pH adjusted with 6.5 µl of 1 M NaOH. Neopterin triphosphate was dephosphorylated by adding 0.5 units of alkaline phosphatase and incubating for a further 60 minutes. The reaction was terminated with 12.5 µl of 1 M acetic acid. The sample was centrifuged and neopterin produced measured by HPLC. A major problem has been the source of alkaline phosphatase. Several phosphatases were tested and found to have GTP cyclohydrolase activity which made the determination of endogenous activity impossible. The following alkaline phosphatases were found to be unsuitable: alkaline phosphatase (grade1) from calf intestine, lyophilized alkaline phosphatase (grade1) from calf intestine and alkaline phosphatase (grade3) suspension from calf intestine obtained from Boehringer Mannheim GmbH and alkaline phosphatase from calf intestine obtained from Sigma Chemical Company.

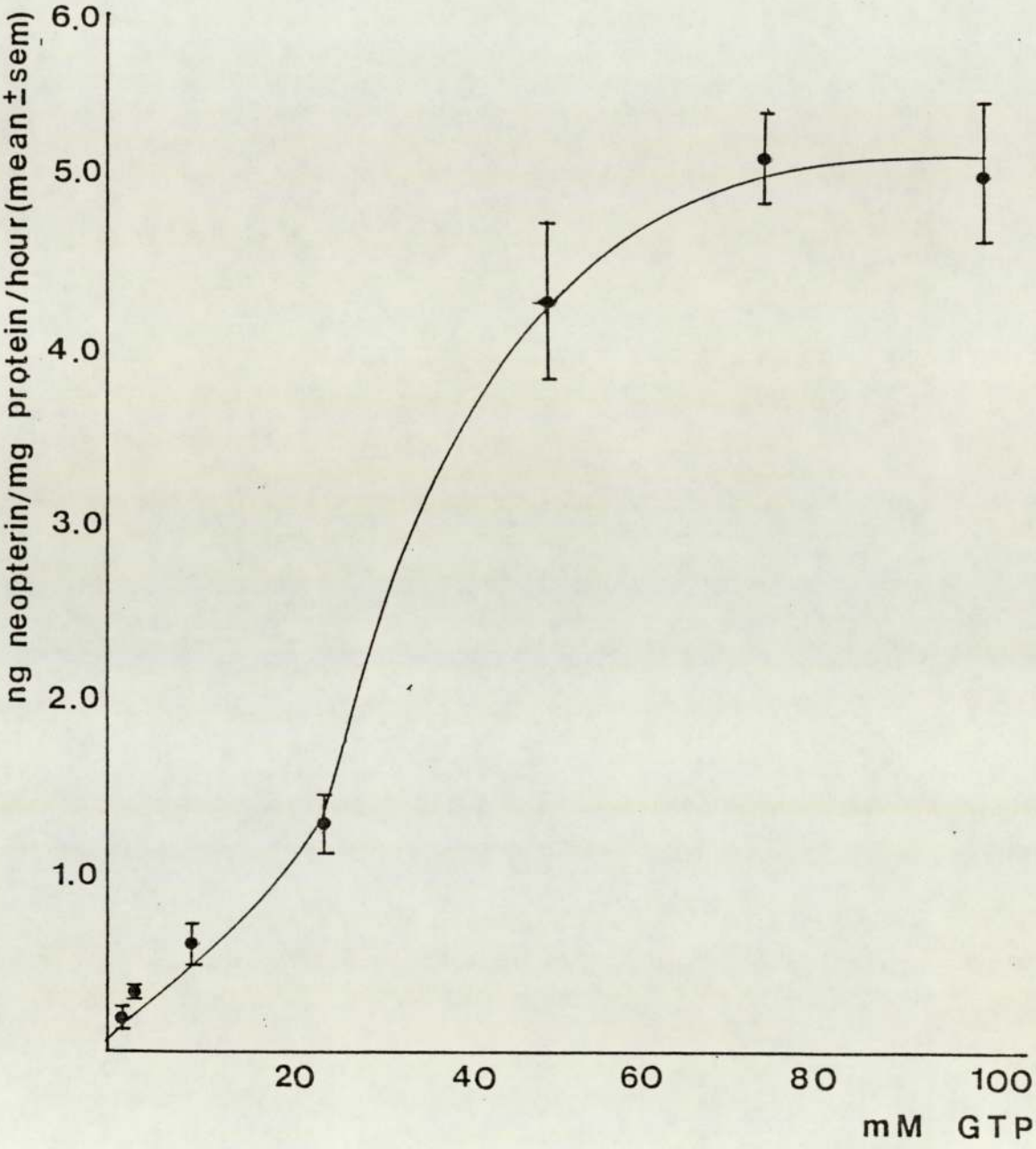


Figure 2.2 GTP cyclohydrolase activity in rat brain preparations as a function of GTP concentration. n=5

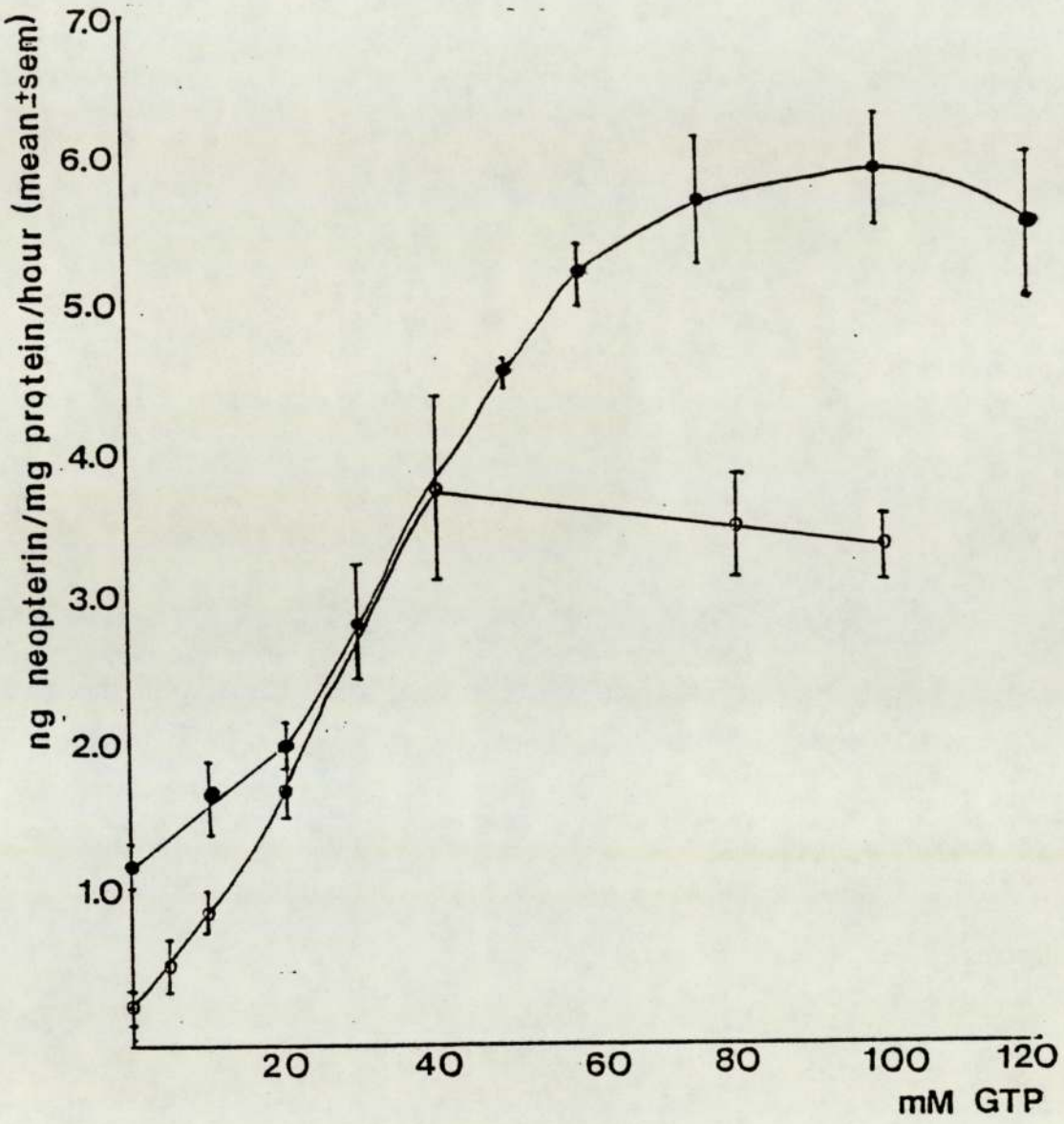


Figure 2.3 GTP cyclohydrolase activity in human frontal cortex preparations as a function of GTP concentration (Patient C.B o——o. Patient J.H ●——●. n=5)

Alkaline phosphatase, type III, from *E. coli* (P4252, Sigma Chemical Company) was found to be the most satisfactory and has been used in the major portion of this work. Unfortunately later batches of this phosphatase were found to be contaminated with GTP cyclohydrolase activity: when the assay was carried out in the absence of enzyme preparation neopterin, or a compound which co-chromatographs with neopterin on HPLC analysis, was formed. The quantity of this compound was greater than that produced by GTP cyclohydrolase *in vitro* making the determination of endogenous GTP cyclohydrolase activity impossible. Both GTP and alkaline phosphatase were required for the synthesis of this compound. Due to the difficulty in obtaining a pure source of alkaline phosphatase later measurements of GTP cyclohydrolase activity were made in the absence of alkaline phosphatase. 50 µl of the desalted enzyme preparation was incubated with 0.1 M GTP in a total volume of 62.5 µl for 90 minutes at 37° C and following acid/ iodine oxidation the sample was assayed for neopterin (Figure 2.4). Tissue preparations assayed in this way are indicated in the text.

2.2.5 Sepiapterin reductase assay

The method used was based on that of Katoh (1971) except that Tris buffer pH7.4 was used. Preliminary studies to determine the optimum pH, substrate concentration and cofactor concentration confirmed that the conditions described by Katoh (1971) are satisfactory for the measurement of sepiapterin reductase activity in rat brain preparations. The incubation medium contained 50 µl of enzyme, 50 µM sepiapterin, 100 µM NADPH and 0.085 M Tris buffer, pH 7.4 in a total volume of $1cm^3$. The enzyme source was omitted from blanks. The decrease in absorbance at 420nm was measured and the results are expressed as nmol sepiapterin consumed/mg protein/minute. The extinction coefficient of sepiapterin at 420nm is $10.2 \times 10^3\ M^{-1}cm^{-1}$ (Katoh 1971). The optimum substrate concentration for the measurement of sepiapterin reductase in human brain tissue was 50 µM sepiapterin.

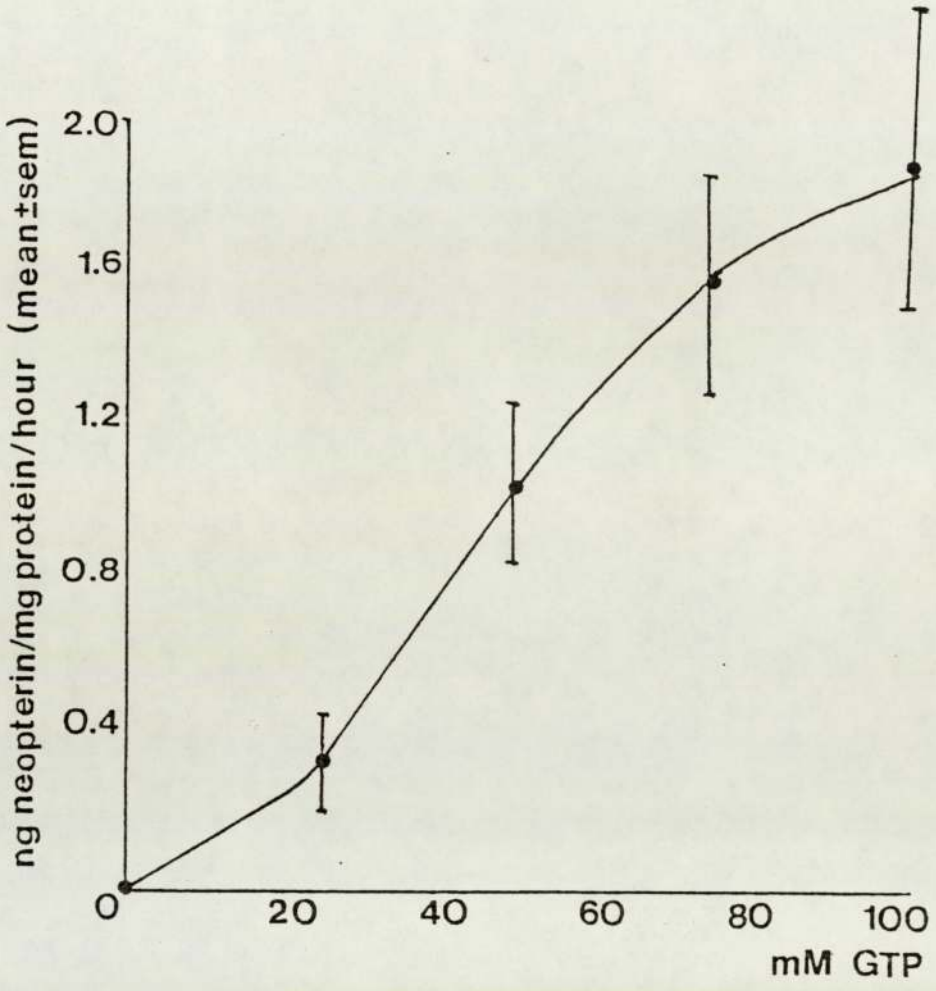


Figure 2.4 GTP cyclohydrolase activity in rat brain preparations as a function of GTP concentration, determined without the use of alkaline phosphatase. n=3

2.2.6 Determination of tissue biopterin

The method used was based on that of Fukushima and Nixon (1980). 1g of tissue was homogenised in $3cm^3$ of 0.1 M HCl and $1cm^3$ of 20% trichloroacetic acid. The homogenate was centrifuged at 40000 rpm for 5 minutes (MSE superspeed centrifuge, Measuring and Scientific Equipment Ltd.). 500 µl of iodine solution (0.5% I_2/1% KI) was added to $2cm^3$ supernatant and incubated at room temperature, in the dark, for 1 hour. Oxidation was terminated with ascorbate. Total tissue biopterin was measured by HPLC.

Table 2.1 Clinical status of control patients (code C) and SDAT patients (code D)

Code number	Sex	Age years	Postmortem delay (hours)	Cause of death
Group A Temporal cortex (Br20/21)				
C241	M	71	57	
C236	M	72	69	Acute myocardial ischaemia
C253	F	74	144	Aspiration of vomitus
C238	F	74	24	Pulmonary embolism
C244	M	74	49	
C254	F	76	47	Cerebral infraction
C257	F	83	48	Bronchopneumonia
C258	F	85	87	Congestive cardiac failure
C278	F	87	73	
C240	F	89	72	
mean ±SD	7F,3M	77±6	67±32	
Group B Temporal cortex (Br20/21)				
D61	F	71	72	
D39	M	71	32	
D45	F	74	50	Bronchopneumonia
D60	F	76	19	Bronchopneumonia
D50	M	80	36	
D59	F	82	22	Congestive cardiac failure
D52	M	87	28	
D38	F	90	11	
D51	F	97	43	
mean±SD	6F,3M	80±10	35±18	
Group C Frontal cortex (Br9)				
C236	M	72	69	Acute myocardial ischaemia
C238	F	74	24	Pulmonary embolism
C254	F	76	47	Cerebral infraction
C256	M	79	89	
C242	F	79	63	Bronchopneumonia
C257	F	83	48	Bronchopneumonia
C258	F	85	87	Congestive cardiac failure
C249	F	85	44	
C267	F	87	79	
C278	F	87	73	
mean±SD	8F,2M	81±5	62±21	

Table 2.1 continued

Code number	Sex	Age years	Postmortem delay (hours)	Cause of death
Group D Frontal cortex (Br9)				
D39	M	71	32	
D40	F	72	20	
D48	F	74	11	
D60	F	76	19	Bronchopneumonia
D59	F	82	20	Congestive cardiac failure
D38	F	90	11	
mean ±SD	5F,1M	78±7	19±8	
Group E Locus coeruleus				
C270	F	72	65	Pulmonary embolism
C236	M	72	81	Acute myocardial ischaemia
C242	F	79	63	Bronchopneumonia
C262	F	87	35	Bronchopneumonia
C266	F	91	62	*E. coli* septicaemia
mean±SD	4F,1M	80±9	61±16	
Group F Locus coeruleus				
D33	F	72	28	Bronchopneumonia
D35	M	73	27	
D56	F	80	67	
D54	F	87	26	Bronchopneumonia
D32	M	90	58	
mean±SD	3F,2M	80±8	41±20	

Samples provided by Dr. Reynolds MRC Brain Bank Cambridge.

Table 2.2 Clinical staus of control patients (code C) and Huntington's disease patients (code H)

Code number	Sex	Age years	Postmortem delay (hours)
Group C			
Temporal cortex			
C383	M	59	48
C263	M	62	98
C379	M	63	64
C375	M	69	66
C311	F	72	93
mean±SD	1F,4M	65±5	73±20
Group H			
Temporal cortex			
H311	M	59	48
H307	M	61	53
H305	M	65	22
H301	F	68	52
H296	M	71	34
mean±SD	1F,4M	65±5	42±13

Samples provided by Dr. Reynolds, MRC Brain Bank Cambridge.

Table 2.3 Clinical status of control patients including 4 patients on peritoneal dialysis.

Code number	Sex	Age Years	Postmortem delay (hours)	Duration of dialysis treatment	Cause of death
Group J*					
D.T	F	40	42	2.5 years	Septicaemia
V.H	F	53	10.5	8 months	Cardiac arrest
K.G	F	60	48	11 months	Cardiac arrest
A.H	F	62	3	13 years	Septicaemia
Mean ± SD	4F	54±10	25±22		
Group K*					
Mi	M	45	29		Vascular
Ge	M	48	41		Cardiac arrest
Al	M	53	41		Polyarteritis
Co	F	56	40		Pulmonary embolism
Th	M	66	48		Carcinoma
Ho	M	75	62		Carcinoma
Mean±SD	5M,1F	57±11	44±11		

*Frontal and temporal cortex were provided for each patient by Dr. Altmann, London Hospital, Whitechapel

CHAPTER 3

BH_4 METABOLISM IN NORMAL BRAIN, AGING AND DEMENTIA

3.1 INTRODUCTION

3.1.1 Alterations in the monoaminergic systems in the CNS in aging

Aging is associated with a decline in intellectual function often termed 'benign senescent forgetfulness' (Reisberg 1983). There is substantial evidence that the catecholaminergic systems are altered in the aging brain. Dopamine and noradrenaline levels are reduced in several brain regions (Winblad *et al* 1985, Robinson *et al*1977, Yates *et al* 1983). Tyrosine hydroxylase and dopa decarboxylase activity decline with age (Winblad *et al* 1985, Mayeux *et al* 1983) whereas monoamine oxidase activity shows a positive correlation with age (Mayeux *et al* 1983). The disturbances in the catecholaminergic systems are accompanied by cell loss and atrophy of remaining neurons in the substantia nigra and locus coeruleus (Mann *et al* 1980, Tomlinson *et al* 1981, Mann *et al* 1983, Carlsson *et al* 1985). The concentration of the monoamine metabolite homovanillic acid (HVA) in CSF shows a positive correlation with age but HVA turnover declines with age, whereas 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-hydroxyindoleacetic acid do not correlate with age (Stahl 1985).

The serotonergic system has not been extensively examined in the aging brain. It has been reported that 5-HT levels in the brain decline with age (Mackay *et al* 1978, Carlsson *et al*1985). The enzymes involved in the biosynthesis of 5-HT have not been measured in the aging brain.

The role that BH_4 plays in the neurological deficits of aging has not been examined in detail. Leeming and Blair (1980) reported that serum biopterin levels increase with age suggesting that there is a loss of DHPR activity in the aged individual. Lewitt *et al* (1982) reported an increase

in the neopterin:biopterin ratio in CSF with age. This suggests that reduced cofactor availability may contribute to the neurochemical deficits of aging.

3.1.2 Alzheimer's disease (AD)

At least 5% of people over the age of 65 suffer from some form of impaired cognitive and intellectual function, termed dementia, and of these 60% are believed to have Alzheimer's disease (Roth 1980, Gottfries 1985, for a review of the subject refer to the British Medical Bulletin 1986). Alzheimer's disease is a neurological disorder with insidious onset in which there is progressive deterioration of intellectual function. It is difficult to diagnose, especially during the initial stages which are characterized by memory deficits which also occur in normal aging. As the disease progresses the patient becomes increasingly unable to cope with normal daily functions, behavioural changes occur and ultimately results in a state of total incapacitation (Reisberg *et al* 1982). Diagnosis of Alzheimer's disease is usually confirmed postmortem by the presence of numerous senile plaques and neurofibrillary tangles in the neocortex, hippocampus and certain subcortical neurones (Alzheimer 1907). The neuropathological and neurochemical changes of AD occur to a lesser extent in the aging brain suggesting that AD may be a continuum of the normal aging process.

The major atrophic changes in Alzheimer's disease primarily involve the hippocampus and neocortex, particularly the inferior temporal cortex and post-central parietal cortex (Brun 1983). In younger patients degenerative changes occur in the subcortical nuclei including the locus coeruleus and the raphe nuclei (Ishii1966, Tomlinson 1981, Forno 1978). Initial work showed that there is a deficit in the cholinergic system in Alzheimer's disease (Perry *et al* 1977, Bowen 1983). Recent studies demonstrate a deficit in the noradrenergic and serotonergic systems in AD. The presence of neurofibrillary tangles in the raphe nuclei and locus coeruleus implicated the serotonergic and noradrenergic systems in the pathogenesis of Alzheimer's disease (Ishii 1966, Tomlinson 1981, Mann *et al* 1983). There is a loss of neurones from the locus coeruleus in AD compared with age-matched controls

particularly in younger patients (Forno 1978, Tomlinson *et al* 1981, Bondareff *et al* 1982). Remaining cells are atrophied (Forno 1978) and have reduced capacity for protein synthesis (Mann *et al* 1981, 1984). The degenerative changes in the locus coeruleus are accompanied by reductions in dopamine-ß-hydroxylase activity in the cerebral cortex (Perry *et al* 1981, Cross *et al* 1981). Noradrenaline levels are reduced in several brain regions in AD (Rossor *et al* 1984, Arai *et al* 1984).

In contrast, the dopaminergic system appears to be intact in Alzheimer's disease. There is no evidence of cell loss or atrophy of the substantia nigra in AD (Mann *et al* 1980). Dopamine levels in the caudate nucleus are reduced in AD (Winblad 1985), whereas dopamine levels in the hippocampus and neocortex, structures which show the greatest neuropathological changes, are normal in AD patients (Mann *et al* 1980, Arai *et al* 1984).

Several lines of evidence point to serotonergic dysfunction in Alzheimer's disease. There are extensive reductions in 5-HT levels in AD brains (Arai *et al* 1984). 5-HIAA is reduced in the hippocampus and neocortex of SDAT patients (Cross *et al* 1983, Gottfries *et al* 1983, Arai *et al* 1984). Pre- and post-synaptic receptors are diminished particularly in the temporal cortex and hippocampus suggesting that synaptic transmission is limited in AD particularly in younger patients (Bowen *et al* 1983, Crow *et al* 1984).

There is strong evidence that the capacity to synthesize BH_4 is diminished in the CNS in Alzheimer's disease. CSF biopterin levels are reduced in SDAT subjects (Lewitt *et al* 1985, Williams *et al* 1980, Morar *et al* 1983). There is a decrease in biopterin levels in the locus coeruleus and substantia nigra and an increase in the neopterin:biopterin ratio in the temporal cortex in SDAT (Nagatsu *et al* 1986, Barford *et al* 1984). Barford *et al* (1984) reported that the capacity for BH_4 synthesis is lower in the temporal cortex of SDAT subjects compared to age-matched controls. This suggests that cofactor insufficiency may contribute to the monoamine deficits of Alzheimer's disease.

3.1.3 Huntington's disease

Huntington's disease (HD) is an autosomal dominant disorder characterized by progressive involuntary choreiform movements and dementia (Huntington 1872). The onset of the disease occurs in the mid 40s although behavioural deficits may occur prior to this. The underlying defect of HD is unknown. The major pathological changes are gross atrophy of the basal ganglia, especially the corpus striatum and, to a lesser extent, the frontal and occipital lobes (Klintworth 1973, Bruyn *et al* 1979). The search for a specific neurochemical deficit has centred on the basal ganglia. The substantia nigra is a major source of innervation to the neostriatum. Several lines of evidence point to dopaminergic involvement in HD. The choreic movements of HD can be alleviated with substances which diminish dopaminergic activity in the basal ganglia. Conversely substances which enhance dopaminergic activity aggravate chorea (Barbeau 1973). Tyrosine hydroxylase activity is increased in the corpus striatum of HD patients (Bird 1980). Stahl *et al* (1985) reported reduced HVA levels in CSF but normal dopamine turnover. Spokes (1980) reported that dopamine levels are increased in the basal ganglia and substantia nigra but suggested that in view of the atrophy of the basal ganglia there is probably a net loss of dopamine in HD. The cholinergic and gamma-aminobutyric acid (GABA)systems are substantially reduced in HD (Barbeau 1979, Bruyn *et al* 1979, Spokes 1980). It is suggested that there is overactivity of the dopaminergic system relative to the cholinergic and GABA systems in the basal ganglia. The imbalance of the neurotransmitter systems leads to the neurological deficits of HD.

BH_4 metabolism in HD has not been examined in depth. Williams *et al* (1980) reported that BH_4 levels in the CSF are reduced in HD suggesting that BH_4 metabolism is altered in HD brain.

This report examines the biosynthetic and salvage pathways for BH_4 in the aging brain, Alzheimer's disease and Huntington's disease in order to clarify the role of BH_4 in these neurological disorders.

3.2 METHODS

The clinical status of all subjects and the methods used have been previously described (Chapter 2). GTP cyclohydrolase activity was determined with the use of alkaline phosphatase. The measurement of DHPR activity in Brodmann area 9 of control and SDAT subjects was carried out by Dr. F. Al-Salihi.

3.2.1 Statistical analysis

Correlation coefficients were calculated using the least squares method. Data for BH_4 synthesis and DHPR activity from control and SDAT brains were analysed by one-way analysis of variance. Comparison between brain types was then made by t-test (Snedicor and Cochran 1980). Data for GTP cyclohydrolase and sepiapterin reductase were analysed by Student's t-test. Dr. C. Q. Mountjoy (St. Andrews Hospital, Northhampton) provided data on the correlation between BH_4 metabolism in the locus coeruleus and noradrenaline levels in the cerebral cortex (Brodmann areas 10, 21, 24), average locus coeruleus cell counts and dementia score.

3.3 RESULTS AND DISCUSSION

Individual values are presented in tables 3.9 and 3.10.

3.3.1 BH_4 metabolism in the aging brain

The first study on the effect of age on BH_4 synthesis by human brain preparations indicated that BH_4 synthesis declined with age in the temporal cortex ($r = -0.523$, $P < 0.05$, $n=16$. Patient groups A, G, J). and frontal cortex ($r = -0.59$, $P < 0.05$, $n=14$. Patient groups C and J. Anderson *et al* 1987). BH_4 synthesis by brain preparations was determined for a further 6 subjects (Subject group K). Within this second group there was a trend towards decreased BH_4 synthesis with age in the frontal cortex ($r = -0.62$, $n=6$) but not in the temporal cortex ($r=$

-0.30, n=6). Combining data from the two groups suggests that overall BH_4 synthesis in the frontal cortex and temporal cortex is unaltered in aging (Table 3.1).

We reported that DHPR activity declines with age in the temporal cortex ($r= -0.575$, $p<0.01$, n=19. Subject groups A, G, J) and frontal cortex ($r= -0.722$, $P < 0.01$, n=14. Subject groups C and J). This has been confirmed by subsequent analysis. DHPR activity shows a good linear correlation with age in the temporal cortex ($r= -0.57$, $P < 0.01$, n=24. Subject groups A, G, J, K) (Figure 3.1. Table 3.1). Subject V.H. was excluded from the analysis of DHPR activity and age in temporal cortex as the value of 1102 nmol/mg protein/minute lies outside the 99% confidence interval for the predicted value. If V.H. is included in the analysis there is still a good correlation between age and DHPR activity ($r= -0.573$, $P< 0.01$, n=25). DHPR activity declines linearly with age in the frontal cortex ($r= -0.74$, $P<0.01$, n=20. Subject groups C, J, K. Table 3.1). Further analysis of the data suggests that the relationship between age and DHPR in the frontal cortex is best described by a quadratic equation ($r= -0.795$, n=20, $P < 0.001$. Table 3.1. Figure3.2). DHPR activity in the frontal cortex preparations was relatively constant between 40-65 years and declined thereafter.

GTP cyclohydrolase activity in the temporal cortex was determined for a small group of subjects. There was no evidence of a decline in activity with age ($r=0.296$, n=9. Subject groups A and J).

The first study on sepiapterin reductase activity in the temporal cortex indicated a non-significant decline in activity with age ($r= -0.471$, n=9. Patient groups A and J. Anderson *et al* 1987). An extended study has demonstrated a deficit in sepiapterin reductase activity in the aging temporal cortex ($r= -0.568$, $P < 0.05$, n=15. Patient groups A, J, K. Table 3.1. Figure 3.3). Sepiapterin reductase activity in the frontal cortex did not decline with age ($r=0.0$. Patient groups J, K. Table 3.1). However, it should be noted that 7 of the 9 subjects in this group were below age 60.

Table 3.1. Correlation between enzyme activity and age in temporal cortex and frontal cortex preparations.

	Groups	Age range Years	n	r	p
Temporal cortex					
BH_4 synthesis	A,G,J,K	40-87	22	-0.285	ns
DHPR	A,G,J,K	40-89	24	-0.570	0.01
GTP cyclohydrolase	A,J	40-85	9	0.296	ns
Sepiapterin reductase	A,J,K	40-85	15	-0.568	0.05
Frontal cortex					
BH_4 synthesis	C,J,K	40-87	20	-0.243	ns
DHPR	C,J,K	40-87	20	-0.795	0.001
Sepiapterin reductase	J,K	40-87	10	0.0	ns

Data analysed by least squares method. ns: not significant

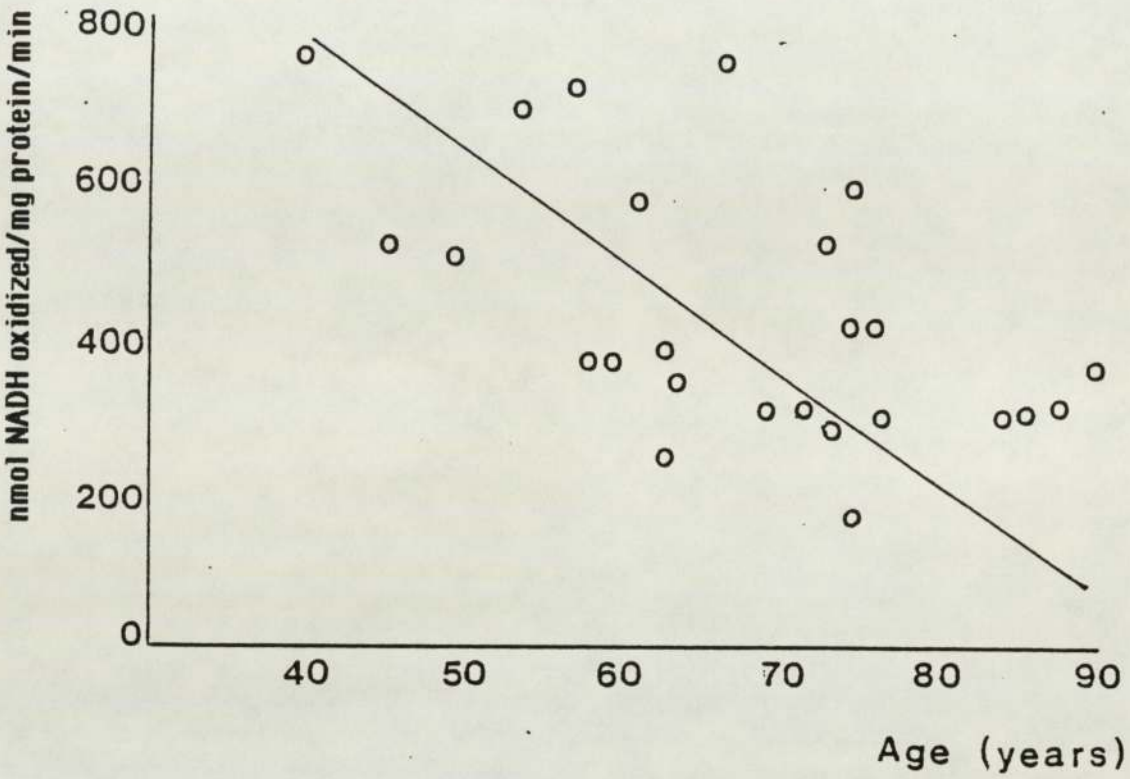


Figure 3.1 DHPR activity in temporal cortex preparations correlates with age

y=-7.47x+952 r=-0.57 P<0.01 n=24

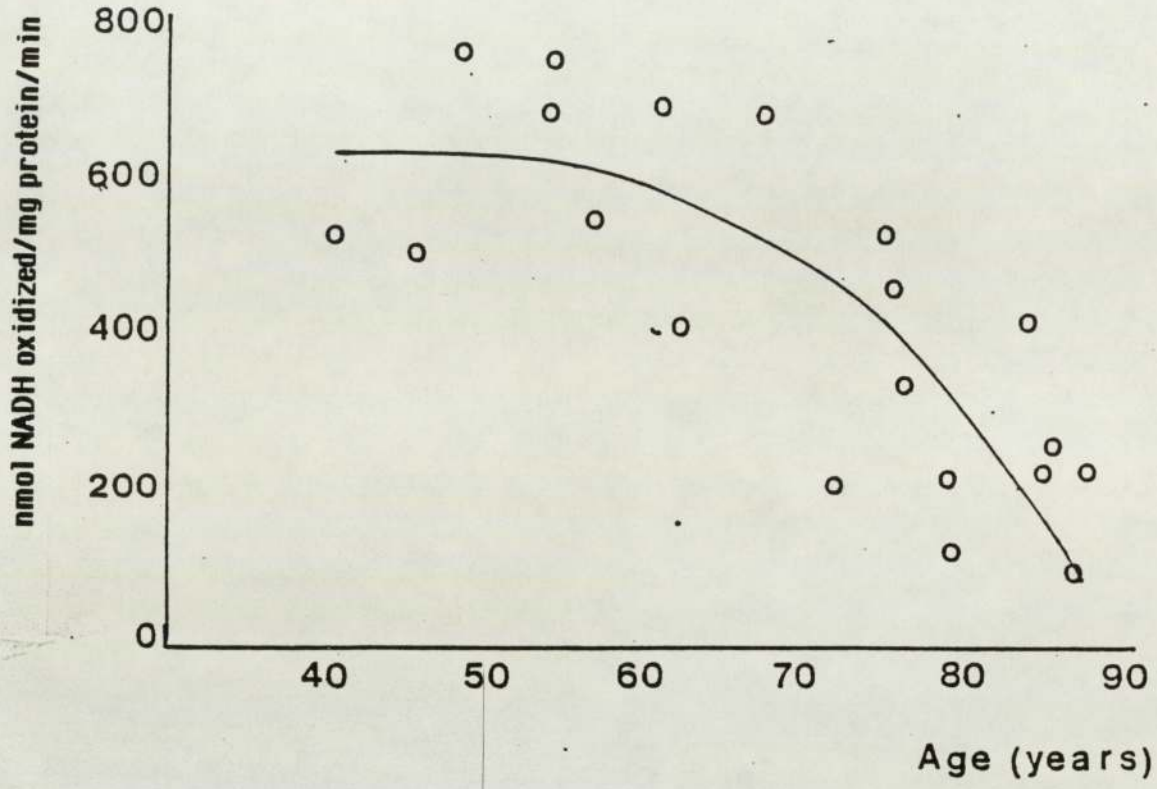


Figure 3.2 DHPR activity in frontal cortex preparations shows a curvilinear relationship with age

$y=-134.8+31.4x-0.317x^2$ $r=-0.795$ $P<0.001$ $n=20$

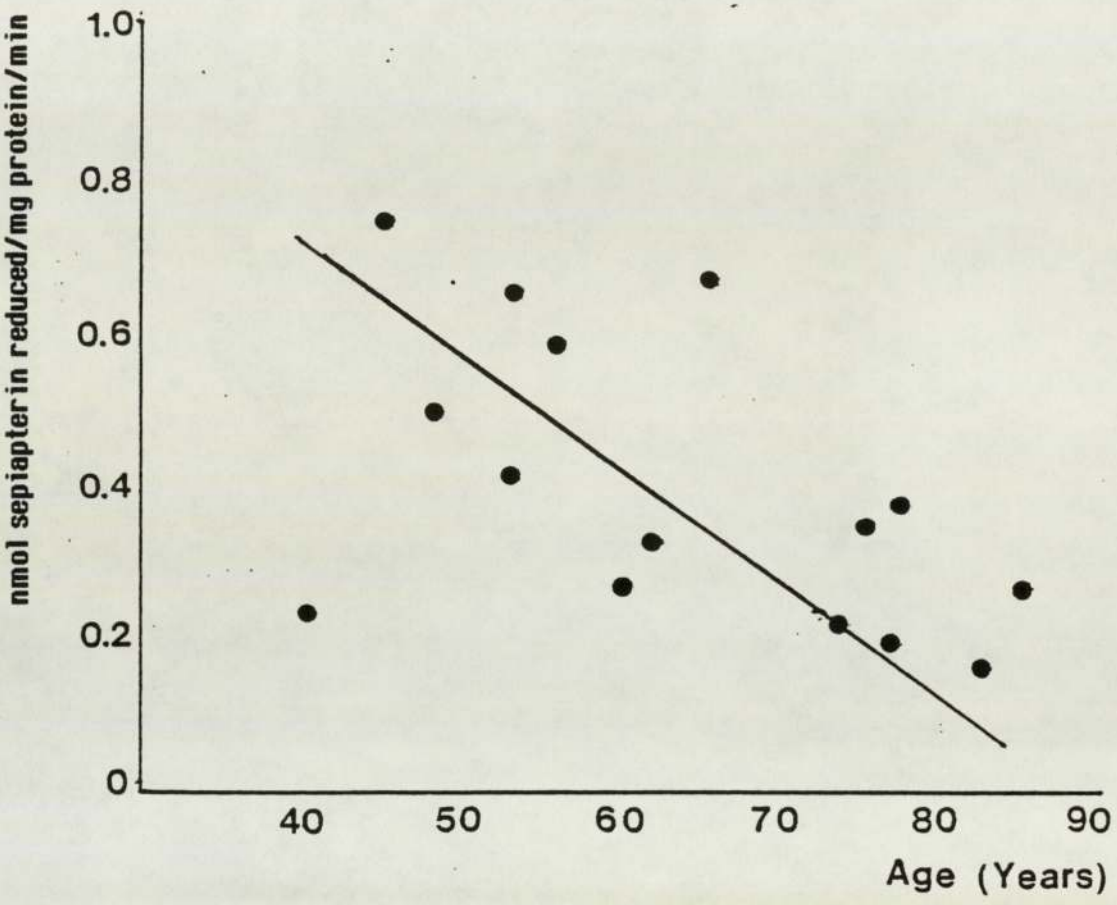


Figure 3.3 Sepiapterin reductase activity correlates with age in human temporal cortex preparations

$y=-0.007x+0.84$ $r=-0.568$ $P<0.05$ $n=15$

3.3.2 A comparison of regional BH_4 metabolism

Table 3.2 contains a summary of BH_4 metabolism in frontal cortex (Brodmann area 9), temporal cortex (Brodmann areas 20/21) and locus coeruleus preparations of control subjects. DHPR activity was similar in the frontal cortex and the temporal cortex. Both areas had significantly less activity than the locus coeruleus ($P < 0.001$). There was no significant difference in overall BH_4 synthesis in the frontal and temporal cortices. BH_4 synthesis in the neocortex was significantly lower than that in the locus coeruleus ($P < 0.001$). GTP cyclohydrolase activity in the temporal cortex was not significantly different from that in the locus coeruleus. There is evidence of a relationship between DHPR activity and sepiapterin reductase activity in the temporal cortex ($r= 0.61$, $P < 0.05$, $n=11$. Patient groups A, J and K. excluding V.H. Figure 3.4)

3.3.3 BH_4 metabolism in Huntington's disease

BH_4 synthesis and DHPR activity in temporal cortex (Brodmann area 21) preparations of Huntington's disease patients was similar to that in age-matched control subjects (Table 3.3).

3.3.4 BH_4 metabolism in SDAT

There was no difference in soluble protein content in frontal cortex (Brodmann area 9) and temporal cortex preparations of SDAT and control subjects (Table 3.4). All activities were measured on a protein base-line. BH_4 synthesis and DHPR activity in SDAT patients was similar in the frontal cortex, temporal cortex and locus coeruleus (Tables 3.4 and 3.5 respectively). BH_4 synthesis was significantly reduced in the locus coeruleus in the SDAT group compared to the control group ($P < 0.01$). BH_4 synthesis was also reduced in the temporal cortex but this did not reach statistical significance. DHPR activity in the neocortex and locus coeruleus of SDAT subjects was similar to that in the

control group. GTP cyclohydrolase activity was unaltered in the temporal cortex and locus coeruleus in SDAT (Table 3.6). Sepiapterin reductase activity in the temporal cortex was similar in SDAT and control subjects (Table 3.7).

BH_4 synthesis, DHPR activity and GTP cyclohydrolase activity in locus coeruleus preparations (SDAT and control subjects combined) did not correlate with noradrenaline levels in Br21 and Br24, average locus coeruleus counts or dementia score. DHPR activity and GTP cyclohydrolase activity declined as noradrenaline levels in Br10 increased, however the sample size was small (Table 3.8., courtesy of Dr. C.Q. Mountjoy, St. Andrews hospital, Northhampton).

Table 3.2. BH_4 metabolism in frontal cortex (Br9), temporal cortex (Br20/21) and locus coeruleus preparations.

	Frontal cortex	Temporal cortex	Locus coeruleus
DHPR activity Mean (SD, n) nmol NADH/mg protein/minute	304* (130, 10)	348* (112, 10)	583 (224, 5)
BH_4 synthesis Mean (SD,n) ng biopterin/mg protein/hour	0.82* (0.66, 10)	0.26* (0.18, 7)	9.38 (10.06, 5)
GTP cyclohydrolase Mean (SD, n) ng neopterin/mg protein/hour	nd	0.20 (0.17, 5)	1.64 (1.7, 5)
Sepiapterin reductase Mean (SD, n) nmolsepiapterin/mg protein/minute	nd	0.24 (0.06, 5)	nd
Age range	72-87	71-89	72-91

Data analysed by one-way analysis of variance (details given in tables 3.5 and 3.6).
*value is significantly lower than the corresponding value for the locus coeruleus $P < 0.001$.
nd: not determined

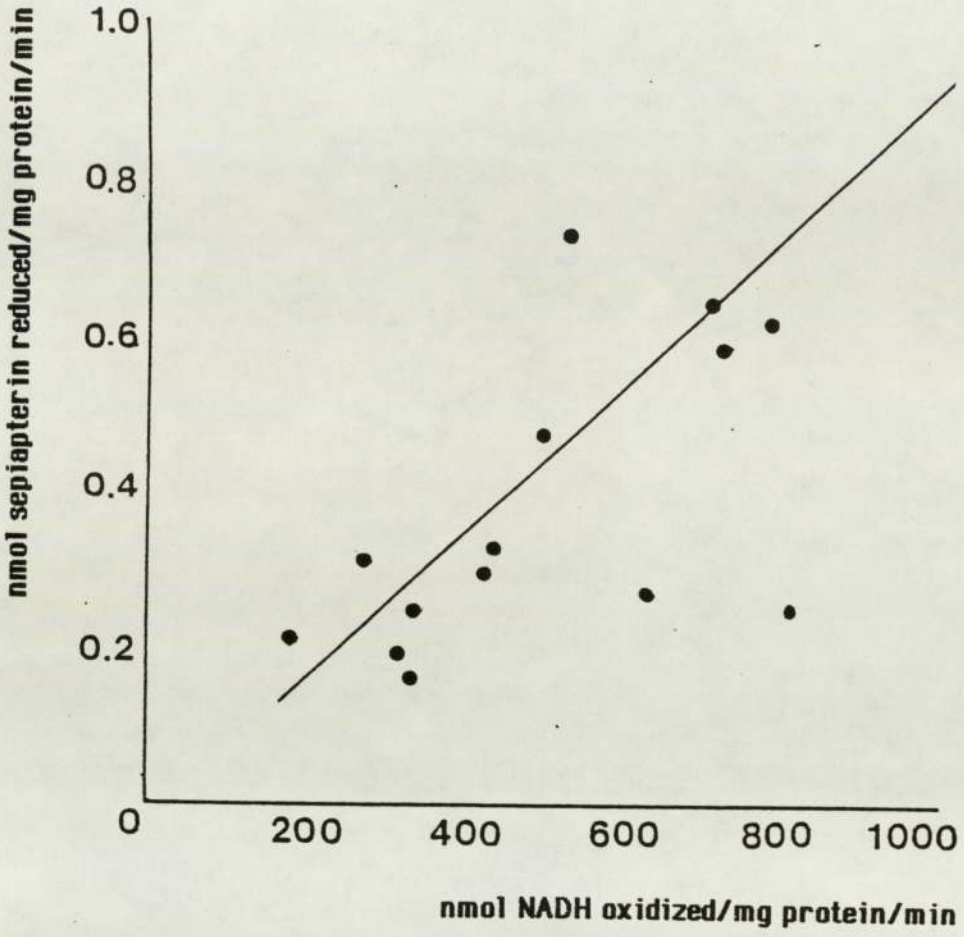


Figure 3.4 DHPR correlates with sepiapterin reductase activity in human temporal cortex preparations

$y=0.000598x+0.103$ $r=-0.61$ $P<0.05$ $n=14$

Table 3.3. Overall BH_4 synthesis and DHPR activity in Brodmann area 21 preparations of Huntington's disease patients and age-matched controls.

	Control	Huntington's disease
BH_4 synthesis: mean ± SD(n) ng biopterin/mg protein/hour	1.52 ± 1.15 (5)	1.44 ± 0.7 (5)
DHPR: mean ± SD(n) nmol NADH/mg protein/minute	404 ± 82 (5)	444 ± 117 (5)
Age: mean ± SD Years	65 ± 5	65 ± 5

Table 3.4. BH_4 synthesis in the frontal cortex (Br9), temporal cortex (Br20/21) and locus coeruleus of SDAT patients and age-matched controls.

	Age range	ng protein/g tissue mean ± SD	BH_4 synthesis mean ± SD
Frontal cortex			
Control	72-87	38.2 ± 7.5 (10)	0.82 ± 0.66(10)
SDAT	71-90	33.3 ± 4.8 (6)	0.97 ± 0.47 (6)
Temporal cortex			
Control	71-89	40.8 ± 18.4 (10)	0.26 ± 0.18(7)
SDAT	71-97	43.2 ± 24.9 (9)	0.12 ± 0.13 (7)
Locus coeruleus			
Control	72-91	nd	9.38 ± 10.06 (5)
SDAT	72-90	nd	3.23 ± 4.02 (5)*
Error mean square			4.9613

Analysis of variance for BH_4 synthesis gave $F=4.96$ (5,34 DF. $P < 0.005$).

*Significant decrease in BH_4 synthesis in the locus coeruleus of the SDAT group compared with the control group. $P < 0.01$.

BH_4 synthesis expressed as ng biopterin/mg protein/hour

Table 3.5. DHPR activity in frontal cortex (Br9), temporal cortex (Br20/21) and locus coeruleus preparations from SDAT patients and age-matched controls.

	Age range Years	DHPR activity: mean ± SD (n) nmol NADH/mg protein/minute
Frontal cortex		
Control	72-87	304 ± 130 (10)
SDAT	71-90	404 ± 40 (6)
Temporal cortex		
Control	71-89	348 ± 112 (10)
SDAT	71-97	438 ± 164 (9)
Locus coeruleus		
Control	72-91	583 ± 224 (5) *
SDAT	72-90	579 ± 241 (5)
Error mean square		23634.64

Analysis of variance for DHPR activity gave $F=3.79$ (5,39 DF, $P < 0.01$).
* DHPR activity in the locus coeruleus is significantly greater than that in the frontal and temporal cortices. $P<0.001$

Table 3.6. GTP cyclohydrolase activity in temporal cortex and locus coeruleus preparations of SDAT subjects and age-matched controls.

	Age (years) mean ± SD	ng neopterin/mg protein/hour: mean ± SD (n) Temporal cortex	Locus coeruleus
Control	80 ± 9	0.20 ± 0.17 (5)	1.64 ± 1.70 (5)
SDAT	80 ± 8	0.15 ± 0.22 (4)	1.34 ± 1.37 (5)

Table 3.7. Sepiapterin reductase activity in temporal cortex preparations (Br 20/21) of SDAT subjects and age-matched controls.

	Age (years) mean ± SD	nmol sepiapterin/mg protein/minute mean ± SD
Control	78 ± 5	0.23 ± 0.06 (5)
SDAT	76 ± 5	0.24 ± 0.09 (5)

Table 3.9. Correlation studies on BH_4 metabolism in the locus coeruleus and noradrenergic activity in the neocortex, average locus coeruleus counts and dementia score.

	BH_4 synthesis	DHPR	GTP cyclohydrolase
NA Br10*	0.397 (4)	-0.951 (4)#	-0.987 (4)S
NA Br21*	-0.206 (4)	0.398 (4)	-0.717 (4)
NA Br24*	0.230 (10)	-0.119 (10)	-0.072 (10)
AVLC@	-0.064 (10)	-0.041 (10)	0.044 (10)
SCORE•	-0.509 (8)	-0.078 (8)	-0.424(8)

*Noradrenaline levels in Brodmann areas 10, 21 and 24
@Average locus coeruleus counts
•Dementia score

Pearson correlation coefficients #P=0.049 SP=0.013
Analysis carried out by Dr. C. Q. Mountjoy

Table 3.9 Individual values for BH_4 synthesis and DHPR activity

Code number	Age (years)	BH_4 synthesis*	DHPR activity^S
Group A			
Temporal cortex			
C241	71	0.19	333
C236	72	–	260
C253	74	0.31	180
C238	74	0.0	432
C244	74	0.28	605
C254	76	0.31	316
C257	83	–	324
C258	85	0.57	323
C278	87	0.13	324
C240	89	–	387
Group B			
Temporal cortex			
D61	71	0.0	459
D39	71	0.33	426
D45	74	0.0	824
D60	76	0.0	243
D50	80	–	489
D59	82	0.11	343
D52	87	332	
D38	90	0.24	446
D51	97	0.18	378
Group C			
Frontal cortex			
C236	72	2.17	245
C238	74	1.14	565
C254	76	0.28	365
C256	79	0.32	255
C242	79	0.54	157
C257	83	0.67	459
C258	85	0.51	300
C249	85	1.77	276
C267	87	0.57	276
C278	87	0.25	137

*ng biopterin/mg protein/hour ^{S}nmol NADH/mg protein/minute

Table 3.9 continued

Code number	Age (years)	BH_4 synthesis*	DHPR activity^S
Group D Frontal cortex			
D39	71	1.22	420
D40	72	1.54	459
D48	74	0.31	379
D60	76	0.63	395
D59	82	0.77	344
D38	90	1.34	427
Group E Locus coeruleus			
C270	72	5.06	850
C236	72	8.81	534
C242	79	26.88	417
C262	87	2.71	335
C266	91	3.46	777
Group F Locus coeruleus			
D33	72	0.01	452
D35	73	9.69	866
D56	80	2.20	746
D54	87	0.0	1.76
D32	90	4.24	253
Group G Temporal cortex			
C383	59	0.58	382
C263	62	1.07	403
C379	63	0.77	358
C375	69	3.39	326
C311	72	1.83	552
Group H Temporal cortex			
H311	59	0.92	422
H307	61	0.63	405
H305	65	1.55	370
H301	68	2.42	373
H296	71	1.66	650

*ng biopterin/mg protein/hour ^{S}nmol NADH/mg protein/minute

Table 3.9 continued

Code number	Age (years)	BH_4synthesis*	DHPR$
Goup J			
Temporal cortex			
D.T	40	2.39	780
V.H	53	2.28	1102
K.G	60	0.35	579
A.H	62	1.90	264
Group J			
Frontal cortex			
D.T	40	1.93	543
V.H	53	1.50	780
K.G	60	0.91	718
A.H	62	2.26	446
Group K			
Temporal cortex			
Mi	45	0.0	531
Ge	48	0.33	508
Al	53	0.68	698
Co	56	0.93	727
Th	66	0.0	769
Ho	75	0.0	432
Group K			
Frontal cortex			
Mi	45	0.28	515
Ge	48	0.88	779
Al	53	0.34	693
Co	56	0.87	568
Th	66	0.0	707
Ho	75	0.0	483

*ng biopterin/mg protein/hour $nmol NADH/mg protein/minute

Table 3.10 Individual values for GTP cyclohydrolase activity and sepiapterin reductase activity.

Code number	Age (years)	GTP cyclohydrolase*	Sepiapterin reductase^{S}
Group A Temporal cortex			
C253	74	0.42	0.22
C238	74	0.04	0.34
C254	76	0.18	0.20
C257	83	0.04	0.17
C258	85	0.30	0.25
Group B Temporal cortex			
D61	71	0.01	0.21
D45	74	0.05	0.38
D60	60	0.48	0.20
D59	82	0.06	0.20
Group E Locus coeruleus			
C270	72	4.12	–
C236	72	0.35	–
C242	79	0.86	–
C262	87	0.19	–
C266	91	2.70	–
Group F Locus coeruleus			
D33	72	1.09	–
D35	73	0.0	–
D56	80	3.48	–
D54	87	1.76	–
D32	91	0.37	–
Group J Temporal cortex			
D.T	40	0.23	0.25
V.H	53	0.34	0.42
K.G	60	0.26	0.28
A.H	62	0.14	0.33

*ng neopterin/mg protein/hour ^{S}nmol sepiapterin/mg protein/minute

Table 3.10 continued

Code number	Age (years)	GTP cyclohydrolase*	Sepiapterin reductase$
Group J Frontal cortex			
D.T	40	0.20	0.28
V.H	53	0.30	0.57
K.G	60	0.25	0.38
A.H	62	0.28	0.29
Group K Temporal cortex			
Mi	45	–	0.75
Ge	48	–	0.49
Al	53	–	0.66
Co	56	–	0.60
Th	66	–	0.64
Ho	75	–	0.36
Group K Frontal cortex			
Mi	45	–	0.40
Ge	48	–	0.90
Al	53	–	0.60
Co	56	–	0.66
Th	66	–	0.53
Ho	75	–	0.52

*ng neopterin/mg protein/hour $nmol sepiapterin/mg protein/minute

3.4 SUMMARY

DHPR activity and overall BH_4 synthesis in human brain preparations from elderly subjects showed regional selectivity: the activity in the locus coeruleus was significantly greater than that in the neocortex. DHPR activity in the temporal cortex fell linearly with age. DHPR activity showed a curvilinear relationship with age in the frontal cortex, thus DHPR activity was relatively constant until the mid 60s and then declined with age. The data suggests that BH_4 synthesis does not fall with age in the neocortex but further analysis is required to confirm this effect. Sepiapterin reductase activity showed a negative correlation with age in the temporal cortex. There was no evidence of an age-effect on sepiapterin reductase activity in the frontal cortex, however the majority of the subjects examined were between 40-65 years. It is possible that a curvilinear relationship between age and sepiapterin reductase activity exists in the frontal cortex. Sepiapterin reductase activity correlated with DHPR activity in the neocortex. GTP cyclohydrolase activity in temporal cortex preparations did not correlate with age.

There was no evidence of a defect in the biosynthetic pathway or salvage pathway in temporal cortex preparations from Huntington's disease patients. Overall BH_4 synthesis was significantly diminished in the locus coeruleus of SDAT patients. DHPR activity in the frontal cortex, temporal cortex and locus coeruleus was unaltered in SDAT. GTP cyclohydrolase activity in temporal cortex and locus coeruleus preparations and sepiapterin reductase activity in temporal cortex preparations was similar in SDAT and control subjects.

CHAPTER 4

THE EFFECT OF ALUMINIUM ON BH_4 SYNTHESIS

4.1 INTRODUCTION

4.1.1 Aluminium neurotoxicity

The neurotoxic action of aluminium is clearly demonstrated by experimentally induced encephalopathy in animals and the dialysis dementia syndrome in man. When aluminium is injected into the CNS of susceptible animals a progressive encephalopathy results which is similar to Alzheimer's disease (AD) in clinical course and neuropathology (Crapper McLachlan and De Boni 1980). Aluminium induced encephalopathy is characterized by neurofibrillary degeneration, 'dying back' of dendrites, reduced nucleolar size and RNA content (Crapper McLachlan and De Boni 1980). Similar changes occur in AD, however the neurofilaments characteristic of AD are distinct from those of aluminium induced encephalopathy (Wisniewski *et al* 1980).

The role of aluminium in progressive dialysis encephalopathy has been extensively reviewed (Sideman and Manor 1982, Arieff *et al* 1979). Aluminium is absorbed from the small intestine, but normal subjects are able to eliminate most aluminium from the body (Alfrey 1983). Aluminium accumulates in body tissues of uremic patients (Arieff *et al* 1979, Alfrey 1986). In the mid 1970s several centres reported that some patients on long-term renal dialysis developed a progressive, usually fatal, neurological disorder (Burks *et al* 1976, Alfrey 1972, Chokroverty *et al* 1976). Dialysis encephalopathy was associated with the accumulation of large amounts of aluminium in body tissues including the brain (Arieff *et al* 1979). Two major sources of aluminium were identified. Dialysate fluid from centres in which dialysis dementia occurred was shown to contain high levels of aluminium (Review by Wills and Savory 1985). Secondly, uraemic patients are administered aluminium-containing phosphate binding gels in order to control serum phosphate levels (Alfrey *et al* 1986, Sideman and Manor 1982).

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Dialysis dementia is not accompanied by the neurofibrillary degeneration characteristic of AD or experimental encephalopathy suggesting that aluminium exerts its neurotoxic action through different processes in this disorder. Further, brain aluminium levels are also increased in diseases which affect the blood-brain barrier suggesting that factors other than the accumulation of aluminium in brain tissue are also involved in the pathogenesis of dialysis dementia (Arieff *et al* 1979).

Neurochemical analysis of dialysis dementia brains is limited. Perry *et al* (1985) reported that MHPG, homovanillic acid, and 5-hydroxyindole acetic acid in the frontal cortex, a region in which aluminium levels were raised, are normal suggesting that catecholaminergic and serotonergic activity is not impaired in dialysis dementia.

4.1.2 Aluminium and Alzheimer's disease

Several groups have considered the role of aluminium in the pathogenesis of Alzheimer's disease but direct evidence that aluminium is a causative agent in AD is lacking (Perl 1983, Crapper McLachlan and De Boni 1980). Aluminium levels are reported to be normal (McDermott *et al* 1979) or increased in AD brain (Crapper *et al* 1976). The conflicting data seems to be a consequence of varying sample size (Crapper McLachlan *et al* 1980). Crapper *et al* (1976) reported that aluminium levels are highest in those regions showing greatest neuropathological change. Regional brain aluminium levels similar to those which induce encephalopathy in animals occur in AD (Crapper *et al* 1976). Further work established that aluminium is localised in neurones which contain neurofibrillary tangles, NFT (Perl and Brody 1980). Candy *et al* (1986) reported the presence of aluminosilicates in the senile plaque core. Whether aluminium accumulation represents non-specific absorption by a degenerating system with no pathological consequences or is a causative agent in neurofibrillary degeneration is unclear. Aluminium binds to chromatin in the nucleus of affected cells suggesting that aluminium may interfere with normal neuronal processes and ultimately with protein synthesis and cell metabolism (De Boni *et al* 1980). Aluminium also accumulates within NFT-bearing cells of aged individuals (McDermott *et al* 1979). Mann (1983) proposed that

degenerative changes occurring in the locus coeruleus with age would lead to inadequate control of brain homeostasis such that toxic substances such as aluminium could gain entry to and accumulate within brain tissue. The accumulation of aluminium in the brain may precipitate AD or predispose the individual to AD upon further metabolic insult.

4.1.3 Aluminium and BH_4 metabolism

The effect of aluminium on BH_4 metabolism has been briefly examined. Aluminium inhibits DHPR *in vitro* and *in vivo* (Leeming and Blair 1979, Brown 1981, Dhondt and Bellahsene 1983). Dhondt *et al* (1982) reported an increase in neopterin and biopterin levels in serum of uraemic patients on maintenance dialysis indicating that BH_4 metabolism is impaired in chronic uraemia. This report examines the effect of aluminium on BH_4 metabolism in the rat brain *in vitro* and *in vivo*. BH_4 biosynthesis and DHPR activity in the frontal and temporal cortex from four uraemic patients on peritoneal dialysis is also examined.

4.2 METHODS

The clinical status of patients on maintenance dialysis is described in Table 2.3. Data on serum aluminium levels was provided by Dr. Altmann, London Hospital, Whitechapel.

4.2.1 Dialysis of sepiapterin reductase

The enzyme preparation was dialysed at 4°C for 20 hours against 0.1 M Tris buffer, pH 7.4. The dialysis sac contained 100 µl of enzyme preparation, 3.4 cm^3 of buffer and 0.2 M $Al_2(SO_4)_2 16H_2O$. The control contained no added aluminium.

4.2.2 *In vivo* studies

Group 1 rats were dosed orally with 0.5 cm^3 H_2O containing 0.53 mmoles of $Al(OH)_3$ in suspension twice daily for 7 days (50mg Al/kg

body wt/day). Control animals received 0.5 cm^3 of water twice daily for 7 days. Both test and control animals were restrained with ether prior to receiving the appropriate dose.

Group 2 rats were fed rat and mouse breeding diet containing 1mg $Al(OH)_3$/g diet for 6 weeks. Animals consumed approximately 16 g food per day (30 mg Al/kg body weight/ day). Control rats were fed rat and mouse breeding diet with no added aluminium.

Both groups were allowed free access to food and water.

All other methods were as described in chapter 2.

4.2.3 Statistics

Paired t-test was used to analyse data obtained from *in vitro* studies. Student's t-test was used to examine data obtained from *in vivo* studies. Correlation coefficients were calculated using the least squares method.

4.3 RESULTS AND DISCUSSION

4.3.1 BH_4 metabolism in neocortex preparations of renal dialysis patients

Overall BH_4 synthesis, DHPR, GTP cyclohydrolase and sepiapterin reductase activities were measured in temporal cortex and frontal cortex preparations of 4 subjects receiving peritoneal dialysis treatment. Data is given in tables 4.1 and 4.2. Serum aluminium levels correlate with the duration of dialysis treatment ($r=0.96$, $n=4$, $P<0.05$) and are greater than values for normal subjects which are reported to be less than 10µg/l (Alfrey 1983). There is a trend towards lower DHPR activity with age in the temporal cortex ($r= -0.55$) but not in the frontal cortex. As serum aluminium levels increase DHPR activity tends to decrease in the temporal cortex ($r= -0.64$) and the frontal cortex ($r= -0.84$). The decrease in DHPR activity with increasing aluminium levels in the temporal cortex may reflect the effect of age on DHPR activity.

Table 4.1. BH_4 metabolism in temporal cortex preparations from renal dialysis patients.

Code number	Serum Al µg/l	BH_4 synthesis*	DHPR^S	GTP# cyclohydrolase	Sepiapterin$ reductase
K.G	10	0.35	579	0.26	0.28
V.H	30	2.28	1102	0.34	0.42
D.T	45	2.39	780	0.23	0.25
A.H	105	1.90	264	0.14	0.33

Table 4.2. BH_4 metabolism in frontal cortex preparations from renal dialysis patients.

Code number	Serum Al µg/l	BH_4 synthesis*	DHPR^S	GTP# cyclohydrolase	Sepiapterin$ reductase
K.G	10	0.91	718	0.25	0.38
V.H	30	1.50	780	0.30	0.57
D.T	45	1.93	543	0.20	0.28
A.H	105	2.26	446	0.28	0.29

*ng biopterin/mg protein/hour
#ng neopterin/mg protein/hour
^{S}nmol NADH/mg protein/minute
$nmol sepiapterin/mg protein/minute

Overall BH_4 synthesis in the frontal cortex increased with increasing aluminium levels ($r = 0.90$). There were no clear trends for GTP cyclohydrolase and sepiapterin reductase activity with respect to age or aluminium levels.

4.3.2 The effect of aluminium on BH_4 metabolism in rat brain *in vitro*

GTP cyclohydrolase activity was determined using alkaline phosphatase (P4252, Sigma chemical company) in the assay. GTP cyclohydrolase activity and overall BH_4 synthesis in the presence of 1×10^{-3} M $Al_2(SO_4)_2 16H_2O$ were similar to control values (Table 4.3). Aluminium inhibited sepiapterin reductase (Figure 4.1). 2×10^{-4} M $Al_2(SO_4)16H_2O$ resulted in a 40% reduction in enzyme activity ($P < 0.01$. Table 4.3). The enzyme preparation was incubated with 2×10^{-4} M $Al_2(SO_4)16H_2O$ at 37°C for 90 minutes. There was no increase in enzyme inhibition with time (Table 4.4). Table 4.5 shows the effect of dialysis on aluminium inhibition of sepiapterin reductase. 2×10^{-4} M $Al_2(SO_4)16H_2O$ reduced sepiapterin reductase activity to 50% of the control value. Following dialysis sepiapterin reductase activity in the Al-treated enzyme preparation was similar to the control value.

4.3.2 The effects of aluminium on BH_4 metabolism in the rat brain *in vivo*

Group 1. There was no significant difference in total biopterin content, overall BH_4 synthesis, sepiapterin reductase or DHPR activity between the control group and the Al-treated group (Table 4.6).

Group 2. Overall BH_4 synthesis, GTP cyclohydrolase activity and sepiapterin reductase activity were similar in the Al-treated group and the control group (Table 4.7). GTP cyclohydrolase activity was determined without the use of alkaline phosphatase. There was a significant increase in DHPR activity in the Al-treated group compared

with the control group ($P < 0.05$. Table 4.7).

DHPR activity correlated with sepiapterin reductase activity in brain preparations of control rats. ($r=0.717$, $P < 0.01$, $n=28$. Figure 4.2).

Table 4.3. The effect of aluminium on BH_4 metabolism in rat brain *in vitro*

	Control Mean ± SD (n)	Test Mean ± SD (n)
BH_4 biosynthesis ng biopterin/mg protein/hour	0.39 ± 0.09 (5)	0.47 ± 0.15 (5)#
GTP cyclohydrolase ng neopterin/mg protein/hour	0.56 ± 0.21 (6)	0.72 ± 0.34 (6)#
Sepiapterin reductase nmol sepiapterin/mg protein/minute	0.90 ± 0.18 (5)	0.53 ± 0.14 (5)*S

1×10^{-3} M $Al_2(SO_4)16H_2O$ * 2×10^{-4} M $Al_2(SO_4)16H_2O$

S Significantly lower than the control value. $P < 0.01$

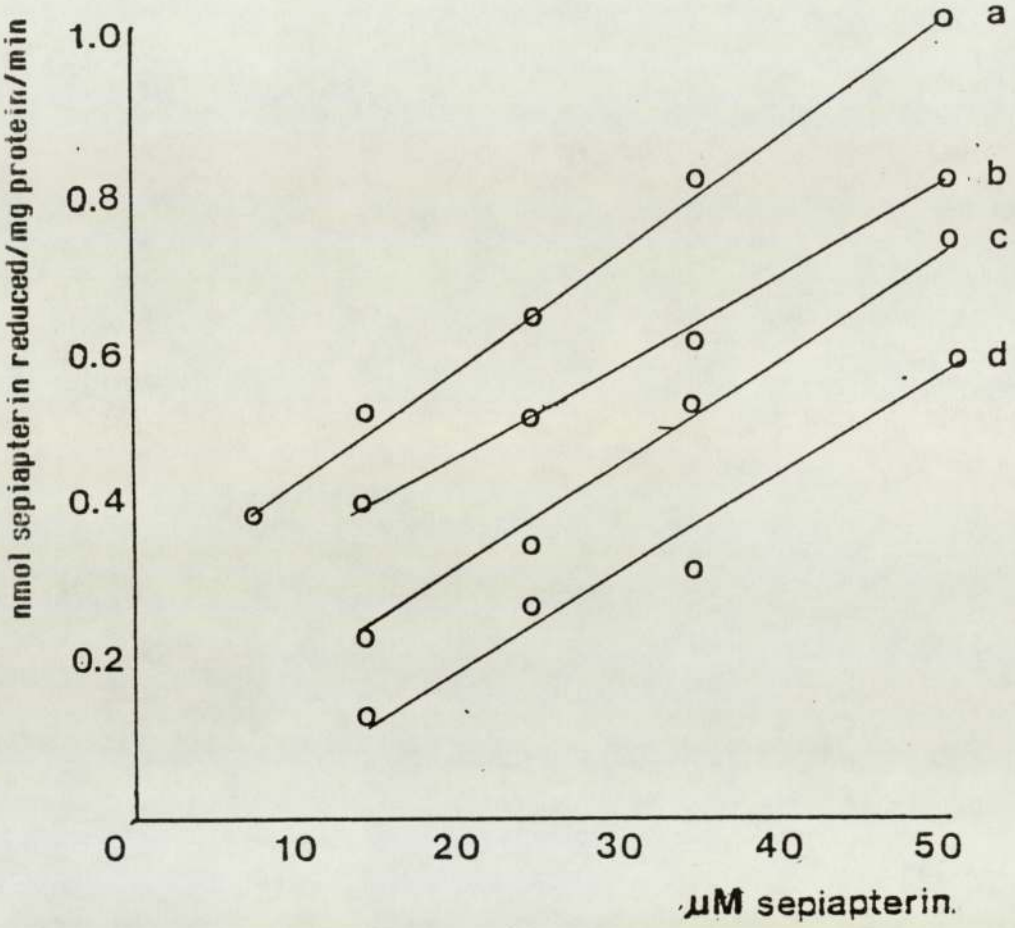

Figure 4.1 Aluminium inhibition of sepiapterin reductase *in vitro*

a.0 b.$1x10^{-4}M$ c.$1.5x10^{-4}M$ d.$2x10^{-4}M$ $Al_2(SO_4)_3 16H_2O$

Table 4.4. Aluminium inhibition of sepiapterin reductase *in vitro* over a 90 minute period.

Time Minutes	Sepiapterin reductase activity nmol sepiapterin/mg protein/minute (n=2)	
0	0.37	0.24
20	0.42	0.41
40	0.42	0.41
60	0.51	0.30
90	0.51	0.24
Control value	0.87	0.85

Table 4.5 Aluminium inhibition of sepiapterin reductase *in vitro* is removed by dialysis treatment.

Sepiapterin reductase activity: mean $\pm$ SD (n=5)
nmol sepiapterin/mg protein/minute

Before dialysis Control	Test #	After dialysis Control	Test #
1.00 ± 0.19	0.48 ± 0.15*	0.56 ± 0.05	0.54 ± 0.03

2×10^{-4} M $Al_2(SO_4)16H_20$

* significantly lower than the control value. $P<0.01$

Table 4.6 The effect of aluminium on BH_4 metabolism *in vivo* Group 1.

	Control Mean ± SD (n=6)	Test Mean ± SD (n=6)
Total biopterin ng biopterin/g tissue	66 ± 12	53 ± 16
BH_4 synthesis ng biopterin/mg protein/hour	0.33 ± 0.40	0.53 ± 0.29
Sepiapterin reductase activity nmol sepiapterin/mg protein/minute	0.79 ± 0.08	0.77 ± 0.07
DHPR activity nmol NADH/mg protein/minute	220 ± 12	208 ± 24

Test group received 50 mg Al/kg body wt/day for 7 days.

Table 4.7. The effect of aluminium on BH_4 metabolism *in vivo* Group 2.

	Control Mean ± SD (n=5)	Test Mean ± SD (n=6)
BH_4 synthesis ng biopterin/mg protein/hour	0.33 ± 0.31	0.43 ±0.39
GTP cyclohydrolase * ng neopterin/mg protein/hour	1.41 ± 0.12	1.54 ± 0.25
Sepiapterin reductase nmol sepiapterin/mg protein/minute	0.85 ± 0.06	0.85 ± 0.11
DHPR nmol NADH/mg protein/minute	343 ± 57	414 ± 36 S

Test animals received 30 mg Al/kg body wt/day in the diet for 6 weeks.

*assay carried out without the addition of alkaline phosphatase

S Test value is significantly higher than the control value. $P < 0.05$.

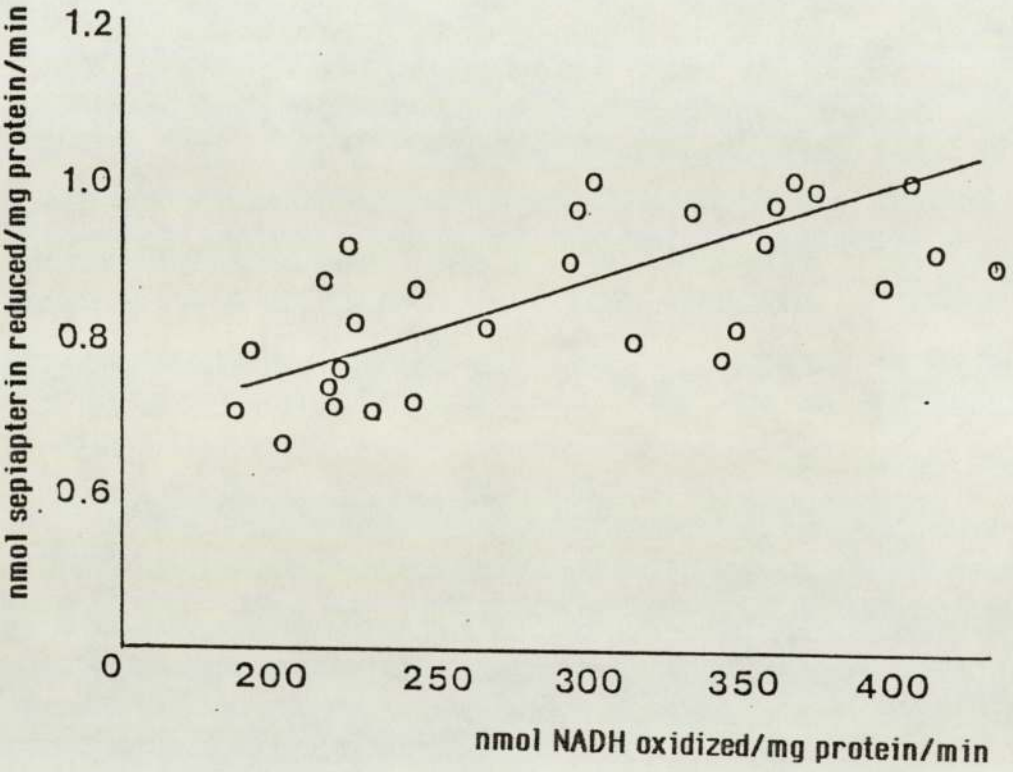

Figure 4.2 DHPR correlates with sepiapterin reductase activity in rat brain preparations

$y=0.00126x+0.512$ $r=0.717$ $P<0.01$ $n=28$

4.4 SUMMARY

Aluminium levels in serum from dialysis patients were greater than reported normal values and increased as the duration of dialysis treatment increased. There was a trend towards decreased DHPR activity in the temporal cortex and frontal cortex of patients on peritoneal dialysis as serum aluminium levels increased. The loss of DHPR activity in the temporal cortex is probably an age effect. DHPR activity in the frontal cortex is constant over the age range of these patients indicating that aluminium may be inhibiting DHPR activity in this region. Overall BH_4 synthesis in the frontal cortex increased as serum aluminium levels increased.

Aluminium reversibly inhibited sepiapterin reductase *in vitro*. Overall BH_4 synthesis and GTP cyclohydrolase were not inhibited by aluminium *in vitro* or *in vivo*. Sepiapterin reductase activity was similar in brain preparations from control and aluminium treated rats. DHPR activity was unaltered or increased in brain preparations from rats receiving aluminium compared to control animals. DHPR activity correlated with sepiapterin reductase activity in rat brain preparations.

CHAPTER 5

DISCUSSION

The central role of BH_4 in neurotransmitter biosynthesis and the neurological consequences of inherited disorders of BH_4 metabolism point to the importance of BH_4 for normal neurological function (Introduction). The aim of this study was to establish normal levels of overall BH_4 synthesis, GTP cyclohydrolase, sepiapterin reductase and DHPR activity in human and rat brain preparations, to determine whether BH_4 metabolism is altered in dementia and to examine the effects of a neurotoxin, aluminium, on BH_4 metabolism.

5.1.1 DHPR activity in human brain preparations

DHPR activity was similar in the frontal and temporal cortices of elderly control subjects but was significantly higher in the locus coeruleus compared to the neocortex (Table 5.1). The locus coeruleus is a major source of noradrenergic neurones (Mann 1983). The regional variation in DHPR activity is in agreement with reports that BH_4, and presumably the enzymes involved in its metabolism, is primarily located in catecholaminergic neurones (Bullard *et al* 1978, Hennings and Rembold 1982, Levine *et al* 1981).

The first study on DHPR activity in aging indicated that DHPR activity declines with age in the frontal cortex ($r= -0.721$, $P < 0.01$, $n=14$) and the temporal cortex ($r= -0.573$, $P < 0.01$, $n=19$) (Anderson *et al* 1986). This relationship was substantiated when DHPR activity in brain preparations from a further 6 subjects was included in the analysis : DHPR activity fell with age in the frontal cortex ($r= -0.74$, $P < 0.01$, $n=20$) and the temporal cortex ($r= 0.57$, $P < 0.01$, $n=24$. Figure 5.1). Further analysis of the data suggested that the relationship between age and DHPR activity in the frontal cortex is best described by a curve ($r= -0.80$, $P < 0.001$, $n=20$. Figure 5.2). DHPR activity in the frontal cortex was relatively constant until the mid 60s and then fell with age.

Table 5.1. DHPR activity in human brain preparations

	Frontal cortex	Temporal cortex	Locus coeruleus
DHPR activity Mean (SD, n) nmol NADH/mg protein/minute	304 (130, 10)	348 (112, 10)	583 S (224, 5)
Age (years) mean ± SD	81 ± 5	77 ± 6	80 ± 9

S DHPR activity in the locus coeruleus is significantly greater than that in the frontal and temporal cortices. $P < 0.01$. Data was analysed by one-way analysis of variance.

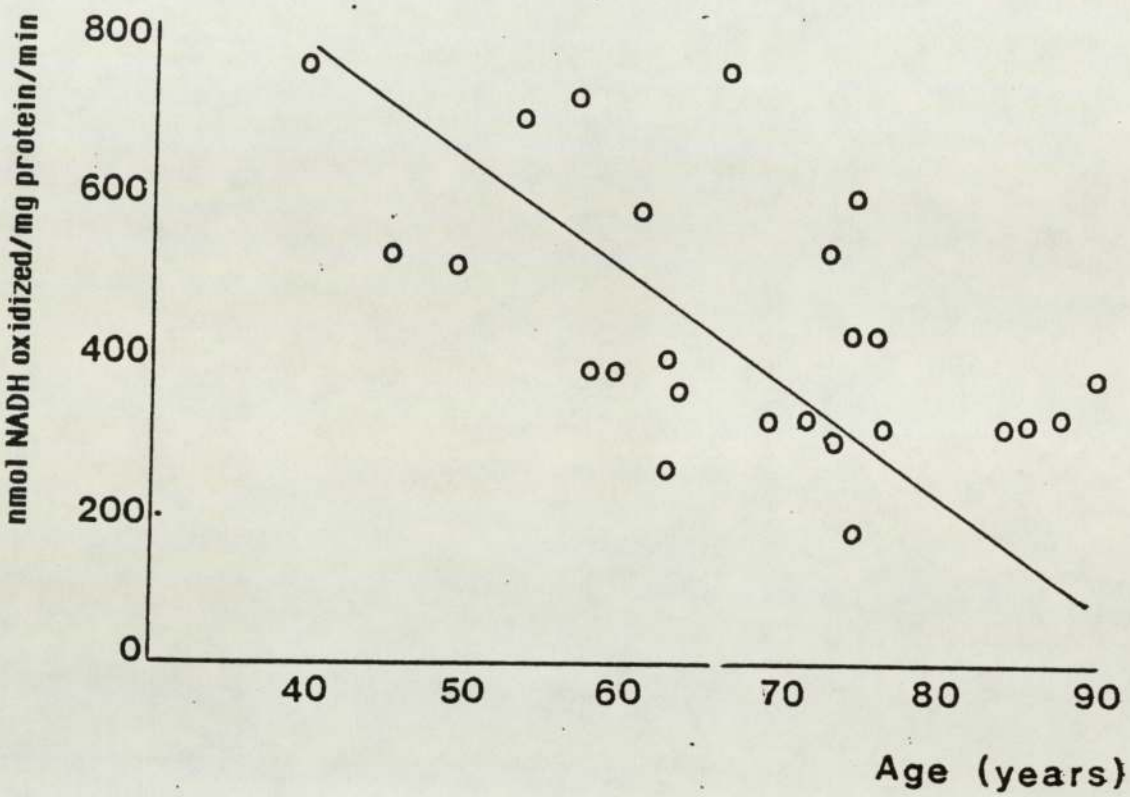

Figure 5.1. DHPR activity declines with age in the temporal cortex

$y = -7.47x + 952$ $r = -0.57$ $P < 0.01$ $n=24$

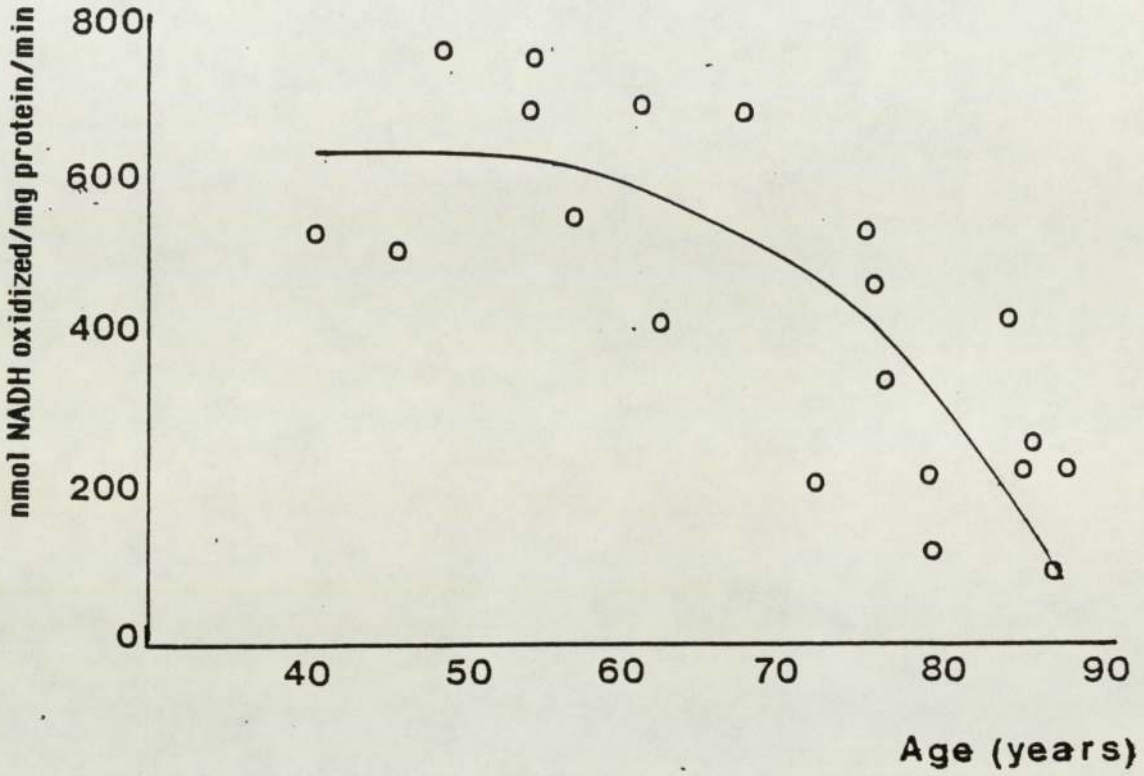

Figure 5.2. There is a curvilinear relationship between age and DHPR activity in the frontal cortex.

$y = -134.8 + 31.4x - 0.317x^2$ $r = -0.795$ $P < 0.001$ $n=20$

Leeming and Blair (1980) suggested that the increase in serum biopterin levels with age may be due to loss of DHPR activity. This study conclusively demonstrates a decline in DHPR activity in the human neocortex with age.

Two lines of evidence support the proposal that low DHPR activity will contribute to the neurological impairment characteristic of old age. Firstly, inherited disorders of DHPR deficiency are characterised by low catecholamine and indoleamine levels and severe neurological dysfunction (Kaufman 1975, Firgaira *et al* 1981). This disorder serves as a model for the situation in extreme old age in which DHPR activity may reach zero (estimated to be approximately age 127 $\pm$ 9 years for the temporal cortex and 98 $\pm$ 12 years for the frontal cortex). Secondly, Altmann and coworkers (personal communication) measured DHPR activity in blood samples from patients on renal dialysis before and after desferrioxamine treatment. They also determined the performance on a psychometric test, designed to detect mild degrees of dementia, before and after desferrioxamine treatment. They found that desferrioxamine treatment resulted in an increase in DHPR activity. Further, the increase in DHPR activity correlated with improved performance on the psychometric test ($r= 0.616$, $P=0.014$, $n=15$). This suggests that impaired DHPR activity is associated with neurological dysfuntion.

5.1.2 Overall BH_4 synthesis in human brain preparations

BH_4 synthesis in the locus coeruleus was significantly higher than that in the frontal and temporal cortices of elderly subjects, again reflecting the role of the locus coeruleus in noradrenergic function (Table 5.2). An early study suggested that BH_4 synthesis in the temporal cortex ($r=-0.522$, $P<0.05$, $n=16$) and frontal cortex ($r= -0.590$, $P<0.05$, $n=14$) declines with age (Anderson *et al* 1987). A later group of 6 subjects (age range 45-75) suggested a trend towards lower BH_4 synthesis with age in the frontal cortex ($r= -0.615$, $n=6$) but little effect in the temporal cortex ($r= -0.304$, $n=6$). Analysis of all the data suggests that overall BH_4 synthesis in the temporal cortex and frontal cortex is unaltered in aging. Inter assay variablity is a problem in the

Table 5.2. BH_4 synthesis in human brain preparations

	Frontal cortex	Temporal cortex	Locus coeruleus
BH_4 synthesis Mean (SD,n) ng biopterin/mg protein/hour	0.82 (0.66,10)	0.26 (0.16,7)	9.38 **S** (10.06,5)
Age (years) Mean ± SD	81 ± 5	77 ± 6	80 ± 9

Data was analysed by one-way analysis of variance.

S BH_4 synthesis in the locus coeruleus was significantly greater than that in the frontal and temporal cortices. $P < 0.01$.

Table 5.3. GTP cyclohydrolase activity in human brain preparations.

	Temporal cortex	Locus coeruleus
ng neopterin/mg protein/hour Mean ± SD (n)	0.2 ± 0.17 (5)	1.64 ± 1.70 (5)
Age: years. Mean ± SD	78 ± 5	80 ± 9

study of the effect of age on BH_4 synthesis which may be compounded by the provision of tissue samples from two sources and the time interval between analysis of the different groups. Analysis of BH_4 synthesis in the neocortex of the control group K, (age range 45-75 years), provided by Dr. Altmann suggests that there may be a trend towards diminished overall BH_4 synthesis with age in the frontal cortex ($r = -0.615$, $n=6$) but little effect in the temporal cortex ($r = -0.30$, $n=6$). Further the report that BH_4 levels in the CSF fall with age suggests that there is diminished capacity for BH_4 synthesis in the aging brain (Levine *et al* 1979, LeWitt *et al* 1982).

5.1.3 GTP cyclohydrolase and sepiapterin reductase activity in human brain preparations

BH_4 synthesis requires at least three enzymes: GTP cyclohydrolase, 6-pyruvoyl-PH_4 synthase and sepiapterin reductase. We examined GTP cyclohydrolase and sepiapterin reductase activity in order to further characterize BH_4 synthesis in brain preparations.

Mean GTP cyclohydrolase activity in the locus coeruleus of elderly subjects was greater than that in the temporal cortex but the difference was not statistically significant (Table 5.3). GTP cyclohydrolase did not decline with age in the temporal cortex (age range 40-85 years, n=6).

Sepiapterin reductase activity declined with age in the temporal cortex ($r=-0.568$, $P<0.05$, $n=15$, Figure 5.3). There was no age-effect on sepiapterin reductase acitivity in the frontal cortex, however, only one subject in this group was over 66. Sepiapterin reductase activity correlated with DHPR activity in the temporal cortex ($r=0.61$, $P<0.05$, $n=13$) and non-significantly in the frontal cortex ($r=0.591$, $n=10$). Therefore, we suggest a curvilinear relationship, similar to that for DHPR, may exist between age and sepiapterin reductase activity in the frontal cortex: sepiapterin reductase is relatively constant until the 7th decade and then declines with age.

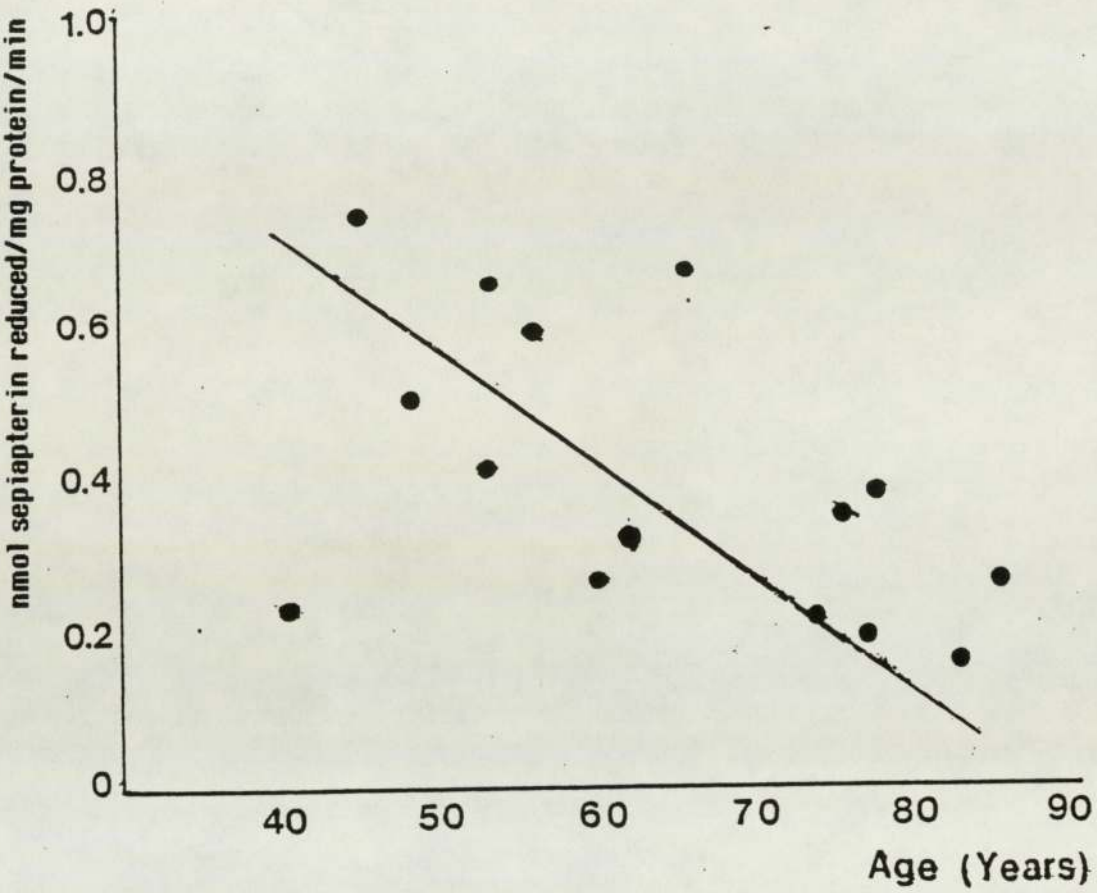

Figure 5.3. Sepiapterin reductase activity declines with age in the temporal cortex.

$y = -0.007x + 0.84$ $r = -0.568$ $P < 0.05$ $n=15$

Although sepiapterin reductase activity in brain tissue is much greater than the overall capacity for BH_4 synthesis we suggest that in old age a large reduction in sepiapterin reductase activity will eventually limit BH_4 synthesis.

The reduction in BH_4 metabolism in the aging neocortex may reflect neurone loss from the substantia nigra and locus coeruleus (Mann *et a.* 1984, Tomlinson *et al* 1981, Mann *et al* 1983, Carlsson *et al* 1985). DHPR activity and sepiapterin reductase activity in the frontal cortex were affected at a later stage than in the temporal cortex. Marcyniuk *et a.* (1986) demonstrated that in SDAT subjects there is a greater loss of neurons which project to the hippocampus and temporal cortex compared with those which project to the frontal cortex. Perhaps an analogous situation occurs in aging so that there is a preferential loss of the neurones which innervate the temporal cortex. The lack of an age-effect on GTP cyclohydrolase suggests that factors other than cell loss are involved in altered BH_4 metabolism in aging. Further, this suggests that there is a lesion on the biosynthetic pathway between NH_2TP and BH_4. Ultimately there will be a reduction in cofactor availability in the aging neocortex leading to impaired noradrenergic and dopaminergic function. This may contribute to the development of neurological dysfunction in the elderly.

5.1.4 BH_4 metabolism in dementia

The results on BH_4 metabolism in the aging brain emphasize the need to select appropriate age-matched subjects when studying BH_4 metabolism in neurological disorders. The incidence of dementia, including Alzheimer's disease, increases with age and many of the neuropathological and neurochemical changes of AD occur to a lesser extent in the normal elderly population (Gottfries 1985, Reisberg 1983). We examined BH_4 metabolism in SDAT brain preparations and age-matched controls. The major difference found in this study was a severe loss of the capacity to synthesize BH_4 in the locus coeruleus of SDAT subjects (Table 5.4). This

Table 5.4. BH_4 synthesis is significantly reduced in the locus coeruleus preparations of SDAT subjects.

	BH_4 synthesis: mean ± SD (n) ng biopterin/mg protein/hour	
	Control	SDAT
Frontal cortex	0.82 ± 0.66 (10)	0.97 ± 0.47 (6)
Temporal cortex	0.26 ± 0.18 (7)	0.12 ± 0.13 (7)
Locus coeruleus	9.38 ± 10.06 (5)	3.23 ± 4.02 (5) [S]

Control and SDAT groups were age-matched.

[S] BH_4 synthesis in the SDAT group is significantly lower than that in the control group. $P < 0.01$. Data was analysed by one-way analysis of variance.

supports the report of reduced biopterin levels in the locus coeruleus of SDAT subjects (Nagatsu *et al* 1986). It has been reported that BH_4 synthesis is reduced in the temporal cortex of SDAT patients (Barford *et al* 1984). This study showed a 50% decrease in overall BH_4 synthesis in the temporal cortex of SDAT subjects compared with control values but the difference did not reach statistical significance (Table 5.4). The lack of a deficit in BH_4 synthesis in the frontal cortex is in agreement with reports that the frontal cortex is relatively well preserved in SDAT (Rossor *et al* 1984, Brun 1983). Further, a recent report suggests that neurones which project to the temporal cortex from the locus coeruleus are more severly affected in SDAT than those which project to the frontal cortex (Marcyniuk *et al* 1986).

The loss of BH_4 synthesis in SDAT subjects may reflect neurone loss. However, there was no difference in DHPR, sepiapterin reductase or GTP cyclohydrolase activity between SDAT and control subjects. We suggest that there is a specific reduction in the capacity to synthesise BH_4 in SDAT. The site of this lesion is between NH_2TP and BH_4, possibly at the level of 6-pyruvoyl-PH_4 synthase. The reports that neopterin levels in the CSF of SDAT subjects are within normal limits, whereas there is a significant reduction in biopterin levels supports this proposal (Morar *et al* 1983, Le Witt *et al* 1985). Cattell and coworkers (personal communication) reported that the neopterin:biopterin ratio is increased in SDAT subjects compared to age-matched controls. The same group measured visual evoked potentials in these subjects. Presenile dementia is characterised by delayed flash P2 component but normal latency P1 and pattern VEP components (Wright *et al* 1984). Cattell and coworkers found a significant correlation between urinary N:B ratio and the flash P2-P1 latency difference in SDAT subjects, demonstrating that deficits in BH_4 synthesis have neurological sequelae.

5.1.5 BH_4 metabolism in Huntington's disease

Huntington's disease is characterised by progressive involuntary

choreiform movements invariably accompanied by dementia. There was no evidence of altered DHPR activity or BH_4 synthesis in temporal cortex preparations from HD subjects compared with control subjects (Table 5.5). The report that BH_4 levels in CSF are decreased in HD (Williams *et al* 1980) may reflect loss of BH_4 synthesis capacity in subcortical structures such as the basal ganglia, which show the greatest neuropathological changes in HD. Normal BH_4 metabolism in HD suggests that the deficit in BH_4 synthesis in the temporal cortex in SDAT subjects is specific to the disease process rather than a secondary consequence of the dementing condition.

5.1.6 Overall BH_4 synthesis and DHPR activity in Down's Syndrome

Down's syndrome is a genetic disorder characterised by mental retardation of unknown origin. Down's syndrome patients show progressive and accelerated loss of intellectual function with age which is invariably accompanied by Alzheimer type changes in the brain of subjects over the age of 35. The noradrenergic and serotonergic systems are impaired in middle-age Down's syndrome patients compared to age-matched controls (Mann *et al* 1985, Wisnieski and Wisnieski 1983). Eggar (Personal communication) measured DHPR activity (288 $\pm$ 77 n mol NADH/mg protein/min) and BH_4 synthesis (0.043 $\pm$ 0.07 ng biopterin/mg protein/hr) in temporal cortex preparations from 3 Down's syndrome patients, age 50 years. DHPR activity in the temporal cortex lies below the age-predicted value of 619 $\pm$ 129 nmol NADH/mg protein/minute (mean $\pm$ standard error) indicating that DHPR activity is diminished in the temporal cortex in Down's syndrome. Aziz *et al* (1982) reported that serum biopterin levels in Down's syndrome are greater than control values and suggested that this may be due to increased oxidation of BH_4. We propose that the increase in serum biopterin levels may reflect a decrease in DHPR activity in the cell which may contribute to the neurological deficits of Down's syndrome.

Table 5.5. BH_4 synthesis and DHPR activity are unaltered in the temporal cortex in Huntington's disease.

	Control	Huntington's disease
BH_4 synthesis: mean ± SD (n) ng biopterin/mg protein/hour	1.52 ± 1.15 (5)	1.44 ± 0.7 (5)
DHPR: mean ± SD (n) nmol NADH/mg protein/minute	404 ± 82 (5)	444 ± 117 (5)
Age: mean ± SD (years)	65 ± 5	65 ± 5

5.1.7 BH_4 metabolism in brain preparations from dialysis patients

BH_4 metabolism was examined in temporal and frontal cortex preparations of 4 subjects who had received peritoneal dialysis treatment (Table 5.6). The effect of age on DHPR activity in these subjects reflects the pattern of the larger group of subjects: DHPR activity tended to fall with age in the temporal cortex ($r = -0.55$) but not in the frontal cortex ($r = 0.02$). There was a trend towards diminished DHPR activity in the frontal cortex ($r = -0.86$) and temporal cortex ($r = -0.64$) as serum aluminium levels increased. The decrease in DHPR activity in the temporal cortex may be an age-effect, whereas DHPR activity in the frontal cortex is constant over this age range suggesting that aluminium may be inhibiting DHPR in this region. Overall BH_4 synthesis in the frontal cortex increased as aluminium levels increased ($r = 0.90$). Further, overall BH_4 synthesis in the frontal cortex and temporal cortex was greater than that in the control group. Dhondt *et al* (1982) reported an increase in serum neopterin and biopterin levels in uraemic patients on maintenance dialysis and suggested that an efflux of BH_2 from the cell due to diminished DHPR activity resulted in a compensatory increase in BH_4 synthesis. Our results suggest that aluminium inhibition of DHPR in the frontal cortex causes a reduction in cofactor availability in the cell which may result in stimulation of the biosynthetic pathway as proposed by Dhondt *et al* (1982).

Few studies on the regional distribution of aluminium in the brain have been carried out. Perry *et al* (1985) reported high levels of aluminium in the frontal cortex of haemodialysis patients. Further, they reported a significant reduction in choline acetyl transferase activity in the frontal cortex but not in the temporal cortex of these subjects. It may be that the frontal cortex is particularly vulnerable to changes in ureamia.

Altmann and coworkers (personal communication) measured blood DHPR activity and performance on a psychometric test by patients on maintenance dialysis before and after treatment with the aluminium

Table 5.6. DHPR activity and BH_4 synthesis in neocortex preparations of renal dialysis subjects.

Subject	Age	Serum Al	DHPR activity [S]		BH_4 synthesis [*]	
	Years	µg/l	frontal cortex	temporal cortex	frontal cortex	temporal cortex
K.G	60	10	718	579	0.91	0.35
V.H	53	30	780	1102	1.50	2.28
D.T	40	45	543	780	1.93	2.39
A.H	62	105	446	264	2.26	1.90

[S] nmol NADH/mg protein/minute
[*] ng biopterin/mg protein/hour

chelating agent desferrioxamine. They found a significant correlation between the increase in DHPR activity following treatment and improved performance on the psychometric test suggesting that impaired DHPR activity is associated with neurological dysfunction. Thus there is a growing body of evidence that BH_4 metabolism is altered in subjects on maintenance dialysis.

Aluminium levels in serum from patients receiving dialysis treatment increased with increasing duration of treatment ($r=0.96$, $n=4$, $P<0.05$), confirming previous reports (Masselot *et al* 1978, Sideman and Manor 1982). Mann (1983) proposed that degenerative changes in the locus coeruleus in aging may lead to inadequate control of homeostasis such that toxic substances such as aluminium may enter the CNS. Aluminium levels in brain tissue are reported to increase with age (McDermott *et al* 1979). We hypothesize that prolonged exposure to aluminium, such as in tap water, may result in the accumulation of aluminium in body fluids and tissues. Increased aluminium levels may contribute to the deficit in DHPR activity in the temporal and frontal cortices which we found to occur with age (Figures 5.1 and 5.2).

5.1.8 The effect of aluminium on BH_4 metabolism in the rat

Aluminium reversibly inhibited sepiapterin reductase *in vitro* (Table 5.7). Leeming and Blair (1979) and Brown (1981) reported that aluminium inhibits DHPR *in vitro* We extended these studies to examine the effect of oral administration of $Al(OH)_3$ on BH_4 metabolism *in vivo* BH_4 synthesis, GTP cyclohydrolase and sepiapterin reductase activity were similar in control and Al-treated rats. DHPR activity in brain preparations was either unaltered or increased in rats receiving $Al(OH)_3$ (Table 5.8). These results contrast with the report by Dhondt *et al* (1982) that DHPR activity was significantly lower in tissues of hamsters receiving 3% $Al_2(SO_4)_3$ in drinking water for 16 days compared with control animals. This may reflect species differences and/or differences in the mode of administration of aluminium. It would be more informative to correlate enzyme activity in brain preparations

Table 5.7. Aluminium inhibits sepiapterin reductase *in vitro*

Sepiapterin reductase activity: mean ± SD (n=5)
nmol sepiapterin/mg protein/ minute

Before dialysis		After dialysis	
Control	Test	Control	Test
1.00 ± 0.19	0.48 ± 0.15*	0.56 ± 0.05	0.54 ± 0.03

Samples were dialysed for 20 hour, at 4°C against 0.1 M Tris buffer pH 7.4.
* value is significantly lower than the control value. P<0.01.

Table 5.8. The effect of oral administration of $Al(OH)_3$ on DHPR activity in rat brain preparations.

DHPR activity: nmol NADH/mg protein/minute. Mean ± SD (n)

Group 1		Group 2	
Control	Test[S]	Control	Test[#]
220 ± 12 (6)	208 ± 24 (6)	343 ± 57 (5)	414 ± 36 (6)*

[S] 0.1% w/w $Al(OH)_3$ in diet for 6 weeks
[#] 0.53 mmol $Al(OH)_3$ in suspension i.g, twice daily for 7 days

* value is significantly greater than the control value, P<0.05. Data was analysed by Student's t-test

with serum or brain aluminium levels. This data is not available at the present time.

These results suggest that oral administration of aluminium does not impair BH_4 metabolism in the brain under normal conditions. However, uraemic rats accumulate more aluminium in body tissues than control rats receiving equivalent doses of aluminium. This study does not exclude the possibility that aluminium impairs BH_4 metabolism in uraemia.

FURTHER WORK

1. Development of an assay for the measurement of 6-pyruvoyl-tetrahydropterin synthase activity in human brain preparations in order to confirm that enzyme activity is reduced in SDAT and to clarify the effect of age on the biosynthetic pathway.

2. Neurochemical alterations associated with aging and SDAT show regional selectivity suggesting that BH_4 metabolism in several regions of the human brain ought to be examined.

3. Examination of BH_4 metabolism in other cortical dementias in order to determine the specificity of the defect.

4. This report suggests a deficit in DHPR activity in the temporal cortex in Down's syndrome. A more detailed study of BH_4 metabolism in Down's syndrome is required to assess the role of BH_4 in the neurological deficits of this disorder.

5. An extended study on the effects of aluminium on BH_4 metabolism is required to clarify the role of aluminium in altered BH_4 metabolism particularly in relation to the neurological dysfunction which may be associated with renal dialysis treatment.

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