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TETRAHYDROBIOPTERIN METABOLISM IN DEPRESSION AND SENILE DEMENTIA OF ALZHEIMER TYPE

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SUMMARY

Excretion of biopterin and the related pteridines neopterin and pterin was measured in urine samples from a group of 76 male and female unipolar and bipolar depressed outpatients receiving lithium therapy, and compared to 61 male and female control subjects. The ratio of neopterin to biopterin excreted (N/B) was significantly higher in the patients than the controls. The significant positive correlation between urinary neopterin and biopterin shown by the controls was absent in the patients, indicating disrupted biosynthesis of tetrahydrobiopterin. Urinary cortisol excretion in depressed patients was similar to controls, implying normal hypothalamus-pituitary-adrenal axis function in these patients. Serum folate was shown to correlate with urinary total biopterin excretion in female unipolar patients.

Two groups of elderly females with senile dementia of Alzheimer type (SDAT) were examined for urinary pteridine excretion. In the first study of ten patients, the N/B ratio was significantly higher than in 24 controls, and the ratio B/B+N significantly lower. A second study of 30 SDAT patients and 21 controls confirmed these findings. However, neopterin correlated with biopterin in both patients and controls, indicating that the alteration in tetrahydrobiopterin metabolism may be different to that shown in depression.

Lithium had no effect *in vivo* or *in vitro* on Wistar rat brain or liver biosynthesis of tetrahydrobiopterin at a range of concentrations and duration of dosing period, showing that lithium was not responsible for the lowered biopterin excretion by depressed patients.

No significant effects on tetrahydrobiopterin metabolism in the rat were shown by the tricyclic antidepressant imipramine, the anticonvulsant sodium valproate, the vitamin folic acid, the anticatecholaminergic agent αmethylparatyrosine, the synthetic corticosteroid dexamethasone, or stimulation of natural cortisol by immobilisation stress.

Scopolamine, an anticholinergic drug, lowered rat brain pterin which may relate to the tetrahydrobiopterin deficits shown in SDAT.

KEYWORDS

Tetrahydrobiopterin Neopterin Depression

Lithium Senile dementia of Alzheimer type

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ABBREVIATIONS

ACh(E)	Acetylcholine (esterase)
ACTH	Adrenocorticotrophic hormone
AD	Alzheimer's disease
ADH	Antidiuretic hormone
ADP/ATP	Adenosine di/triphosphate
B	Biopterin
BDI	Beck depression inventory
BH_2	7,8-dihydrobiopterin
BH_4	5,6,7,8-tetrahydrobiopterin
CAT	Choline acetyltransferase
CDR	Clinical dementia rating
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
CRF	Corticotropin-releasing factor
CSF	Cerebrospinal fluid
DA	Dopamine
DHFR	Dihydrofolate reductase
DHPR	Dihydropteridine reductase
$DMPH_4$	6,7-dimethyl-5,6,7,8-tetrahydropterin
DR	Doctor's rating
DST	Dexamethasone suppression test
EC	Enzyme commission
ECT	Electroconvulsive therapy
FH_4	Tetrahydrofolate
GABA	γaminobutyric acid
GTP	Guanosine triphosphate
GTP-CH	Guanosine triphosphate cyclohydrolase
HCl	Hydrochloric acid
5HIAA	5-hydroxyindoleacetic acid
HPA	Hypothalamus-pituitary-adrenal
HPLC	High pressure liquid chromatography
5HT	5-hydroxytryptamine (serotonin)
HVA	Homovanillic acid
L-dopa	L-3,4-dihydroxyphenylalanine
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor

ABBREVIATIONS (continued)

MHPG	3-methoxy-4-hydroxyphenylglycol
MID	Multi-infarct dementia
α MPT	α methylparatyrosine
MRC	Medical Research Council
NA	Noradrenaline
ND	Not determined
NH	Dihydroneopterin
NS	Not significant
NTPH	Dihydroneopterin triphosphate
pKU	Phenylketonuria
qBH	Quinonoid dihydrobiopterin
SD	Standard deviation
SDAT	Senile dementia of Alzheimer type
SEM	Standard error of the mean
TCA	Trichloroacetic acid
UK	United Kingdom
US	United States (of America)
VEP	Visual evoked potential
VMA	Vanillylmandelic acid

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INTRODUCTION

1.1 STRUCTURE OF TETRAHYDROBIOPTERIN

Pteridines are aromatic heterocyclic compounds which have in common the pyrazino-(2,3-d)-pyrimidine ring system (Purrmann, 1940a,b):

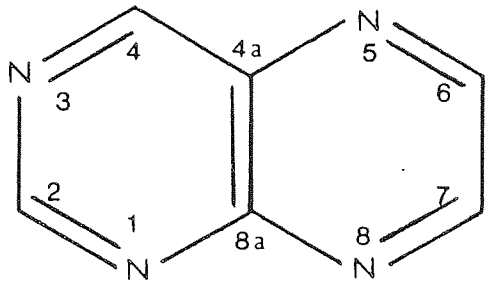


Figure 1.1 Numbering of the pteridine structure

Most naturally occurring pteridines have a substituted amino (NH_2) group in the 2 position and a keto (C=O) group in the 4 position. Tetrahydrobiopterin (BH_4) is a biologically active pteridine with the structure as in figure 1.2:

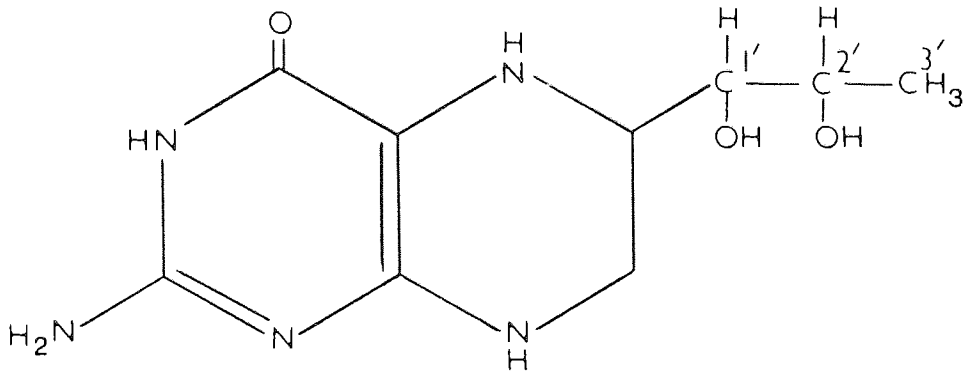


Figure 1.2 L-erythro-5,6,7,8-tetrahydrobiopterin

Tetrahydrofolate (FH_4) is a related compound. It is the reduced form of folic acid and is a conjugated pteridine, with one or more p-aminobenzoylglutamates linked to the basic ring at position 6. The oxidised form is folic acid (figure 1.3).

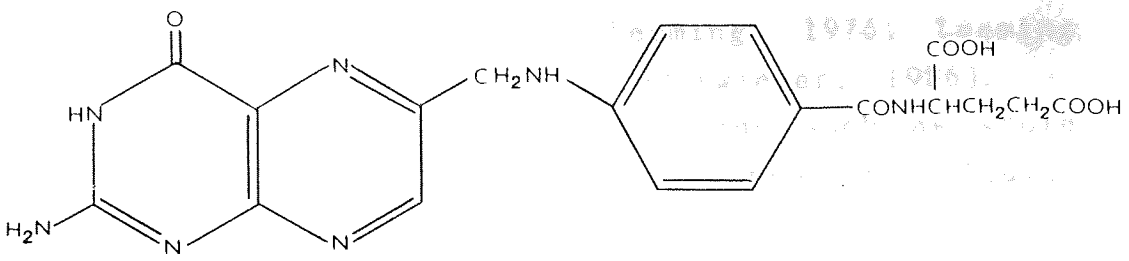


Figure 1.3 Folic acid

Folates can therefore be mono- or polyglutamates according to the added glutamic acid residues. Although there are similarities in the structures of these two pteridines, they have differing roles in intermediate metabolism. BH_4 is a cofactor for several aromatic amino acid monooxygenases (hydroxylases) while FH_4 has an unrelated function in one-carbon transfer reactions.

1.2 NATURAL OCCURRENCE OF TETRAHYDROBIOPTERIN

The first pteridines to be identified (xanthopterin, isoxanthopterin and leucopterin) were discovered in butterfly wings (Purrmann, 1940a,b), leading to the assumption that the major role for these compounds was non-dynamic, as pigments. Since then, BH_4 and its fully oxidised species biopterin has been found in microorganisms and plants, as well as such diverse animal phyla as fish, amphibia, reptiles and mammals; in mammals, pteridines have been shown in the tissues of the mouse, rat, dog and monkey as well as man (review Sawada and Nagatsu, 1987; Nichol et al (1985) Tissue distribution varies with species. Lower levels of biopterin are found in rat brain for example (Baker et al, 1974), whereas monkey brain pteridine levels are comparable to humans (Duch et al, 1984a). In man, BH_4 has been demonstrated in normal tissues such as the liver, gut, kidney, adrenals and brain (Haberle

et al, 1978; Blakley, 1969; Leeming, 1976; Leeming and Blair, 1980; Hasler and Niederwieser, 1986). Detectable amounts are found in fluids such as whole blood, serum, breast milk, and cerebrospinal fluid. Comparatively copious amounts are excreted daily in the urine (Stea et al, 1980). Endogenous BH_4 synthesis was established in 1974 by Fukushima and Shiota.

The related compound FH_4 has been known since 1941 when Mitchell and co-workers isolated a substance from spinach leaves which they named folic acid; Angier et al (1946) synthesised pteroylglutamic acid and proved it identical to the natural product. Other groups soon isolated reduced substituted derivatives of folic acid from mammalian liver such as 5-formyltetrahydrofolate (Bond et al, 1949) and 5-methyltetrahydrofolate, 5-CH_3THF (Donaldson and Keresztesy, 1959). 5-CH_3THF and 10-formylTHF are the predominant plasma and urinary folates and represent transport forms. Polyglutamates of these folates form the bulk of intracellular folates and are the principal coenzymes. Mammals have lost the capacity to synthesise the molecular skeleton of folates (but not unconjugated pteridines such as BH_4) de novo. Gut microflora are unable to prevent serum folate deficiency in man fed a low folate (5 μg/day) diet indicating that there was no net folate synthesis useful to man (Herbert, 1962; Hoffbrand et al, 1971). Dietary folate is the sole source of folate available to man. Folates are found in most foodstuffs including green vegetables, milk, eggs and liver, but may be rapidly destroyed in cooking, leading to an inadequate intake (Herbert, 1962).

1.3 BIOSYNTHESIS OF TETRAHYDROBIOPTERIN

In the initial step in the biosynthesis of BH_4 the enzyme guanosine triphosphate cyclohydrolase (GTP-CH; EC 3.5.4.16) converts the purine guanosine triphosphate

(GTP) via an Amadori rearrangement (Hodge, 1955) to D-erythro-7,8-dihydroneopterin triphosphate ($NTPH_2$) and formate (Burg and Brown, 1968). The reaction is proposed to proceed via imidazole ring opening cleaving the bond C8-N9, followed by loss of C8 as formate. Ring closure of the deoxypentulose derivative yields $NTPH_2$, figure 1.4. A report by Gal *et al* (1978) that this process was a two-enzyme step in rat brain has been discounted (Curtius *et al* 1983a). $NTPH_2$ can be oxidised and hydrolysed non-enzymatically to dihydroneopterin (NH_2) and neopterin (N) which can be excreted in the urine of some mammals, including man (figure 1.5). Only $NTPH_2$ and not NH_2 or N will serve as a substrate for BH_4 synthesis. In those organisms which retain the capacity to synthesise FH_4, $NTPH_2$ is also the first intermediate in the process (Brown, 1971).

1.3.1 De novo biosynthesis of tetrahydrobiopterin

Initial suggestions were that $NTPH_2$ was dephosphorylated and proceeded via a series of dihydropterin intermediates to BH_4. Evidence from Fukushima (1970) showed that [^{14}C]guanosine administration to tadpoles resulted in [^{14}C]sepiapterin and [^{14}C]biopterin formation, and *in vivo* and *in vitro* studies demonstrated that sepiapterin could be converted to dihydrobiopterin, BH_2, (Kapatos *et al*, 1982; Smith and Nichol, 1983). It was assumed that the reductase for dihydrofolate (DHFR: EC 1.5.1.3) would complete the biosynthesis of BH_4. However, Stone (1976) showed that massive doses of the DHFR inhibitor trimethoprim had little effect on BH_4 levels in rat liver, and so presumably the biosynthesis of BH_4 did not involve reduction of BH_2. Another study by Nichol *et al* (1983) involved a mutant strain of Chinese hamster ovary cells (DUKX-B11) which were totally deficient in DHFR; BH_4 biosynthesis was normal. It is now thought that the pathway from $NTPH_2$ to BH_4 comprises tetrahydropterin intermediates only, and

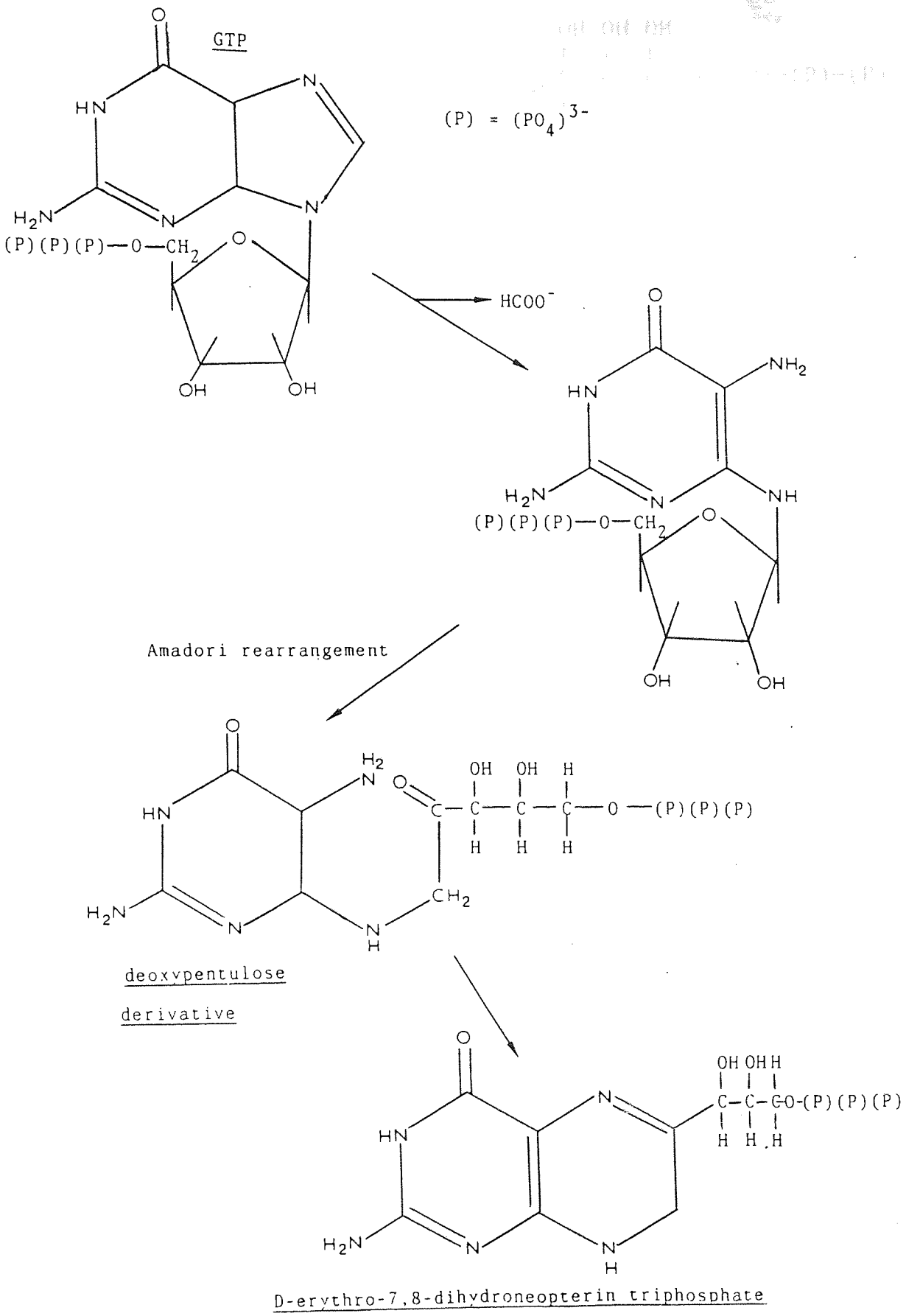


Figure 1.4 Conversion of guanosine triphosphate to dihydroneopterin triphosphate

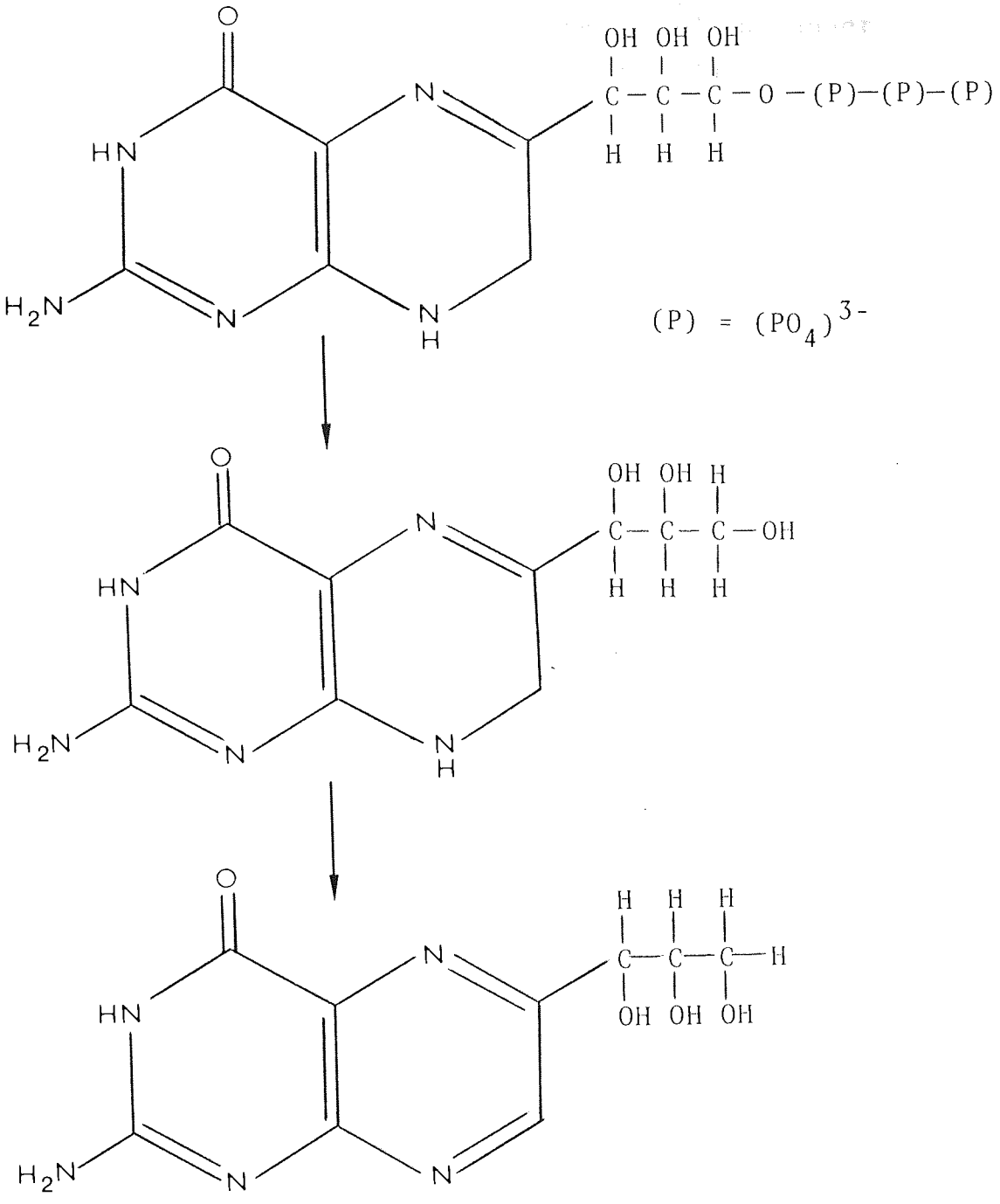


Figure 1.5 Conversion of dihydroneopterin triphosphate to dihydroneopterin and neopterin

that the production of sepiapterin and BH_2 arises from oxidation of these labile intermediates. Using anaerobic high pressure liquid chromatography (HPLC), electrochemical and ultraviolet spectroscopic techniques, various groups have provided evidence for a magnesium (Mg^{2+})-dependent enzyme process whereby $NTPH_2$ is converted directly to 6-pyruvoyltetrahydropterin, 6-PTP (Switchenko et al, 1984; Smith and Nichol, 1984; Heintel et al, 1984). This enzyme has been designated as phosphate eliminating enzyme (PEE) by Heintel's group, and pyruvoyltetrahydropterin synthase by Smith and Nichol (1984). Another enzyme, 6-PTPreductase, converts 6-PTP to 6-lactoyl-5,6,7,8-tetrahydropterin which can be further reduced by the same enzyme or sepiapterin reductase (EC 1.1.1.153) to BH_4, as in figure 1.6. The intermediate 6-lactoyl-5,6,7,8-tetrahydropterin can be oxidised non-enzymatically in air to sepiapterin. Nichol et al (1985) propose that 6-PTP reductase be known as biopterin synthase. Metabolites of the biosynthesis of BH_4 therefore include neopterin and sepiapterin. Neopterin derived from $NTPH_2$ is abundant in tissues and fluids from man and monkey (Fukushima and Nixon, 1980; Duch et al, 1984a) but is much lower in dog liver (Duch et al, 1984a) and until recently was thought to be completely absent from the rat (Duch et al, 1984a). This indicates that the rate-limiting step for the synthesis of BH_4 may be species-specific: in man and higher apes it is at the level of PEE, and at the GTP-CH stage in lower mammals such as the rat.

1.3.2 'Salvage' of tetrahydrobiopterin

As BH_4 is metabolised during its cofactor activity for monooxygenases it is oxidised to a form of BH_2 known as quinonoid dihydrobiopterin (qBH_2). This form of BH_2 would rapidly tautomerise to 7,8-dihydrobiopterin ($7,8\text{-}BH_2$) and be lost from the cell were

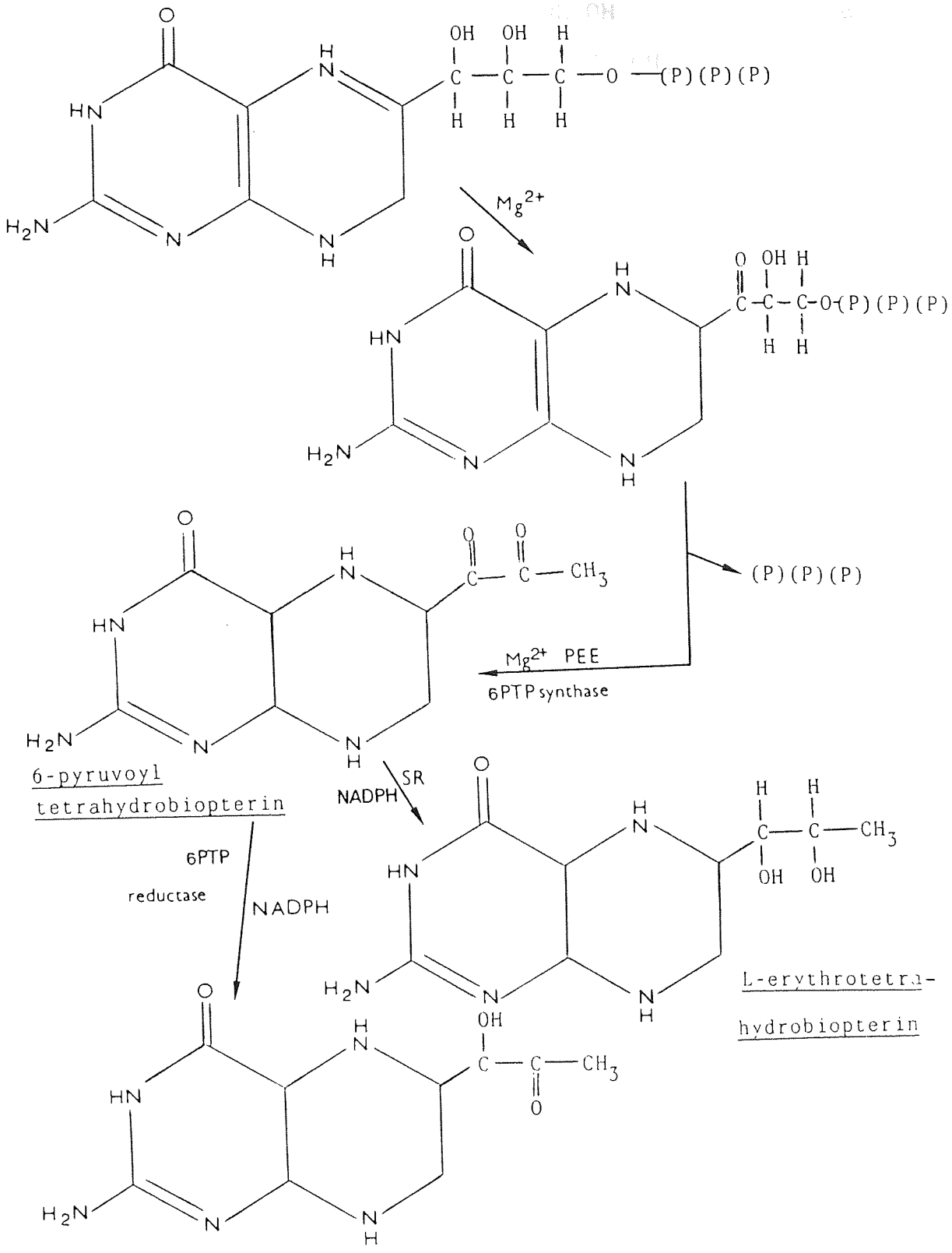


Figure 1.6 Proposed conversion of $NTPH_2$ to BH_4.
(Nichol et al 1985)

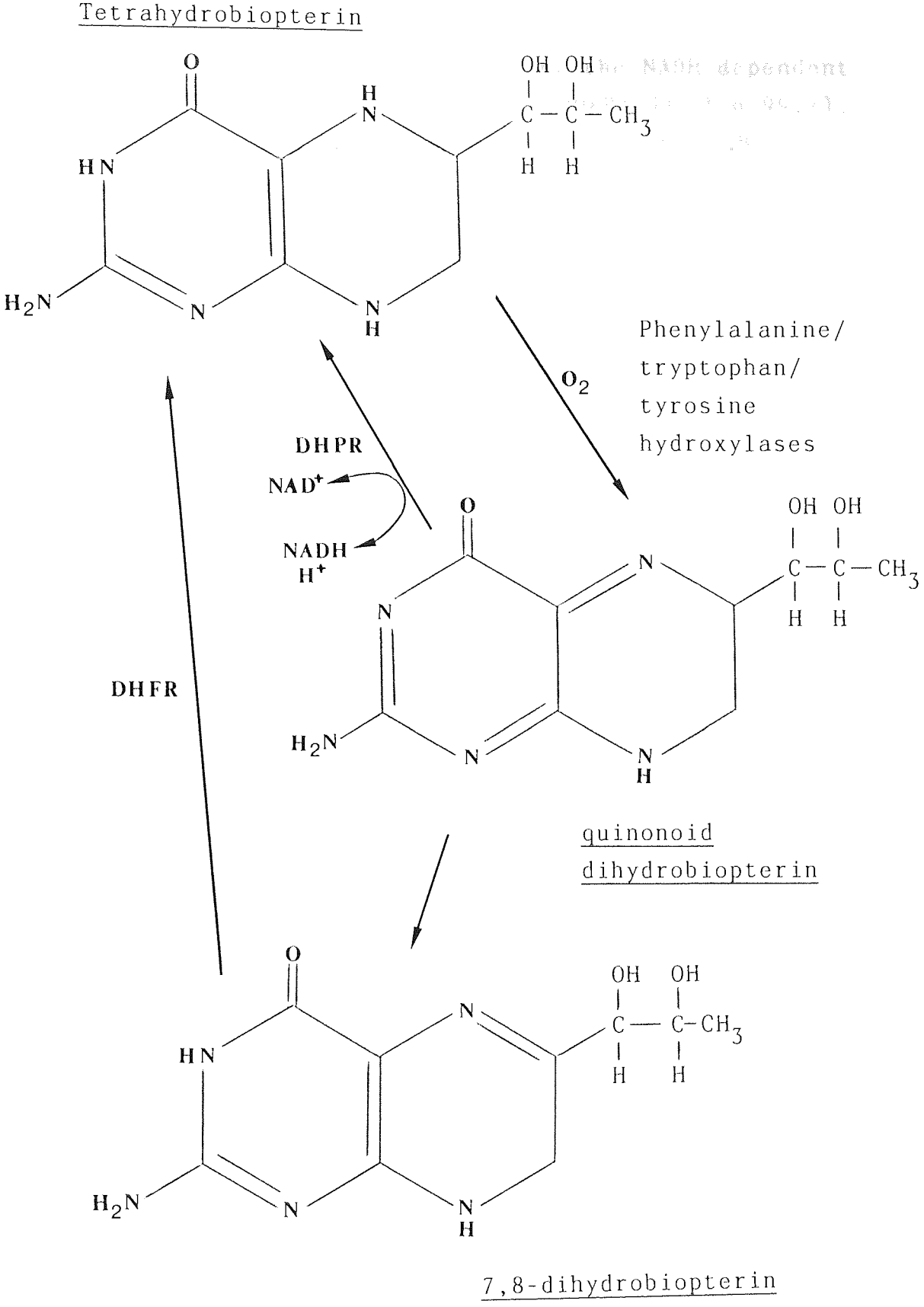


Figure 1.7 Salvage of tetrahydrobiopterin by DHPR and DHFR

it not for the 'salvage' activity of the NADH-dependent enzyme dihydropteridine reductase (DHPR; EC 1.6.99.7), figure 1.7. DHPR activity is specific for qBH_2 and not $7,8\text{-}BH_2$ (Gready, 1985). $7,8\text{-}BH_2$ can be reduced by DHFR back to BH_4 (Milstein and Kaufman, 1983), figure 1.7, although low levels of this enzyme are found in the brain (Blakley, 1969).

1.4 Tetrahydrofolate biosynthesis

Nimmo-Smith et al in 1948 showed that bacterial preparations could synthesise folate-like compounds when incubated with p-aminobenzoic acid (PABA, figure 1.8)

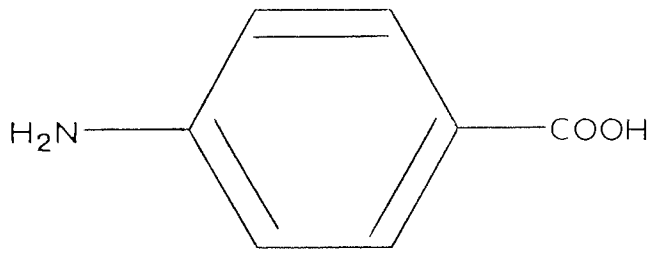


Figure 1.8 p-Aminobenzoic acid

and later Shiota (1959) tested several pteridines and found that $6\text{-}CH_2OH\text{-}7,8\text{-}H_2$pterin was the most effective at promoting synthesis in Lactobacillus plantarum in the presence of PABA, ATP and magnesium ions. Jones and Brown in 1967 established that dihydroneopterin triphosphate, an intermediate in BH_4 biosynthesis, was converted via dihydroneopterin aldolase (EC 4.1.2.25) to this aldol pteridine, and thus showed that tetrahydrofolate biosynthesis was endogenous in microorganisms. Inhibition of some of the steps of folate biosynthesis has formed the molecular basis of action of some classes of antibiotics such as sulfonamides (Nimmo-Smith, et al, 1948; Brown, 1962) which are structural analogues of PABA. The active coenzymes in one-carbon transfer reactions are polyglutamate

folates formed in mammalian cells by the action of pteroylpolyglutamate synthetase (McBurney and Whitmore, 1974) in a reaction summarised as follows:

$$FH_4(Glu)_n + \text{L-Glu} + ATP \longrightarrow FH_4(Glu)_{n+1} + ADP + P$$

1.5 ANALYSIS OF TETRAHYDROBIOPTERIN

Tetrahydrobiopterin is an essential nutrient for the trypanosomid insect parasite Crithidia fasciculata and thus a microbiological bioassay for measurement of this reduced pteridine has been established (Baker et al, 1974; Leeming et al, 1976).

Recent methods include gas chromatography-mass spectrometry (Kuster et al, 1984), radioimmunoassay (Nagatsu et al, 1979) and high pressure liquid chromatography (HPLC). HPLC is becoming the most widely used method for determination of pteridines in diverse substances such as biological fluids and tissues, and also foodstuffs. Separation can be achieved using simple C_{18} reversed-phase (RP) columns and elution with an isocratic solvent solution. Pteridines separate on RP columns according to polarity: for example neopterin with three (OH) groups is rapidly eluted whereas pterin with no polar side chain is usually the last pteridine to elute. Reduced species can be detected by coupling the separating column to an electrochemical detector, and similarly oxidised species can be measured fluorimetrically. Using fluorescence detection, μg/pmol amounts of pterins can be measured. Reduced species can easily be converted by chemical oxidation methods (manganese dioxide; acid/iodine/iodide mixes) to fluorescent species. Stea et al (1980) and Howells and Hyland (1987) have outlined the basic techniques in HPLC separation of pteridines.

The related compound FH_4 is likewise a growth factor for several microorganisms including Streptococcus faecalis (Bird et al, 1945) and Pediococcus

cerevisiae (Pfiffner et al, 1946) and Lactobacillus caseii remains the bioassay method of choice (Bird and M^{C} Glohon, 1972).

1.6 TETRAHYDROBIOPTERIN IN HEALTH AND DISEASE

1.6.1 Metabolic role of tetrahydrobiopterin

BH_4 is the specific cofactor for a variety of oxygen transfer reactions, most importantly in the synthesis of the catecholaminergic and indoleaminergic neurotransmitters dopamine, noradrenaline and serotonin.

1.6.1.1 Catecholamine neurotransmitter synthesis

BH_4 is the natural cofactor for the conversion of the amino acid phenylalanine to tyrosine via phenylalanine hydroxylase (phenylalanine 4-monooxygenase, EC1.14.16.1) (Kaufman, 1958; Kaufman, 1963). Tyrosine is hydroxylated to L-3,4-dihydroxyphenylalanine (L-dopa) by tyrosine hydroxylase (tyrosine 3-monooxygenase, EC 1.14.16.2) (Nagatsu et al, 1964) in a rate-limiting step (Levitt et al, 1964) for the eventual synthesis of dopamine and noradrenaline, figure 1.9. The catabolism of these neurotransmitters is shown in figures 1.10 and 1.11.

1.6.1.2 Indoleamine neurotransmitter synthesis

BH_4 is the cofactor for tryptophan hydroxylase (tryptophan 5-monooxygenase, EC 1.14.16.4) catalysing the hydroxylation of the amino acid tryptophan to 5-hydroxytryptophan (Hosoda and Glick, 1966) in a rate-limiting step for serotonin (5-hydroxytryptamine) synthesis (Costa and Meek, 1974). The biosynthesis is outlined in figure 1.12. The catabolism of serotonin is shown in figure 1.13.

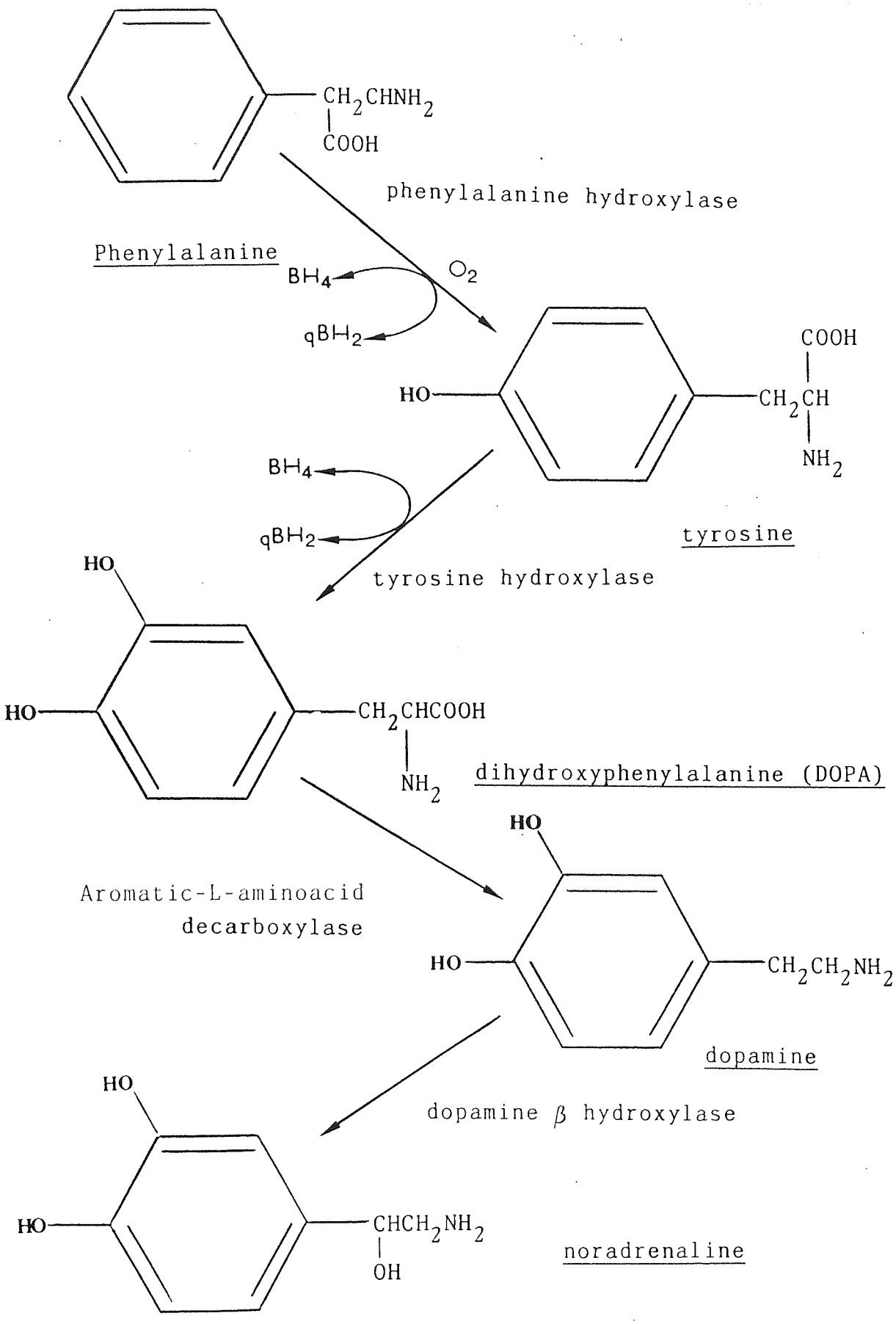


Figure 1.9 Cofactor role of BH_4 in the biosynthesis of dopamine and noradrenaline

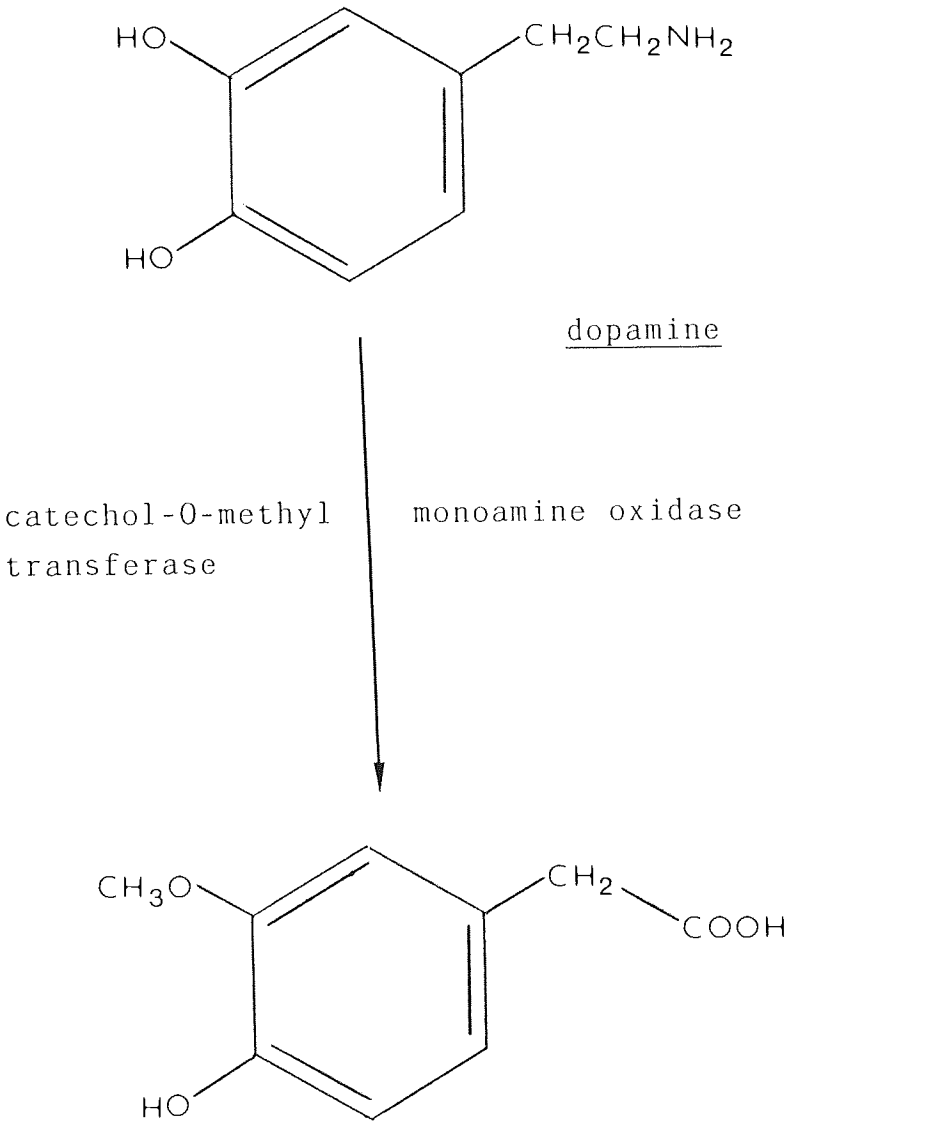


Figure 1.10 Catabolism of dopamine

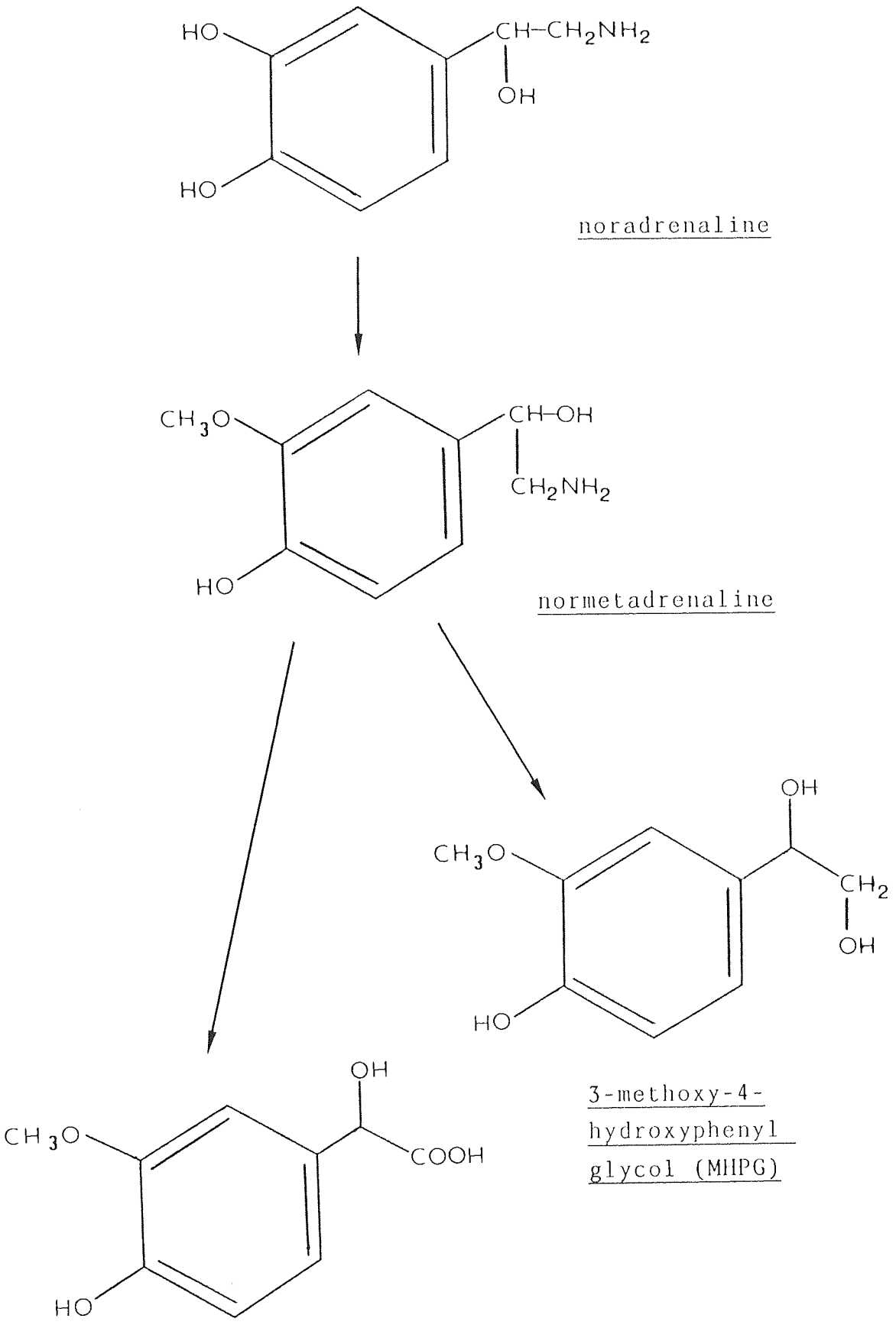
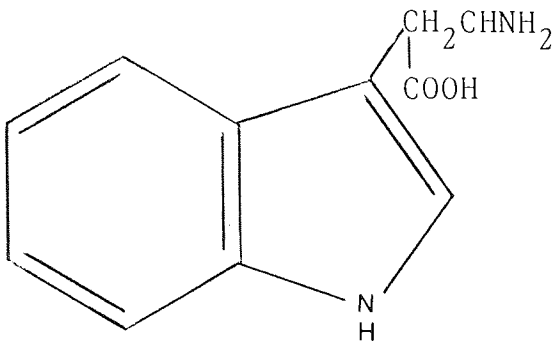
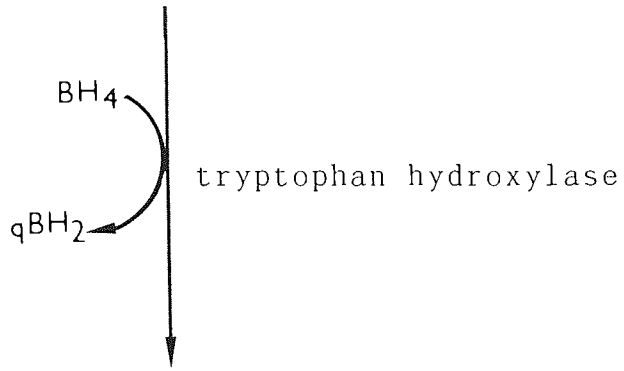
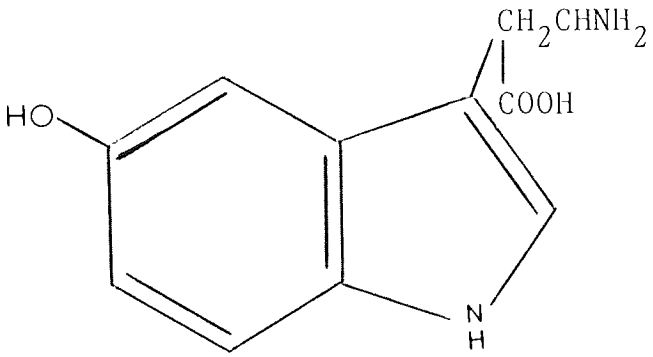


Figure 1.11 Catabolism of noradrenaline

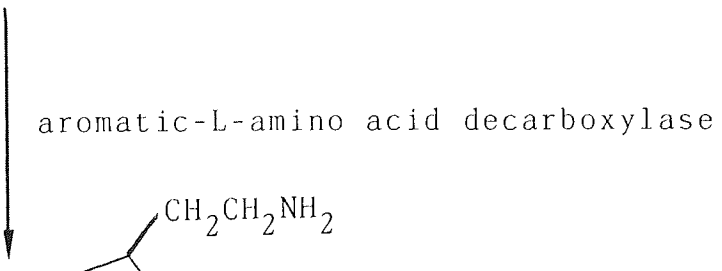


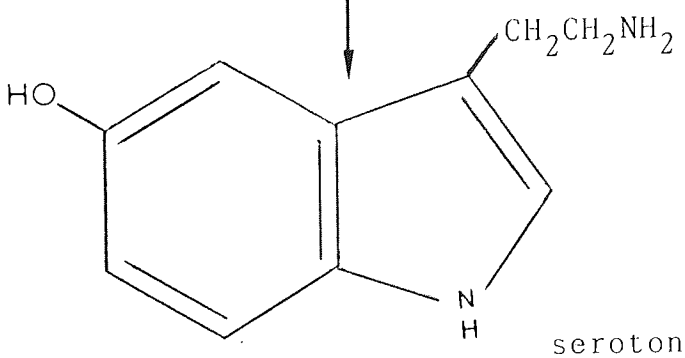
Tryptophan





5-hydroxytryptophan

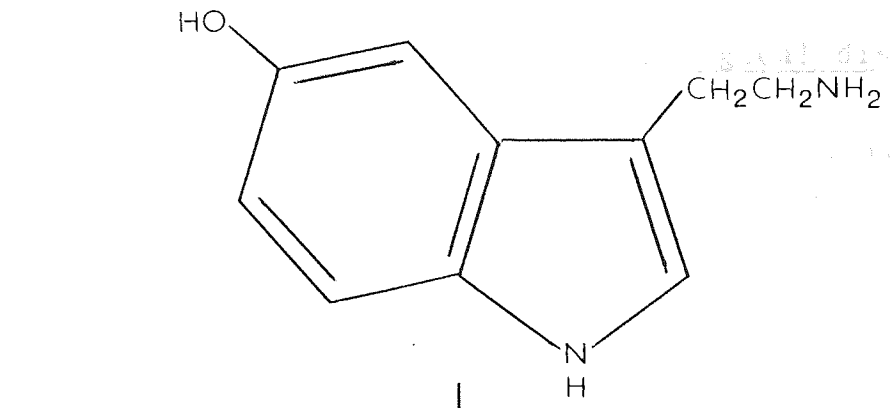




serotonin

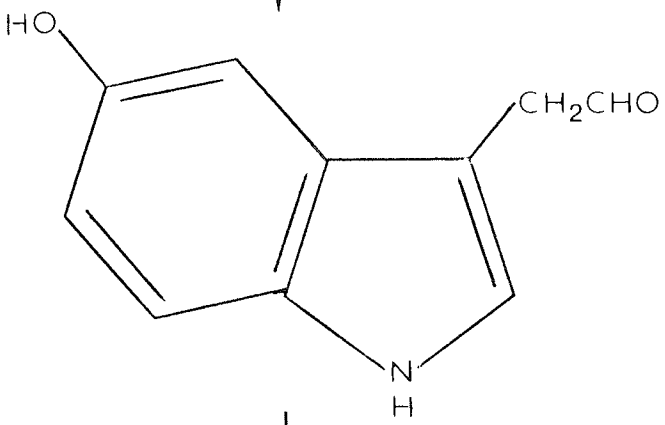
(5-hydroxytryptamine)

Figure 1.12 Cofactor role of BH_4 in biosynthesis of serotonin



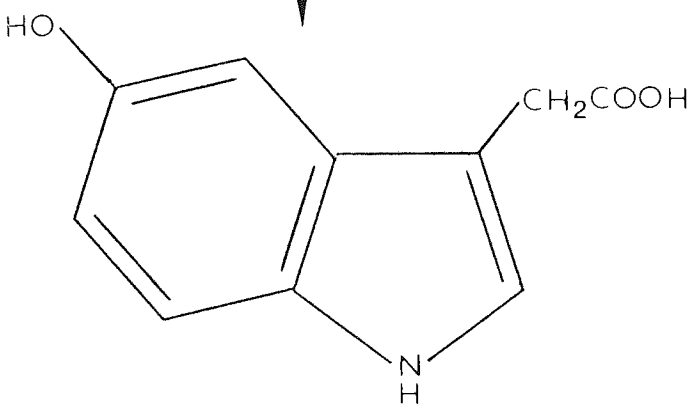
serotonin

monoamine oxidase MAO



5-hydroxyindole-acetaldehyde

aldehyde dehydrogenase



5-hydroxyindole-acetic acid

Figure 1.13 Catabolism of serotonin

1.6.2 Tetrahydrobiopterin in neurological disease

It is beyond the scope of this thesis to review in depth the various neurological and psychiatric disease states in which deficits in BH_4 metabolism have been noted. However, a brief description of the role of tetrahydrobiopterin in the pathologies of atypical phenylketonuria, Down's syndrome and Parkinson's disease serves to emphasise the importance of BH_4 in neurological disorders.

1.6.2.1 Classical and atypical phenylketonuria

Folling in 1934 reported a failure of the enzyme phenylalanine hydroxylase to convert phenylalanine to tyrosine in some children, leading to hyperphenylalaninaemia and urinary excretion of ketone bodies. Phenylketonuria (PKU) has since been shown to be transmitted by an autosomal recessive gene. Children with PKU have decreased stature and head size, and reduced skin pigmentation. Mental retardation is invariably present, and often epilepsy and electroencephalogram wave and spike activity characteristic of petit mal epilepsy. This metabolic lesion can be detected at birth, and treatment is by dietary restriction of phenylalanine. However, in approximately 2% of cases observed, there was no response to dietary control and this subgroup was termed 'malignant' (now called atypical) phenylketonuria (Danks *et al*, 1978). It was established that in these instances there is reduced or absent biopterin cofactor activity, due to diminished *de novo* synthesis (Leeming *et al*, 1976) including an absence of PEE (Niederwieser *et al*, 1985) or lowered salvage via DHPR (Rey *et al*, 1977). Screening for this atypical variant is now performed routinely (Matalon, 1984).

1.6.2.2 Down's syndrome

Down's syndrome is the commonest chromosomal abnormality, present in one out of every six hundred live births. It is caused by autosomal trisomy 21. Patients with Down's syndrome have a high incidence of congenital heart lesions, cataracts and umbilical hernias. The skull is bradycephalic and the eyes are typically slanted. Various degrees of mental retardation are present, the I.Q. being low, in the 25-49 range, with a downward trend on increasing age. Serum biopterin (Aziz et al, 1982) and BH_4 synthetic capacity of the brain (Blair et al, 1984a; Anderson et al, 1986) has been shown to be decreased in Down's syndrome which may contribute to the gross neurological defects seen in this condition.

1.6.2.3 Parkinson's disease

Parkinson's disease (PD) is a disturbance of motor function which affects predominantly males in the age group 50-70 years. It is characterised by slowing and enfeeblement of emotional and voluntary movement, muscular rigidity and tremor. Parkinsonism is caused by cellular degeneration in the basal ganglia. The most consistent pathological lesion is that of loss of melanin pigment and degeneration of neurones in the substantia nigra and the nigrostriatal dopamine pathway. This reduction can be be down to 25% of normal values. The traditional treatment of PD was by anticholinergic drugs to reduce tremor. However, as it was observed that dopamine given orally did not reach the CNS, precusor treatment was instigated, with L-dopa being successfully employed in 75% of cases where bradykinesia is present. As BH_4 is essential for dopa and dopamine synthesis, a speculative role exists for BH_4 in the treatment of parkinsonism. Curtius et al (1984) report that some symptoms of PD

have been successfully alleviated by tetrahydrobiopterin therapy.

1.7 AFFECTIVE DISORDERS

The term 'affect' is used for the emotional or feeling aspect of mental life, or mood, and the clinical states where a particular affect is involved are called affective disorders. An individual with an affective disorder may show mood changes into a state of depression or mania or both. Mood changes may be very variable in severity. Affective disorders are commonly recurrent.

1.7.1 Symptoms of depression

Classic cases of depression present the following triad of symptoms (based on Sim, 1974): depressed mood, psychomotor retardation, and morbid thought content with feelings of unworthiness or guilt.

Depressed mood can develop slowly or suddenly. In mild to moderate cases, there is a lack of interest and concentration. The depressed mood is often worse in the mornings. Irritability is a feature. Very noticeable is the fact that the patient is easily moved to tears, especially when contrasted to previous 'cheerful' nature. In severe cases, the patient looks miserable and can be agitated. He or she complains that there is no joy in life, life is not 'worthwhile', and the patient does not wish to carry on. There are often clear signs that the patient is contemplating suicide.

Psychomotor retardation may initially show as tiredness, slow gait and loss of libido. There is indecision, and performance of simple acts may require intense concentration. In severe cases, this retardation may result in stupor and mutism.

Morbid thoughts of unworthiness and guilt are felt by the patient. Patients refer to 'letting people

down' and a feeling of inadequacy. Guilt is often felt over minor incidents. Suicidal ideation is common and can lead to overt suicidal behaviour in severe cases.
Other features are also associated with depression. Sleep is often impaired, with characteristic early waking followed by insomnia. Appetite may be increased in mild depression with a slight weight gain, but is more usually a dramatic weight loss in severe cases. Somatic complaints include fatigue and tension symptoms such as pain in the head, neck and back. Hypochondriasis is often present, and can arise from feelings of guilt. Patients may indicate that the disease they believe they are suffering from is of a socially reprehensible nature, for example syphilis , or will result in a slow and painful death.

1.7.2 Symptoms of mania

Mania without an alternating state of depression is much rarer than depression alone. Mania may just develop out of a natural state of well-being, or may follow an attack of depression. The following symptoms and signs are usually present (Sim, 1974):
Mood is usually elated, but can give way to rudeness and hostility. Transient features of depression, for example weeping, may also occur.
Motor activity is increased from a mild increase in normal activity to wild hyperactivity and exhaustion.
Thought processes and content are characteristic in that the patient shows flights of ideas and delusions of grandeur. Speech becomes verbose and circumstantial, often with rhyming. Attention is distractible.
Other features include increased libido which may result in promiscuity. Hyperactivity may lead to weight loss. Marked insomnia is often present. Hypomania is a lesser version of manic attack which may develop into full-blown mania or exhibit depressive features.

1.7.3 Classification of depressive illness

1.7.3.1 Historical origins

People suffering from depression have been recognised as melancholics since the time of the early Greeks. However, in more recent times, affective disorder was described as 'folie circulaire' in the mid 19th century by Farlet. Kraepelin was the first to systematically classify dementia praecox (schizophrenia) and manic depressive psychosis on a sound basis of symptomatology and course of illness. Kraepelin considered psychiatric disturbance to arise solely from an endogenous cause, and virtually excluded external factors from having a bearing on the onset of illness. However, Freud and his followers viewed mental illness from a different approach, in which they considered environmental factors and social interaction were important elements in the development of a psychiatric disorder. These two different views of manic depression have resulted in differences in classification and treatment of the illness (namely pharmacological and psychoanalytical therapy).

1.7.3.2 Clinical classification of depression

Numerous classifications of affective disorder exist and it is beyond the scope of this thesis to describe them all. Only the salient features of the most widely used classifications are briefly described.

1.7.3.2.1 Psychotic-endogenous/neurotic-exogenous classification

Psychotic depression is generally of acute onset and is a more severe depression. It may be accompanied by hallucinations. Neurotic depression is milder and may be accompanied by anxiety and hysteria.

Exogenous depression is considered to be precipitated by stressful life events. There is a certain amount of heterogeneity in this classification, in which Kendell (1976) considers personality to be an important factor.

1.7.3.2.2 Primary-secondary classification

This classification is mainly used in the United States. Primary depressive illnesses are considered to arise independently of any other psychiatric disorder, whereas secondary affective disorder follows an original psychiatric disturbance such as schizophrenia, alcoholism or personality disorder.

1.7.3.2.3 Bipolar-unipolar classification

In this classification, bipolar affective disorder describes an endogenous illness with both depressive and manic phases, and unipolar describes an endogenous illness with recurrent phases of either depression or mania. Unipolar depression is much more common than unipolar mania. This classification was first described and characterised by Leonhard in 1963.

A patient with unipolar depression must have had at least three episodes of psychotic depression, separated by intervals of complete remission. A diagnosis of bipolar disorder is made after one attack of mania followed by a bout of depression.

The bipolar-unipolar dichotomy is supported by several lines of evidence. Bipolar illness has an earlier age of onset (peak 25 - 29 years) than unipolar illness (peak 40 - 44 years) (Perris, 1966). Differences are also seen between the two groups in terms of personality, biochemistry and pharmacology (Perris, 1966). Untreated patients with bipolar dis-

order can expect to have more affective order episodes than patients with unipolar illness (Angst, 1981). With both bipolar and unipolar recurrance, the frequency of depressed or manic episodes increases with the number of previous episodes (Zis and Goodwin, 1979) and later episodes tend to be more severe (Post et al, 1981). Bipolar illness occurs in individuals with less personality disturbance, and there is a more favourable response to prophylactic lithium than in non-bipolar depression.

Bipolar disorder has been subclassified by Angst (1978) into three groups: predominantly depressed (mD); predominantly manic (Md); and a group suffering from both severe mania and severe depression (MD). Andreasen and Winokur (1979) divided unipolar disorder into three subgroups of familial pure depressive disease; depressive spectrum disease; and sporadic depressive disease.

1.7.3.2.4 International Classification of Disease (ICD)

The most recent revision of the international classification (ICD-9) published by the World Health Organisation (WHO, 1978) contains 16 different sub-categories of affective disorder.

1.7.3.2.5 DSM-III classification

The American Psychiatric Association (1980) have published a third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III). The main subclassification of affective disorder is into major affective disorder; other specific disorders; and atypical affective disorder. A criticism of the DSM-III is that it fails to require a periodic or recurrent course as essential for diagnosis.

1.7.4 Prevalence of depression

Affective disorders are the most common single psychiatric disorder and represent a major public health problem that consumes a large portion of expenditure on mental health. It is estimated that from 13 - 20% of the population in Western countries has some depressive symptoms at any given time (Weissman and Myers, 1978; Wing et al, 1978), and approximately 2 to 3 percent of the population is hospitalised or seriously impaired by affective illness (Goodwin and Jamison, 1984). In particular, bipolar disorder affects 10 out of every 100,000 people per year (Nielsen and Nielsen, 1979). The major social concern of depressive illness is the associated threat of suicide: 15% of depressives commit suicide (Guze and Robins, 1970) and up to 64% of all suicides have some psychiatric disorder, depression being the most common (Barraclough et al, 1974). As the World Health Organisation in 1974 estimated that 0.5 to 1.0% of deaths recorded in Western countries were due to suicide, the damaging potential of depressive illness is enormous.

1.7.5 Causes of depression

This introduction proposes to consider the biochemical origin of endogenous depression only, and not any social or environmental contribution to the development of the illness.

1.7.5.1 Monoamine theory of depression

The monoamine theory of depression concerns events pertaining to neurotransmitter function at the synapse, so this is briefly described here.

The neurotransmitter is usually synthesised from precursors in the cell body and transported down the axon to the neuron ending where it is stored in

vesicles. Release of the neurotransmitter into the synaptic cleft occurs upon electrical depolarisation of the nerve cell. In the synaptic cleft, the neurotransmitter has several options. It can bind to a receptor in the membrane of the presynaptic terminal (re-uptake); it can be metabolised to inactive products in the cleft by one or more enzymes; or it can perform its function as a neurotransmitter by binding to a receptor at the postsynaptic site, the adjacent nerve ending.

For catecholamine neurotransmitters, the biosynthesis of tyrosine from phenylalanine, and the subsequent conversion of tyrosine to dopa requires tetrahydrobiopterin. Dopa is further converted to the neurotransmitters dopamine and noradrenaline. BH_4 is also required for the hydroxylation of tryptophan to 5-hydroxytryptophan before conversion to the indoleamine neurotransmitter 5-hydroxytryptamine, or serotonin. In the synaptic cleft, noradrenergic neurotransmitters are metabolised by monoamine oxidase (MAO) or catechol-O-methyl transferase (COMT) activity, or both. If binding at a postsynaptic site does occur, dopamine will bind to a dopaminergic receptor, and noradrenaline to either an α or β adrenoceptor. For a serotoninergic neuron, 5-hydroxytryptamine can be catabolised after release by MAO in the synaptic cleft or can bind to a postsynaptic receptor. Two serotoninergic receptors are thought to exist on neurons: a $5HT_1$ receptor which will bind radioactive serotonin, and a $5HT_2$ receptor which binds radioactive spiroperidol. Both catecholamine and indoleamine neurotransmitters can be reuptaken by binding at the presynaptic terminal. A separate presynaptic 5HT receptor has been identified (Peroutka and Snyder, 1979) which is responsible for reuptake of the amine. A receptor for the tricyclic antidepressant imipramine is thought to be located near to the 5HT reuptake receptor at

the presynaptic terminal.

The monoamine theory of depression claims that there is a functional deficit of amine neurotransmitters in the brain. A deficit in the catecholaminergic system has been proposed by Schildkraut (1965) and diminished indoleaminergic function by Coppen (1967). Many studies of mood, drug and neurotransmitter interaction in recent years led to the formulation of these hypotheses.

One of the earliest observations connecting monoamines and depression was that the antihypertensive drug reserpine caused depressive symptoms to develop in up to 15% of patients (Muller et al, 1955; Bunney and Davis, 1965) and reserpine was shown to deplete rat brain of monoamines (Shore et al, 1955). Other patients prescribed the antituberculosis drug iproniazid and the major tranquilliser imipramine were shown to have a more cheerful mood and some symptoms of elation (Zeller et al, 1955). Both these drugs increased monoamine functional activity: iproniazid by inhibiting monoamine oxidase activity, and imipramine by preventing reuptake at the presynaptic neuron.

Following these chance observations, there have been many subsequent studies that support the hypothesis that a central deficiency of active amines is causal for the development of depression.

1.7.5.1.1 Brain studies that support the monoamine theory of depression

Levels of 5HT and its metabolite 5-hydroxyindoleacetic acid (5HIAA) have been shown to be lowered in post mortem brains from suicides and depressives (Pare et al, 1969; Lloyd et al, 1974; Birkmayer and Riederer 1975).

1.7.5.1.2 CSF studies that support the monoamine theory of depression

Measurements in CSF obtained post mortem from suicides have shown diminished levels of 5HIAA compared to controls (Asberg et al, 1976; Traskman et al, 1981). In life, lowered CSF 5HIAA levels have been suggested as a vulnerability factor for depression (Ashcroft et al, 1966; Coppen et al, 1972). Van Praag (1982) considers diminished CSF 5HIAA concentration as the best available pointer to a disturbance of central 5HT metabolism in depression. There has also been one report of decreased noradrenaline in CSF from depressed patients (Christensen et al, 1980).

1.7.5.1.3 Peripheral studies that support the monoamine theory of depression

Platelets are non-nucleated discoid structures whose function in the circulation is to maintain primary homeostasis. Some structural aspects of platelets are analogous to those of neurons and the platelet can serve as a limited model for serotoninergic neurons of brain (Stahl, 1985). Many studies have used platelets in investigations into the biochemistry of depression (review Wood and Coppen, 1982). Platelets have serotoninergic receptors similar to the receptors found at the postsynaptic neuron. Depressed patients have diminished 5HT uptake in platelets which is thought to represent the disturbances in the clinical state (Meltzer et al, 1981). Platelets also have imipramine receptors at the surface which are considered to be analogous to 5HT reuptake receptors (Langer et al, 1980). These are shown to have a reduced density in platelets from depressives, presumably as a compensation for insufficient 5HT synthesis (Paul et al, 1982).

Urine studies have mainly focussed on the measurement of the principal noradrenaline metabolite of the CNS, 3-methoxy-4-hydroxyphenyl glycol (MHPG). Maas et al (1972) found decreased urinary excretion of MHPG in depression, and evidence from primates supports the contention that urinary MHPG reflects noradrenaline metabolism in the CNS (Elsworth et al, 1982). Studies of urinary and plasma 5HIAA concentrations are unhelpful due to peripheral serotonin distribution (serotonin has a major role as a vasoconstrictor).

1.7.5.1.3 Pharmacological studies that support the monoamine theory of depression

If, as postulated, there is diminished synthesis and activity of monoamines in depressive illness, any pharmacological agent that increases monoamine concentration should have a net result in alleviating depression.

Tryptophan is a precursor of serotonin synthesis, and decreased levels have been shown in the plasma and CSF of depressives compared to controls (Coppen et al, 1973 a). Supplementing depressives with tryptophan (Coppen et al, 1963) and 5-hydroxytryptophan (Van Praag, 1981) has proved a useful addition to MAOI antidepressant medication.

The action of many antidepressant drugs is briefly described here to show how they promote monoamine activity in neural transmission (from Kruk and Pycock, 1987).

The most widely prescribed drugs for depressive illness are the tricyclics, named after their common structure (see figure 5.2). They are chemically related to the phenothiazines but have antidepressant rather than sedative actions. The pharmacological properties of tricyclics are ascribed to their ability to inhibit the reuptake of monoamines at the presynaptic neuron (Stahl, 1985). Different tricyclics

have different selective effects: desipramine predominantly inhibits reuptake of noradrenaline, clomipramine predominantly inhibits serotonin reuptake, and imipramine and amitriptyline have mixed action.

Monoamine oxidase inhibitors (MAOIs) are drugs that act to prevent catabolism of the neurotransmitters in the synaptic cleft, and therefore prolong their action. These drugs include ipronazide, nialamide and pargyline. Tranylcypromine and phenelzine are MAOIs which also inhibit noradrenaline reuptake. Tranylcypromine releases dopamine as well as blocking its uptake, so has some stimulant (amphetamine-like) reactions as well as antidepressant action. Nomifensine is a non-MAOI, non-tricyclic antidepressant which inhibits the uptake of dopamine.

Pharmacological studies therefore present ample evidence for a functional increase in CNS monoamine concentration after the administration of antidepressant drugs. This may be considered as restoring the monoamine deficits shown in depressed patients to normal levels.

1.7.5.2 The alternative monoamine theory of depression

More recently, criticisms have been levelled at the original monoamine theory of depression which postulated a functional deficit in active amines in depression. The theory has been criticised for its simplistic nature and for other biochemical observations: inhibition of reuptake of amines by antidepressant drugs can be achieved *in vitro* immediately, yet the therapeutic effects of most drugs require 2 - 3 weeks administration (Oswald *et al*, 1972). Sulser (1979) proposed an alternative to the original theory. This postulated that there is a primary monoaminergic hyperactivity in depressive illness. The basis for this theory arises from two observations: the chronic administration of tricyclic antidepressants causes

a decrease in the number (down-regulation) and sensitivity of post synaptic receptors. Peroutka and Snyder (1980) working with rat brain showed that the down-regulation occurred at the same time as the therapeutic effect of antidepressant drugs (after a couple of weeks). Charney et al (1981) claim a diminished sensitivity of postsynaptic β receptors after chronic tricyclic treatment.

The alternative theory has not gained widespread acceptance for several reasons. According to the alternative theory, any agent that reduces the pathological monoamine hyperactivity should function as an antidepressant. Shopsin et al (1976) showed that p-chloroalanine, a 5HT synthesis inhibitor, reversed the antidepressant effect of monoamine oxidase drugs. Electroconvulsive therapy is one of the most effective treatments for endogenous depression, yet it causes an increase (up-regulation) in $5HT_2$ receptors in rat cerebral cortex (Kellar et al, 1981). If, as the alternative hypothesis postulates, increased catecholamines cause depression, any agent that blocks the binding of noradrenaline to a postsynaptic receptor should cause an increase in depressive symptoms. Many patients receive β blockers such as propranolol, yet there are no reports of clinical depression being induced by β blockers. This compares with the depression known to be induced by reserpine which depletes the brain of monoamines.

The traditional monoamine hypothesis is therefore more convincing.

1.7.6 Neuroendocrinology of depression

Disturbances in the neuroendocrine system have long been recognised in depressive illness. The most investigated system has been the interaction between the hypothalamus, pituitary and adrenal glands, the

HPA axis.

The normal functioning of the HPA axis can be summarised as following. Corticotropin-releasing factor, CRF, is produced by the hypothalamus and stimulates the pituitary gland to produce adrenocorticotrophic hormone, ACTH. This has a direct effect on the adrenal cortex to release cortisol. Control of secretion is via a negative feedback loop whereby rising levels of blood cortisol inhibit further production of CRF and ACTH. In healthy adults, plasma cortisol has a circadian rhythm, being highest at 9am and lowest the following 4am. Plasma cortisol is transported bound to cortisol binding globulin, and any non-bound steroid is filtered at the kidney and excreted into the urine.

In some disease states such as Cushing's syndrome and in stressed states, the normal circadian rhythm of cortisol regulation is overidden. In addition, cortisol dysregulation is particularly associated with depression.

Hyperactivity of the HPA axis in depressive illness has been shown many times. Hypersecretion resulting in raised plasma cortisol has been shown in up to 50% of depressives (Sachar et al, 1973; Carroll et al, 1976; Cohen et al, 1984). Other studies showed a rise in CSF cortisol (Stokes et al, 1984) and urinary free cortisol (Rubinow et al, 1984) in depressive illness.

One of the most widely used tests for HPA function is the dexamethasone suppression test, originally devised to diagnose Cushing's syndrome. Dexamethasone is a synthetic structural analogue of natural cortisol. Administration of a small dose of dexamethasone normally results in the suppression of ACTH release, and a secondary decrease in cortisol secretion from the adrenal cortex. It was shown that depressives had an early escape from dexamethasone suppression,

and thus Carroll et al (1981) introduced the standardised (1mg) dexamethasone suppression test (DST) as a diagnostic test for depression. Carroll et al (1981) claim that the DST is highly specific for endogenous depression compared to other psychiatric disturbances, with 50% of depressives showing an abnormal DST. More recently, Coppen et al (1983) found that while the DST is very sensitive to the presence of endogenous depressive illness (81%), its specificity is low (70%). Abnormal DST responses have also been shown in mania (Godwin, 1984), dementia (Raskind et al, 1982), and anorexia nervosa (Abou-Saleh et al, 1985).

It is not clear how the disturbances in the HPA axis and resulting DST abnormalities in depressive illness fit in with the postulated neurotransmitter deficits, but the DST is widely considered to be useful in the diagnosis and prognosis of depression.

1.7.7 Treatment of depression

A detailed review of all the available treatments for depression is beyond the scope of this thesis. The main ways of treating depression are psychotherapy, electroconvulsive therapy (ECT), and drugs. The choice of treatment depends on the diagnosis. Severe endogenous depression would not be treated by psychotherapy. Of the three main therapies, only drug treatment attempts to redress alterations or deficiencies in brain neurotransmitter synthesis and action.

1.7.7.1 Antidepressant medication

There are four main types of antidepressant medication, namely cyclic antidepressants, monoamine oxidase inhibitors (MAOIs), other drugs which are neither cyclic nor MAOIs, and lithium. In general, and in support of the traditional monoamine theory, the drugs considered the most effective in depression are those which

are characterised by their ability to enhance brain serotonin function (Glue et al, 1986).

1.7.7.1.1 Tricyclic antidepressants

Tricyclic drugs such as imipramine, desipramine, nortriptyline and amitriptyline have a structural similarity (see Chapter 5) and are the drug treatment of choice for unipolar depression. They are considered to improve the symptoms of 60 - 70% of depressed patients first taking them (Klerman and Cole, 1965).

The first symptoms to improve, after two to three weeks administration, are sleep patterns and then there is alleviation of appetite disturbance and suicidal thoughts. After four weeks, general mood and activity improves. Amitriptyline and trimipramine have marked sedative effects, and are used for depressed patients with symptoms of agitation. There is also improvement in the accompanying insomnia. Desipramine and protriptyline have mild stimulant actions, and therefore may be useful in depressed patients who are in need of arousal.

The biochemical role of tri- and tetracyclic antidepressants is to prevent reuptake of active amines presynaptically: desipramine inhibits the reuptake of noradrenaline; clomipramine inhibits reuptake of serotonin; and imipramine and amitriptyline may inhibit both.

The main side effects of these drugs are related to their anticholinergic action in that they block muscarininc receptors. These effects include memory disorders, blurring of vision, dry mouth, micturition, constipation, and difficulty in swallowing.

They weakly block α adrenoceptors and cause postural hypotension. They may also cause hypertension due to prolonged stimulation of α and β adrenoceptors following inhibition of noradrenaline reuptake. This causes increased vasoconstriction and cardiac output. Other side effects include palpitations, fine tremor and weight gain. They can precipitate seizures in patients with epilepsy, and seizures have been known to occur with administration of tricyclics in patients with no previous history of epilepsy. In overdose or in combination with drugs such as barbiturates, hypnotics and ethanol, there is serious respiratory depression.

1.7.7.1.2 Monoamine oxidase inhibitors

MAOIs are not much used for bipolar depressed patients, but are best used in atypical depression where there may be extra somatic symptoms, phobias and other neurotic complaints. The most commonly used monoamine oxidase inhibitors are phenelzine and tranylcypromine sulphate.

MAOIs act in the synaptic cleft to inhibit enzymes such as COMT and MAO which would otherwise convert catecholamine neurotransmitters and serotonin to inactive metabolites.

Patients receiving MAOIs must follow a diet that restricts foods containing tyramine. The so-called 'cheese effect' of the interaction of tyramine and MAOIs causes hypertensive crises (White and Simpson, 1981).

1.7.7.1.3 Lithium

Lithium has been known for its therapeutic effects in the treatment of mania since the early studies of Cade in 1949 and Noack and Trautner in 1951, but has

more recently been proposed as an antidepressant (Coppen et al, 1971; Prien et al, 1973).

In 1978, Hullin estimated that one out of every two thousand of the population of the United Kingdom was receiving lithium as treatment for manic-depressive psychosis, representing 25,000 patients. The response of new bipolar disorder patients to lithium is generally good: Yang (1985) found 60% of patients improved and Birch (1982) showed an 80% response. Smigan (1985) found that the response to lithium varied from 63 - 70%, with females responding better than males. The value of lithium as sole medication in unipolar depression has not been fully established: Giannini et al (1986) claim that its best use as an antidepressant is in bipolar disorder. However, it does have an effect in potentiating other antidepressant treatments (section 1.7.7.2).

The biological and pharmacological effects of lithium and their relation to depressive illness are discussed in chapter five.

1.7.7.2 Combination of antidepressant treatments

In some cases, patients with resistant depression are successfully treated with a combination of therapies.

ECT is a rapid and effective treatment for severe endogenous depression, achieving good results in up to 50% of drug-resistant patients (Markowitz et al, 1987).

Controlled investigations have shown that addition of lithium carbonate to the drug regime of patients who have not responded initially to tricyclics produces a clear improvement in 50-60% of cases (De Montigny et al, 1983; Heninger et al, 1983; Worrall, 1986). Lithium supplementation of tranylcypromine has been shown to be successful where other combinations have not resulted in improvement (Price et al, 1985).

1.8 DEMENTIA

Dementia is the global impairment of higher cortical functions, including memory, the capacity to solve the problems of day to day living, the performance of learned perceptuo-motor skills, the correct use of social skills and control of emotional reactions, in the absence of gross clouding of conciousness. The condition is often irreversible and progressive (Royal College of Physicians, 1981).

1.8.1 Features of dementia (from the American Psychiatric Association, DSM III, 1980)

Dementia may be recognised by the following features: there is a loss of intellectual abilities which is severe enough to interfere with social and occupational functioning; the memory is impaired to varying degrees; there may be impairment of abstract thinking and judgement; other disturbances of higher brain functions involving language and complex sequences of action; there may be perceptual impairment and a personality change; and there is often a combination of these features. The patient must present these signs in the absence of delirium or intoxication. Often the patients medical history, a physical examination or laboratory tests will indicate that there exists an organic feature which could have produced dementia.

1.8.2 Diseases causing dementia

Dementia is a syndrome or grouping together of symptoms and not a disease in its own right.

Virtually any brain insult may result in dementia and there are specific diseases or disorders that give rise to dementia. It is recognised that dementia is associated with Down's syndrome, progressive supranuclear palsy, Pick's disease, Creutzfeld-Jakob

disease and kuru, and has been shown in boxers and some renal dialysis patients. Vascular or multi-infarct dementia, MID (Hachinski et al, 1974) is caused by loss of brain tissue after multiple strokes or brain infarcts and is the second most common cause of dementia in the elderly (Jorm, 1987). However, the most common disease causing dementia in the UK and the rest of the developed world is the disease named after Alzheimer.

1.8.3 Senile dementia of Alzheimer type

Alzheimer in 1907 described a woman aged 51 who exhibited all the features now recognised as dementia. The illness was progressive and she died four years later. Autopsy performed by Alzheimer revealed unusual histopathological changes in the brain.

It had long been known that dementia could occur in the elderly (senile dementia) and was generally thought to arise from cerebral atherosclerosis. Patients suffering from dementing illness younger than 65 years were originally described as having presenile dementia or Alzheimer's disease. However, subsequent post mortem studies revealed that there were many similarities between the brains taken from presenile and senile dementia patients, and older patients now tend to be referred to as suffering from senile dementia of Alzheimer type (SDAT). Some clinicians prefer to describe all such demented patients, irrespective of age, as having Alzheimer's disease.

1.8.3.1 Diagnosis and assessment of SDAT

When SDAT is suspected, the patient may be subjected to various tests for tentative confirmation or elimination of dementia.

Two of the most common screening tests for dementia include the Mini-Mental State Examination, MMSE (Folstein et al, 1975) and the Clifton Assessment

Procedures for the Elderly, CAPE (Pattie and Gilleard, 1979). These tests can be performed by nursing or other staff after brief training.

Intelligence and memory can be assessed by the Wechsler Adult Intelligence Scale and the Wechsler Memory Scale tests administered by psychologists.

The ability of a patient to cope with day to day activities can be assessed by a scale such as the Index of Independence in Activities of Daily Living, ADL, performed by occupational therapist or nursing staff familiar with the patients behaviour.

The diagnosis of SDAT is accomplished by psychiatrists who find that the patient conforms to the criteria accepted as dementia. In this, they are aided by standardised interviews, the two most common being the Geriatric Mental State Schedule, GMS (Copeland et al, 1976) and the Comprehensive Assessment and Referral Evaluation, CARE. The Cambridge Mental Disorders of the Elderly Examination, CAMDEX, is a recent development. The standardised examination involves a psychiatric interview with the patient, cognitive tests of the patient, and an interview with a relative or friend of the patient.

1.8.3.2 Cognitive features of SDAT

In SDAT all cognitive functions become impaired, the impairment increasing with the degree of dementia.

Memory deficit Often the first sign of SDAT in a patient is memory loss. Short term, or working memory can be much poorer than controls in even mildly impaired SDAT patients (Jorm, 1987). Long term storage of information appears to be reasonably preserved, although patients have difficulty in encoding new information. Subsequent retrieval of new or stored information may need considerable prompting.

Intellectual deficit Standardised tests such as the Wechsler Adult Intelligence Scale reveal that both verbal and performance intelligence is impaired in SDAT.

Language impairment

Aphasia is common in SDAT, and the patient will often incorrectly name an object with a general term or may describe what the object does (Martin and Fedio, 1983). The use of empty words, irrelevant observations and repetition of words is usual in SDAT speech patterns (Hier et al, 1985). Syntax is generally preserved.

Perceptual impairment

Agnosia is a feature of SDAT. People and objects are clearly seen but not recognised. The patient may be able to recognise a three-dimensional object, but find it harder to identify the same object in a photograph or line drawing (Jorm, 1987).

Visual-spatial impairment

Demented patients show visual-spatial impairment in that they become lost in the rooms of their own home, or in a familiar environment such as at the local shops. They find it difficult to copy drawings or draw an object from memory (Jorm, 1987).

1.8.3.3 Classification of SDAT

SDAT can range from mildly confused states to total disorientation, memory loss and dependence on another for functions such as feeding, dressing and personal hygiene. Hughes et al (1982) therefore devised the Clinical Dementia Rating (CDR) which places people in one of five stages in a sequence ranging from health (CDR 0), questionable dementia (CDR 0.5), mild dementia (CDR 1), moderate dementia (CDR 2) to severe dementia (CDR 3). Features covered include memory, orientation,

judgement and problem solving, community affairs, home and hobbies and personal affairs. Individual SDAT patients progress at different rates along this continuum. Crapper and DeBoni (1980) consider SDAT to generally take 18 months to 19 years from diagnosis to death, with a mean duration of illness of eight years.

1.8.3.4 Risk factors for SDAT

SDAT does not affect the majority of people: there are currently five known risk factors for development of this disease. These are old age; a family history of Alzheimer's disease; head trauma; Down's syndrome; and a family history of Down's syndrome (Jorm, 1987).

1.8.3.5 Prevalence and social impact of SDAT

A problem with surveying the occurrence of SDAT in an elderly population is that AD can often be accompanied by MID, resulting in 'mixed dementia'. However, it is generally agreed that in the Western world, dementia affects approximately 2% of people aged 65 to 70 years, and about 20% of 80+ year-olds (Wilcock, 1988). The frequency of dementia is strongly correlated with age (Nielsen, 1963). Davies (1983) has estimated that the US contains at least 3 million people over 65 years old who are demented to some degree, and Castledine (1984) in the UK states that some 750,000 individuals are suffering from dementia of various causes. Alzheimer's disease in people younger than 65 years has been estimated at less than 0.1% of the 45 - 64 year-old age group (Molsa *et al*, 1985).

The impact of SDAT on society is of increasing importance. In developing countries where many people do not reach the age of 65, SDAT is at present much less of a problem than in the Western world where longer lifespans are more frequent. However, all countries

of the world are experiencing a marked ageing of the population (World Health Organisation, 1982) which will mean that the proportion of elderly (65+ years) and very elderly (80+ years) will be increased. It is in these groups that SDAT is usually found. The ageing population naturally represents an economic burden on the younger, working section of society. The falling birth rate in many Western societies means that there will be less economically active people being able to support the elderly in the future. The demented elderly are also surviving longer due to the development of drugs such as antibiotics for the treatment of infectious diseases. SDAT patients no longer succumb to diseases such as pneumonia which would have caused death forty years ago. The impact on the social and health services is expected to increase substantially in the next three decades. Up to half of all SDAT patients require hospitalisation, and, once admitted, have a much poorer prognosis (in terms of survival and discharge home) than other mentally ill people of the same age (Blessed, 1980). The costs of institutional care, or grants and allowances to relatives caring for SDAT patients at home, are usually met by the state in the UK and will rise as the number of SDAT sufferers in society increases.

1.8.3.6 Alterations in the brain in SDAT

The brain and neurochemical changes that occur in SDAT are considered.

1.8.3.6.1 Histopathology of SDAT

SDAT suspected in life is usually confirmed at autopsy. Alzheimer (1907) was the first to recognise microscopic changes in brain tissue giving rise to senile plaques and neurofibrillary tangles.

Plaques are found in all areas of the brain containing axons. They are seen in silver preparations under the light microscope as irregular masses 15 - 200 μm in diameter with densely staining centres. There is a core of protein called amyloid which is not naturally occurring in the brain. The reason for the formation of these plaques is not yet known.

Neurofibrillary tangles are found in the cell body of the neuron. They can be viewed under the light microscope as abnormally thick ultracytoplasmic fibrils. Electron microscopy enables them to be measured as 22nm long. They may arise from changes in the normal neurofilaments of the neuron (Gajdusek, 1985).

Plaques and tangles in the brain are characteristic of SDAT but also occur in unaffected elderly people. Tomlinson et al (1968) found that 65% of normal people aged over 65 years had some plaques and tangles in the cerebral cortex, although not in large numbers. In SDAT, these structures are abundant and Perry et al (1978) have shown a correlation existing between the numbers of plaques and rating tests which examined cerebral functioning and severity of dementing process. The plaques and tangles are not evenly distributed throughout the brain, but tend to be concentrated in the hippocampus and cerebral cortex.

Other histopathological changes include Hirano bodies and granulovascular degeneration in the hippocampus (Rossor, 1982). There is also a loss of neurons from the locus coeruleus (Bondareff et al, 1981).

The changes in the hippocampus and cerebral cortex are of interest when their specific functions are considered. The hippocampus has a vital role in memory, whereas the cerebral cortex is the centre for complex mental processes including voluntary action, speech, perception, and most of human intelligence.

1.8.3.6.2 Changes in the metabolism of acetylcholine

Acetylcholine is not assayed in necropsy brain as it is extremely labile. The activity of enzymes associated with its metabolism are usually measured. One of the first reports of a deficit in the acetylcholinergic system was that of Pope et al (1964) who showed lowered acetylcholinesterase (AChE) activity in post mortem SDAT brain samples compared to controls. However, the enzyme cholineacetyl transferase (CAT) is more specific for cholinergic neurons, and this was shown to be lowered in post mortem brain SDAT cerebral cortex samples by Davies and Maloney (1976). CAT activity in necropsy cortex correlates with mental test scores (Perry et al, 1978) and reduction in cholinergic neurons (Bowen et al, 1979). In life, cerebrospinal fluid samples are considered most representative of the neurochemical profile of the brain. However, Lal et al (1984) found no differences in AChE activity in lumbar CSF samples from SDAT patients compared to non-demented controls with back pain, and concluded that CSF AChE was not a useful marker for SDAT. Plotkin and Jarvik (1986) have recently reviewed acetylcholine deficits in SDAT.

1.8.3.6.3 Changes in neuropeptides in SDAT

A general decrease in neuropeptides such as substance P (Davies et al, 1982) and corticotropin-releasing factor (DeSouza et al, 1986) has been observed in SDAT brain samples. Somatostatin is found to be reduced in cortical material (Davies et al, 1980; Beal et al, 1985) and there is some evidence that somatostatin neurons are particularly affected by plaques and tangles (Roberts et al, 1985).

1.8.3.6.4 Changes in the metabolism of catecholamines in SDAT

Since the early seventies there have been many studies on catecholaminergic neurons and chemistry in SDAT. Measurements have been made of the active amines dopamine (DA) and noradrenaline (NA), and their respective metabolites homovanillic acid (HVA) for dopamine, and both vanillyl mandelic acid (VMA) and 3-methoxy-4-hydroxy-phenyl glycol (MHPG) for noradrenaline.

The concentration of many catecholaminergic neurotransmitters in the brain does decline with age: Pradhan (1980) reviewed the neurochemical status of ageing brain in normal individuals aged between 15 to 93 at death. Both DA and NA concentrations were decreased in the hypothalamus, with DA also lower in the caudate nucleus, putamen, hindbrain and hippocampus. The dopaminergic marker enzyme dopa decarboxylase had less activity in the caudate nucleus with age, and the activity of the catabolic enzyme catechol-O-methyl transferase was at a higher level in the cortex.

In one of the earliest studies, Gottfries et al (1969) found that HVA was decreased in SDAT CSF and caudate nucleus and went on to show that there was a significant correlation between the degree of intellectual impairment and reduction in levels of HVA in SDAT (Gottfries et al, 1970). Fisher (1972) found that urine levels of HVA were decreased in SDAT. Other studies have confirmed that there is indeed a decrease in cerebrospinal HVA (Gottfries and Roos, 1973; Nakamura et al, 1984; Palmer et al, 1984). The active amine DA has been shown to be reduced in post mortem brain samples from SDAT patients compared to controls (Adolfsson et al, 1979; Gottfries et al, 1983). The activity of dopa decarboxylase has been shown several times to be decreased in SDAT brain at autopsy compared to controls (Bowen et al, 1974; Cross et al, 1981).

Reduced post mortem brain concentrations of noradrenaline have been reported by many groups (Adolfsson et al, 1979; Gottfries et al, 1983; Arai et al, 1984). The locus coeruleus is the main origin for adrenergic projections to the cerebral cortex, and Bondareff et al (1981) have reported a loss of neurons in this region in SDAT. These results imply that there is disturbed noradrenaline metabolism in SDAT: supporting evidence includes lowered MHPG levels in urine samples taken from living SDAT patients (Fisher 1972).

1.8.3.6.5 Changes in the metabolism of indoleamines in SDAT

Less attention has been focussed on the serotoninergic system in SDAT than on other transmitters such as acetylcholine. However, one of the earliest studies showed that the serotonin metabolite 5-hydroxyindole acetic acid (5HIAA) was lower in the CSF of SDAT patients than controls, and the lowest levels were found in the most demented patients (Gottfries et al, 1970). Later studies confirmed the decreases in 5HIAA concentrations both in post mortem CSF (Gottfries et al, 1976) and in samples from living patients (Argentiero and Tavolato, 1980; Soininen et al, 1981). Serotonin itself has been shown to be reduced in post mortem SDAT hippocampus compared to controls by Winblad et al (1985).

Cross et al (1984) compared two groups of autopsy brain samples that had been matched for age of patients and post mortem delay. The abundance of both serotoninergic receptors was significantly reduced in the hippocampus, temporal and frontal cortex of the demented patients. The authors claimed a specificity for SDAT in the reduction of these receptors compared to the other neuropsychiatric conditions of Huntington's and Parkinson's diseases, and schizophrenia.

1.8.3.7 Treatment of SDAT

Two main approaches for the treatment of SDAT have been made: administration of precursor compounds and actual drug therapy.

1.8.3.7.1 Precursor treatment

As there are well documented acetylcholine deficiencies in SDAT, Smith et al (1984a) have given patients the cholinergic precursor lecithin, but with little improvement. Growdon et al (1984) have used foodstuffs enriched with phosphatidylcholine which is the naturally occurring choline in the diet with no appreciable effect. To address indoleamine deficits, Shaw et al (1981) proposed that long-term tryptophan administration would be of benefit to potential SDAT sufferers, but these findings were later disputed by Smith et al (1984b) who claimed that serum tryptophan levels could be effectively raised by giving 3g of tryptophan per day to SDAT patients, but that this did not result in any clinical improvement.

1.8.3.7.2 Drug treatment

The drug treatment of SDAT has been largely unsuccessful. Various approaches have been tried, mainly for alleviation of distress in SDAT (treated with antidepressants and tranquillisers), or to attempt to treat the underlying causes (effect central neurotransmitter stimulation). Hollister (1985) in a review assessing the efficacy of treatments in SDAT listed six classes of drugs including about 30 different agents, and concluded that many were of limited use and needed stricter evaluation. Physostigmine (eserine) is an anticholinesterase which can temporarily clear confusion, but has no long term improvement on memory or the other symptoms of SDAT. Drugs that show the most promise

are alaproclate, a specific inhibitor of serotonin reuptake (Bergman et al, 1983) and tetra-hydro-amino-acridine (Summers et al, 1986)

1.8.3.8 Theories as to the origin of SDAT

Currently there are three theories as to why SDAT develops in an individual, namely a genetic cause, transmission by an infectious agent, and exposure to an environmental toxin, most studies implicating this to be aluminium.

1.8.3.8.1 Genetic theory

Transmission of neurological and psychiatric disease by a single defective gene has been established in Huntington's chorea, and it seems reasonable to assess the likelihood of Alzheimer's disease involving a genetic mechanism. An early report (Heston et al, 1966) suggested an autosomal dominant model of inheritance, but to explain most cases of SDAT it is necessary to assume that many genes are involved (Wright and Whalley, 1984).

Support for the genetic theory comes from a study of Down's syndrome. Most DS individuals who reach middle age show dementia similar to SDAT, and at autopsy their brains show characteristic AD-type plaques and tangles. The development of the tangles is thought to be as a consequence of abnormal neurofilaments, and Heston and White (1978) have suggested that DS and AD can occur in the same families because of some basic defect in these neurofilaments.

Evidence against a genetic origin for SDAT come from monozygotic twin studies. Hunter et al (1972) showed that SDAT developed in only one twin, and Cook et al (1981) showed that there was a ten year difference in onset between twins.

1.8.3.8.2 Infectious agent

There are two known dementias that have been shown to be spread by unconventional slow viruses, indicating that SDAT could also be of viral origin.

Kuru (Gajdusek, 1977) is a progressive extrapyrimidal and cerebellar degeneration with dementia which is endemic in New Guinea. It is thought that cannabalism of kuru victims was responsible for its spread: since cannabalism has been suppressed the incidence has declined. Creutzfeldt-Jakob disease is a rare, rapidly progressive dementia in the elderly which leads to death within 6 months. Both diseases have been shown to be transmitted to chimpanzee by cerebellar innoculation with material from the brains of patients. The spread of Creutzfeldt-Jakob disease is by an unknown cause in man, although there have been reports of transmission by the use of depth electrodes and corneal transplants from infected patients.

A similar transmittable slow virus has been proposed for SDAT. The fact that the disease is usually apparent in advanced age is explained by the long incubation period of the unconventional virus. As yet there are no confirmatory animal experiments.

1.8.3.8.3 Toxic agent

The suggestion that aluminium may be the toxic agent responsible for Alzheimer's disease arose from studies in the early 70's, when Crapper et al (1973) showed that aluminium accumulated in aged brains, and specifically in SDAT brain (Crapper et al, 1976). Another study showed that aluminium intoxication could cause fatal encephalopathy in renal dialysis patients (Alfrey et al, 1976): dementia is associated with this condition which implies that aluminium may be the common factor in this condition and SDAT.

A histopathological study by Perl and Brody (1980) found that in SDAT brain, aluminium was located in the nuclei of neurones specifically affected by neurofibrillary tangles, and not adjacent normal cells.

Aluminium is abundant in the earth's crust and people may be exposed to it in several ways. The use of aluminium utensils is thought to be of minor significance. Shore and Wyatt (1983) have shown that chronic administration of antacids containing aluminium can substantially contribute to the dietary source of aluminium. However, Heyman et al (1984) showed that patients with Alzheimer's disease had not taken more antacids containing aluminium than other people of the same age.

The postulated involvement of aluminium and the development of SDAT remains controversial.

1.9 AIMS OF THE STUDY OF TETRAHYDROBIOPTERIN METABOLISM IN DEPRESSION AND SENILE DEMENTIA OF ALZHEIMER TYPE

As discussed earlier, tetrahydrobiopterin cofactor activity is required for both tyrosine and tryptophan hydroxylase. These enzymes are rate-limiting in the synthesis of dopamine and noradrenaline, and serotonin. As soon as it became apparent that there were deficits in indoleamine and catecholamine neurotransmitters in the psychiatric and neuropathological states of depressive illness and senile dementia of Alzheimer type, attention was directed towards study of BH_4 metabolism.Most studies have been made on brain tissue obtained post mortem, or on CSF and serum taken from living patients. The changes found in BH_4 metabolism in these tissues and fluids are discussed in the introductions to chapters three and four. The development of high pressure liquid chromatography has led to rapid but specific and sensitive discrimination and quantitation of different pteridine species from a variety

of sources. While urinary neopterin has received attention as an immunological marker (Volin et al, 1987; Abita et al, 1986), there have been few measurements made of urinary biopterin derivatives in relation to neurological and psychiatric disease.

1.9.1 Tetrahydrobiopterin in depressive illness

This thesis aims to explore urinary pteridine excretion in a large, well defined group of male and female, unipolar and bipolar, depressed patients. Different aspects of BH_4 metabolism were examined in the rat in the presence of antidepressive drugs (lithium and the tricyclic imipramine); an anticonvulsant (sodium valproate); a neurotransmitter synthesis inhibitor (∝methylparatyrosine); a vitamin related to BH_4 (folic acid); a synthetic steroid (dexamethasone), and physiological stress (by restraint); these studies were performed to complement the results from the clinical findings.

1.9.2 Tetrahydrobiopterin in senile dementia of Alzheimer type

This thesis aims to study urinary pteridine excretion in patients with SDAT: there has been very little research in this field. A related study in the rat was to administer the anticholinergic drug scopolamine as a model for dementia, and to examine possible changes in BH_4 metabolism in rat tissue.

CHAPTER TWO:

MATERIALS AND METHODS

2.1 CHEMICALS

Pterin, biopterin and neopterin were obtained from Dr. B. Shircks, Wettswill, Switzerland. 5-methyltetrahydrofolate was obtained from Eprova Research Laboratories, Switzerland. 5-formyltetrahydrofolate was obtained from Lederle Laboratories Division, Cyanamid of Great Britain Ltd., London U.K. HPLC grade methanol was obtained from Fisons U.K. PLC, Loughborough, England. Iodine was from May and Baker Chemicals; Dagenham, England.
The following compounds were obtained from the Sigma Chemical Company, Poole, England:
Ascorbic acid, lithium carbonate, dexamethasone, imipramine, bovine serum albumin (BSA), guanosine triphosphate (GTP), potassium iodide, reduced nicotinamide dinucleotide (NADH), reduced nicotinamide dinucleotide phosphate (NADPH), 6,7-dimethyl-5,6,7,8-tetrahydropterin ($DMPH_4$), Trizma base (tris), sodium heparin, trichloroacetic acid and scopolamine hydrobromide.
Sodium valproate was a gift from Dr. D. Easter at Sandoz-Labaz Ltd.

2.2 TECHNIQUES

2.2.1 Thin-layer chromatography

Thin-layer chromatography was performed using 0.10mm cellulose MN 300 UV_{254} precoated plastic sheets (Polygram). All solvents were of general purpose grade. Solvent systems were either 5% acetic acid in water or 4:1:5 n-butanol:acetic acid:water.

2.2.2 High pressure liquid chromatography (HPLC)

Separation of pterins was achieved by a Spherisorb octadecylsulphate (ODS) reverse phase column with a particle size of 5 μm and a column size of 25cm x 4.6mm in conjunction with a pre-column packed with CO: Pell

ODS particles (HPLC Technology casing and Whatman packing). Injection onto the column was by a Waters Intelligent Sample Processor (WISP) 710B (Waters Associates). Detection was by a Laboratory Data Control (LDC) Fluorometer at a sensitivity setting of 2. Excitation was at 360nm and emission at 450nm. Solvent used was 5% methanol:95% glass-distilled water and was made up volumetrically and degassed under reduced pressure. It was pumped through the system at a flow rate of 1.0cm^3/minute by an LDC Constametric Model III metering pump. Results were recorded on a JJ dual pen CR652 chart recorder operated at 0.4cm/minute. Figure 2.1 represents typical separation of standards obtained by HPLC, and figure 2.2 represents typical standard curves for biopterin, neopterin and pterin.

2.2.3 Measurement of concentration

The concentrations of all pteridine compounds were determined spectrophotometrically using a Shimadzu Graphicord UV-visible recording spectrophotometer and Shimadzu Graphic Printer (PR-1) according to the following extinction coefficients:

Table 2.1 Extinction coefficients of pteridines

Pteridine	pH	ϵmax x 10^3	λmax (nm)	Source
Biopterin	13	8.3	363	Fukushima & Nixon, 1980
Neopterin	13	8.3	362	Fukushima & Nixon, 1980
Pterin	13	6.6	358	Blakley, 1969
Pterin-6-COOH	13	9.2	365	Blakley, 1969
Pterin-6-CHO	13	24.6	255	Blakley, 1969
5-CHOTHF	13	28.2	282	Blakley, 1969
5-CH_3THF	7	31.7	290	Blakley, 1969
THF	1	20.6	292	Blakley, 1969

Figure 2.1 Typical separation of neopterin, biopterin and pterin by HPLC

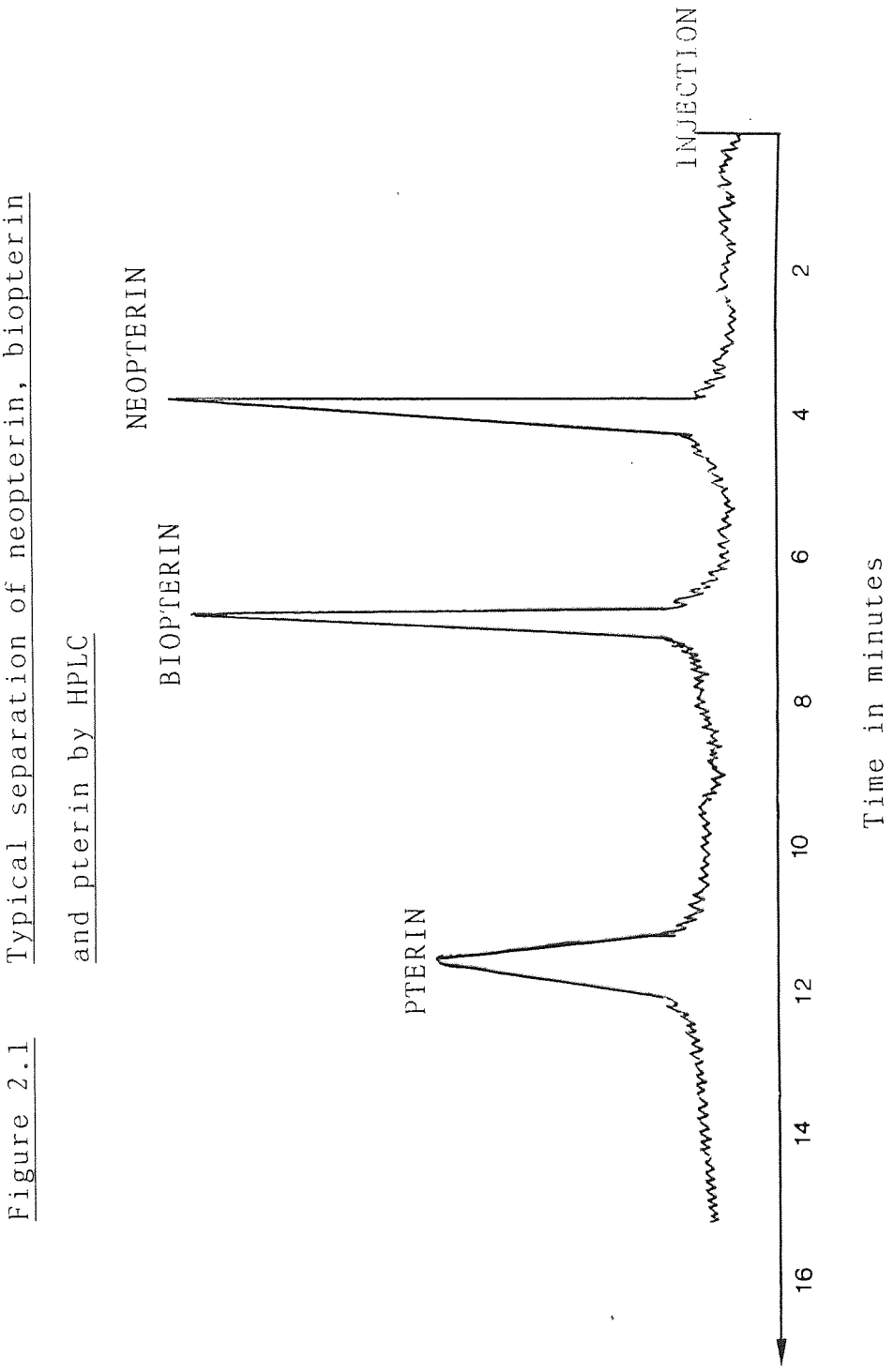
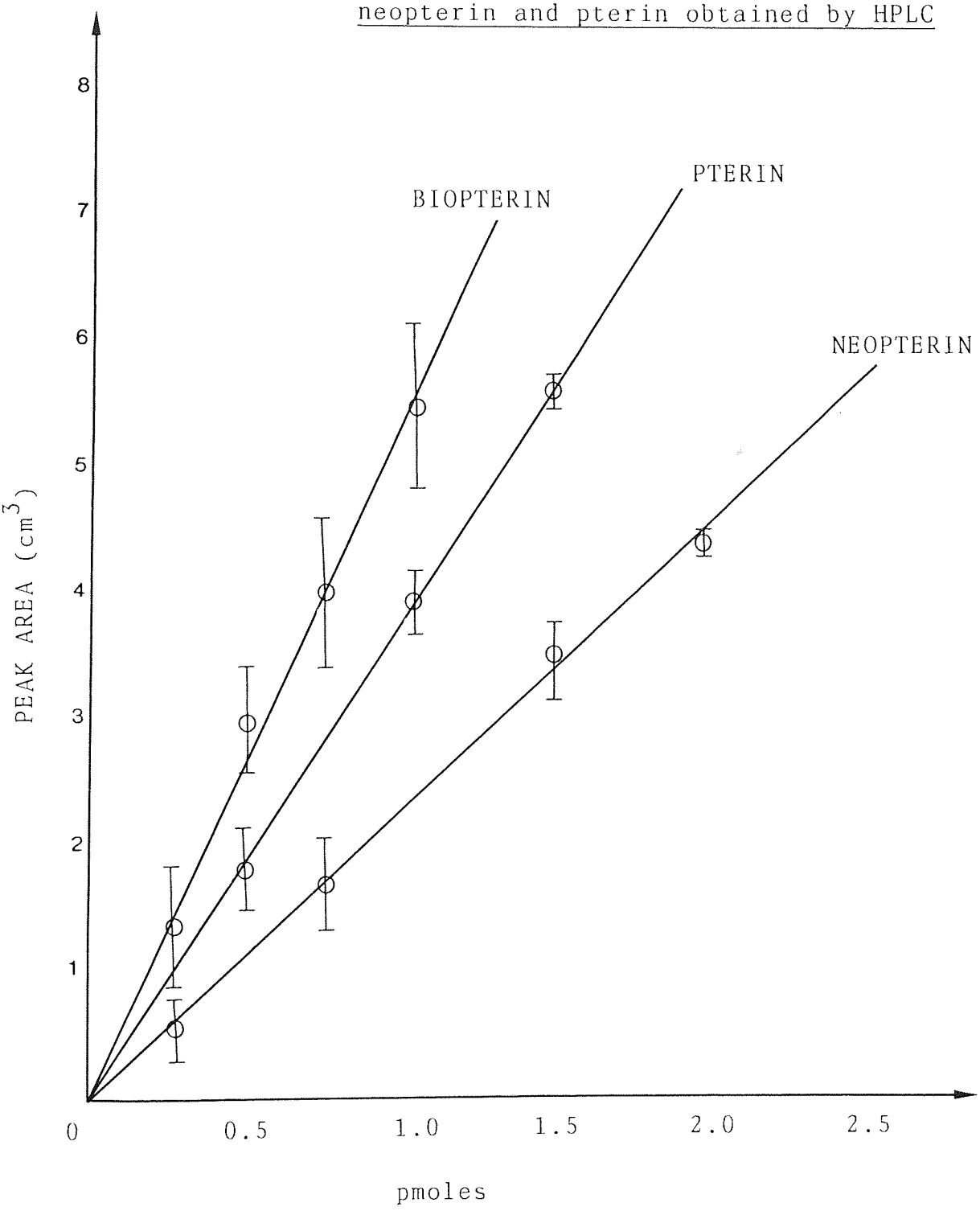


Figure 2.2 Typical standard curves for biopterin, neopterin and pterin obtained by HPLC



Each point represents the mean of 3 determinations ± standard error of the mean

2.2.4 Atomic absorption measurements

Plasma samples

Whole blood for lithium assay by atomic absorption was collected by cardiac puncture and immediately placed in vials treated with sodium heparin (500 units/ml). The plasma was separated after one minutes centrifugation and placed in tubes containing 500 μl of the heparin solution. Before assay, the plasma samples were diluted 1:1 with a caesium/lanthanide diluent.

Tissue samples

Whole brains were removed from experimental animals and lightly macerated. Approximately 1g of brain and liver were used in the acid digest stage.

Acid digest

All acid digests were performed by Mr. R. Davie of the Division of Biomedical Sciences at Wolverhampton Polytechnic. Each sample was placed in a labelled boiling tube in a fume cupboard. 4.0cm^3 of concentrated nitric acid were added to each and the tubes were left for a minimum of 24 hours to digest. After this time, perchloric acid was added according to the method of Birch and Jenner (1973) and the tissue samples diluted to 5.0cm^3 prior to atomic absorption spectrometry.

Spectrometry

Measurements were made using an Instrumentation Laboratory aa/ae 357 Spectrometer with the following settings:

λ	670.8
I	5mA
H	530mV
Band width	0.5nm_3
Uptake rate	5.0cm^3/min

2.3 ASSAYS

2.3.1 Tissue assays

2.3.1.1 Total biopterin in tissue as measured by HPLC

Approximately 1.0g tissue (rat brain or liver) was accurately weighed and made up into a 20% $^{w}/v$ homogenate in a 20% $^{w}/v$ trichloroacetic acid solution to precipitate protein. The suspension was spun for one minute in a Measuring and Scientific Equipment (MSE) bench centrifuge and the supernatant decanted. The volume was measured and $1.0cm^3$ was processed as follows:

$1.0cm^3$ supernatant
$1.0cm^3$ 0.1M HCl
$1.0cm^3$ 6% KI:3% I_2 solution

The tube was covered, mixed and left to stand in the dark at ambient temperature for one hour. Oxidation was terminated by the addition of a few crystals of ascorbic acid until the solution became colourless. Oxidised solutions were injected into the HPLC apparatus as described in section 2.2.2, and identity determined by comparison to the retention times of known standards. Typical traces of pteridine separations from processed rat brain and liver tissue obtained by HPLC are as in figures 2.3a and 2.3b.

2.3.1.2 Dihydropteridine reductase (DHPR) assay

20% $^{w}/v$ tissue homogenates were made up in 1.0M tris/HCl buffer (pH 7.6) and centrifuged at 40,000 rpm for 45 minutes. DHPR was assayed spectrophotometrically by a modified method of Craine et al, 1972:

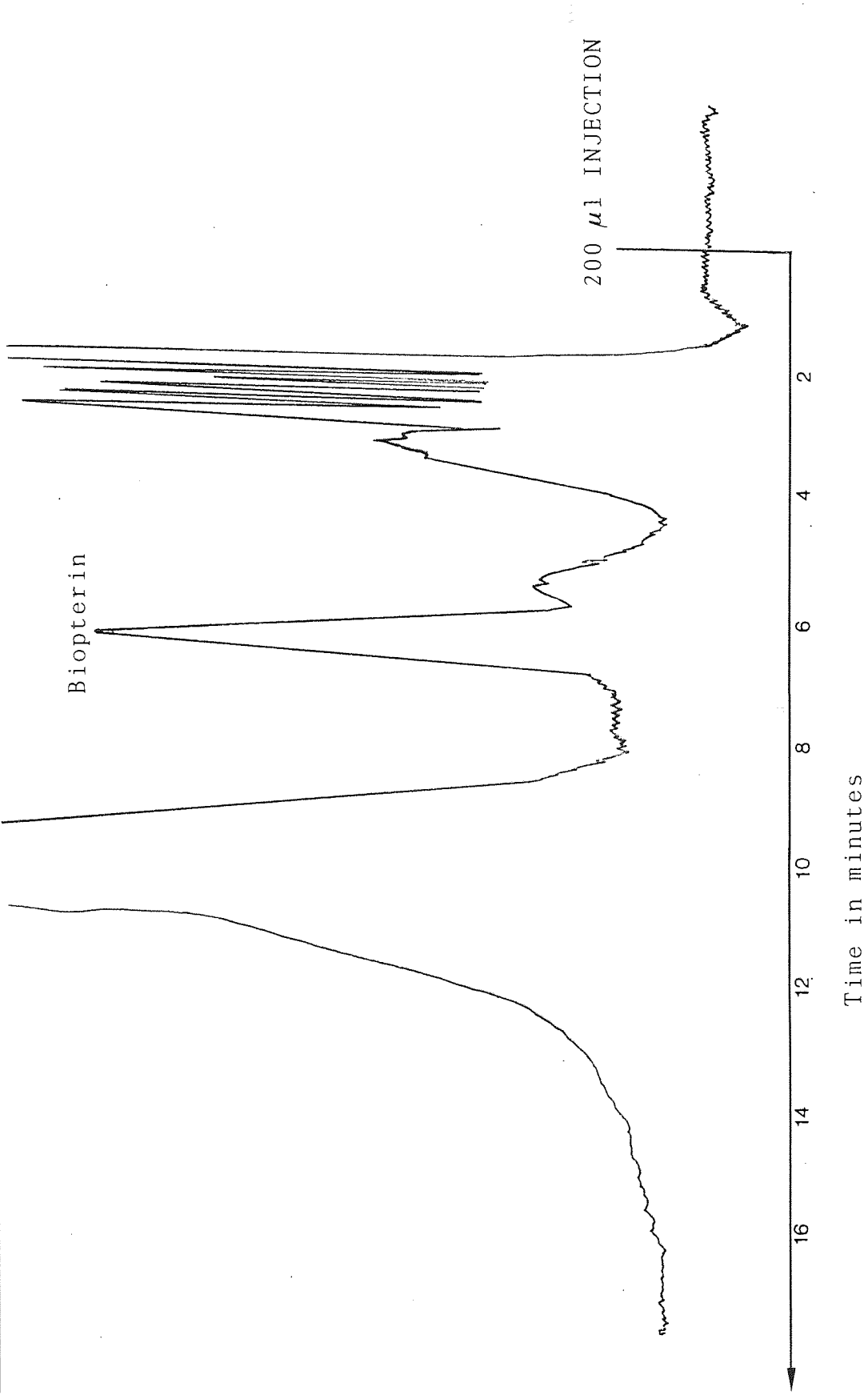


Figure 2.3a HPLC separation of rat brain tissue

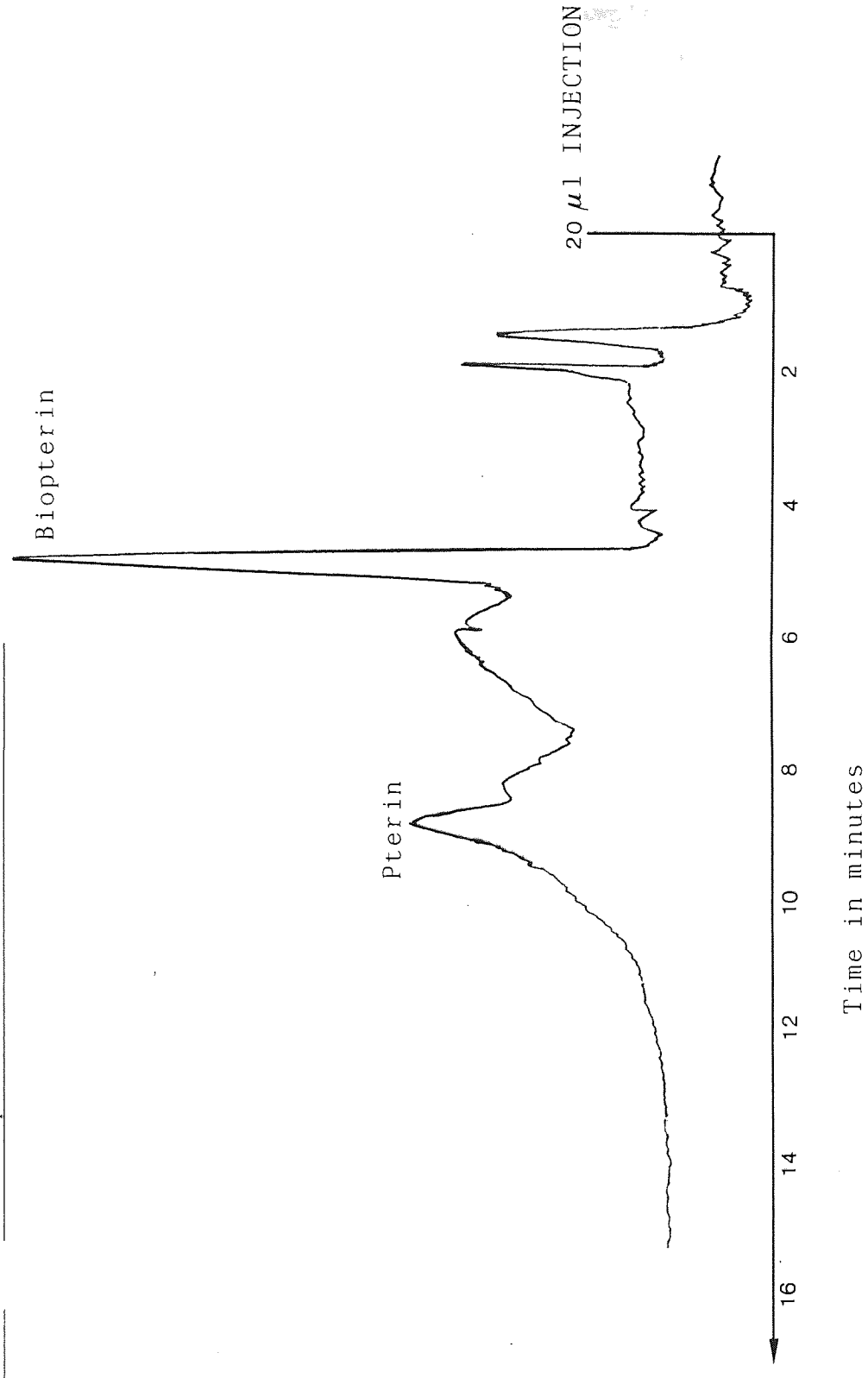


Figure 2.3b HPLC separation of rat liver tissue

Reagent	Reference cell (cm^3)	Sample cell (cm^3)
1.0M tris/HCl, pH 6.8	0.1	0.1
Sodium azide (16.8mg/100cm^3)	0.1	0.1
Horseradish peroxidase (0.8mg/10cm^3)	0.1	0.1
Hydrogen peroxide (58 μl/100cm^3)	0.1	0.1
NADH (3.7mg/10cm^3)	0.1	0.1
Distilled water	0.4	0.38
Tissue supernatant (DHPR)	-	0.02
Mix and leave at 37°C for 90 seconds		
$DMPH_4$ (2.3mg/10cm^3)	0.1	0.1
Mix and leave at 37°C for 30 seconds		

Read change in absorbance at 340nm for 30 seconds.

All reagents except the buffer were freshly prepared. The specific activity of DHPR was expressed as nmol NADH oxidised/minute/mg protein. Protein was assayed by the method of Biuret.

2.3.1.3 Biuret assay for protein estimation

50mg bovine serum albumin were dissolved in 5.0cm^3 distilled water and a standard curve set up. A typical standard curve is shown in figure 2.4. Each point is a single measurement.

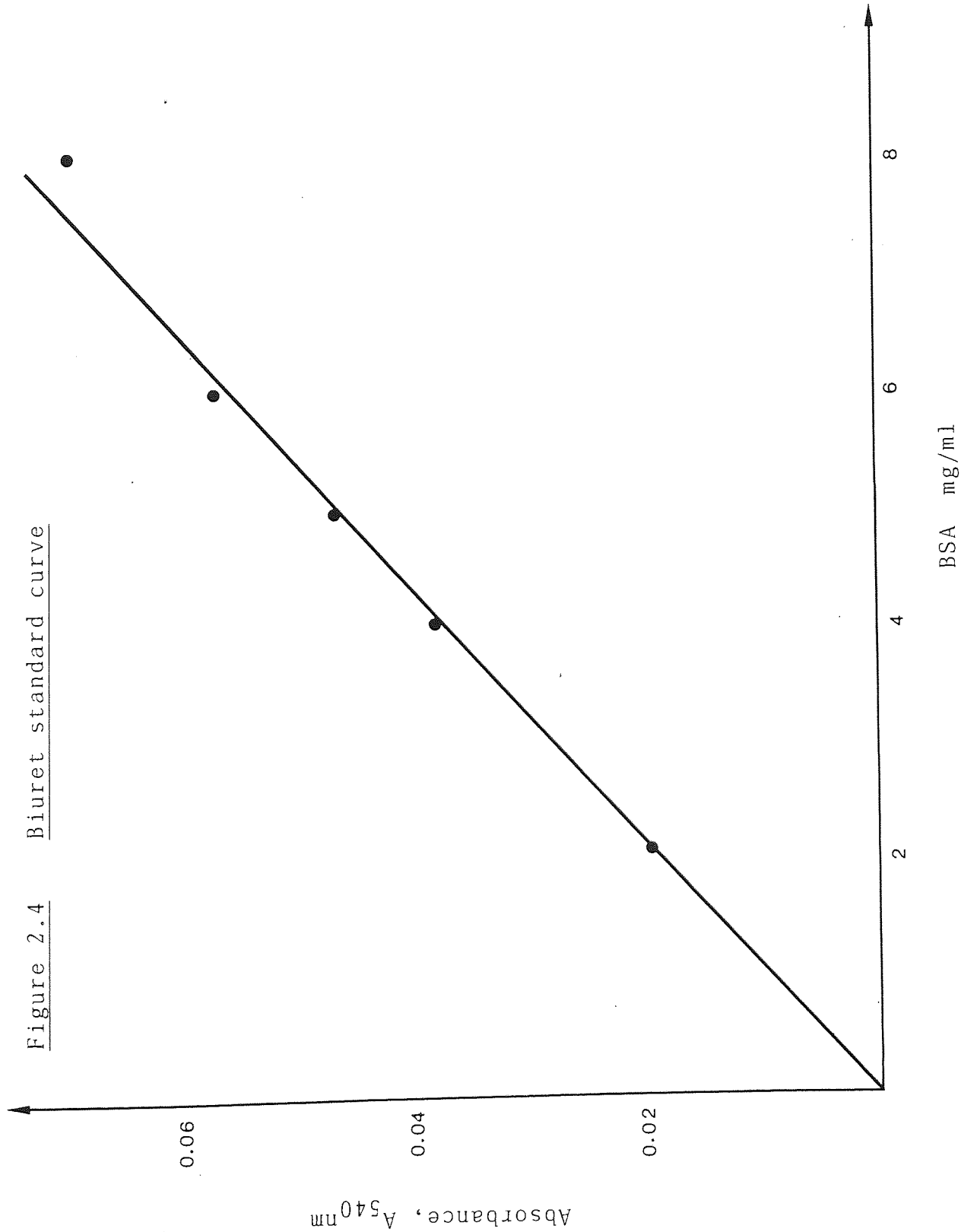


Figure 2.4 Biuret standard curve

Standard curve

Tube	BSA solution cm^3	Distilled water cm^3
1	2	8
2	4	6
3	6	4
4	8	2
5	10	0

Samples and standards were prepared for estimation by the Biuret method as follows:

$0.1cm^3$ sample/standard
$0.4cm^3$ water
$2.0cm^3$ Biuret reagent

Colour was left to develop for 30 minutes and the absorbance at 540nm measured on a Shimadzu Graphicord spectrophotometer as previously described.

2.3.1.4 Tetrahydrobiopterin biosynthesis assay

This assay was based on the method of Fukushima et al (1975). 20% $^{w}/_{v}$ tissue homogenates were made up in 1.0M tris/HCl buffer (pH 7.6) and centrifuged at 40,000 rpm for 45 minutes. The reaction was set up in 4" x $^{1}/_{2}$" Pyrex test tubes as follows:

Reagent	Volume (μl)
75mM tris/HCl pH 8.0	700
6mM GTP (31.39mg in $10cm^3$)	50
3mM $MgCl_2$ (2.03mg in $10cm^3$)	50
3mM NADPH (25.00mg in $10cm^3$)	50
Additives/water	50
Supernatant (added last to initiate reaction)	100
Total volume	1000

All reagents except the buffer were freshly prepared. Blank tubes contained no substrate GTP.

The tubes were covered and incubated in the dark at 37°C in a shaking water bath for 3 hours after which the reaction was terminated by the addition of 2.0ml 0.1M HCl. $1.0cm^3$ of the assay medium was removed into a fresh test tube. One drop of 6% KI:3% I_2 was added and the tube covered and left in the dark at room temperature for one hour. Oxidation was terminated by the addition of crystals of ascorbic acid until the solution became colourless. The solutions were dried to complete dryness for a minimum of 3 hours in a Virtis 10-030 freeze drier (usually overnight). The dried samples were reconstituted with $0.25cm^3$ water and injected into the HPLC system as previously described.

2.3.2 Urine assays

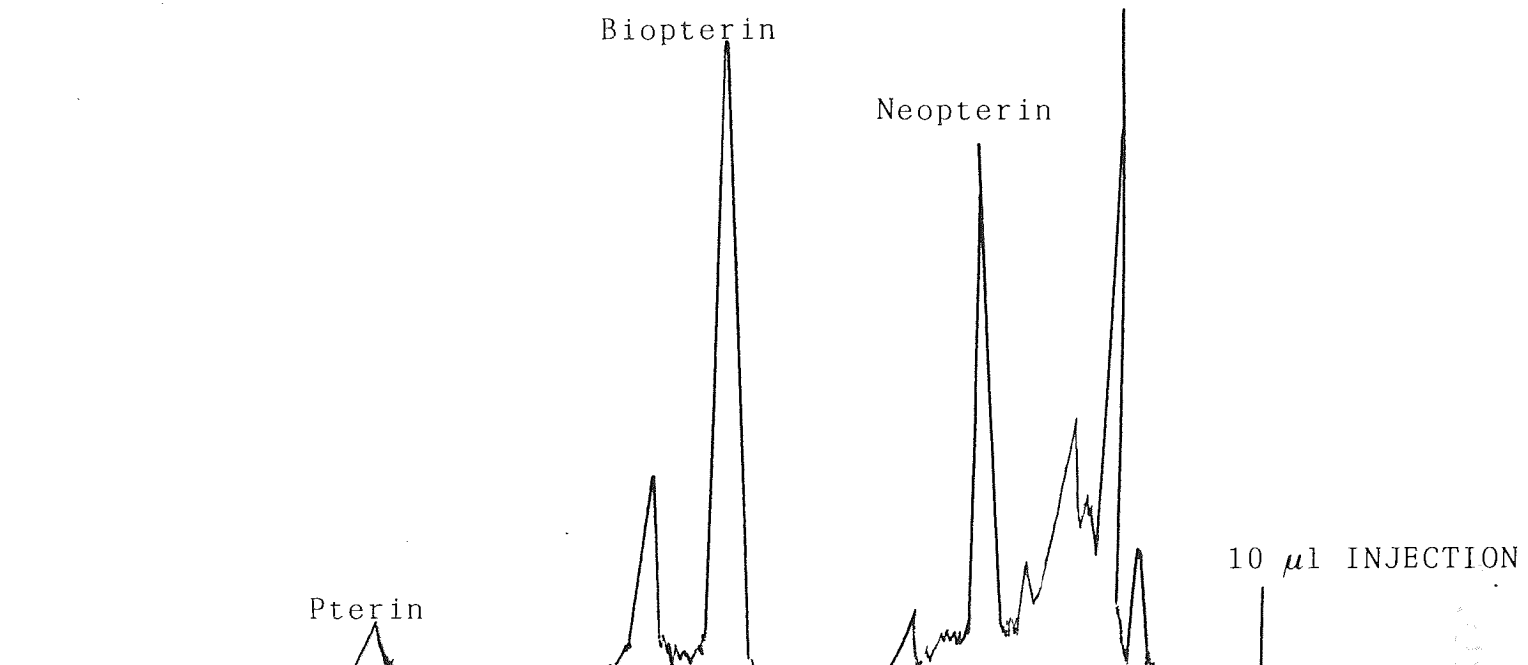
2.3.2.1 Urinary biopterin, neopterin and pterin measurements by HPLC

Neat urine samples were diluted 1 in 8 with water and oxidised with $HCl/KI/I_2$ exactly as for tissue samples. After the addition of ascorbate, the samples were filtered through Millipore "Swinnex" filters fitted with 0.22 μm Sartorius membranes and injected into the HPLC system. Figure 2.5 is an example of a typical HPLC trace from a processed urine sample.

2.3.2.2 Urinary biopterin measurements by Crithidia fasciculata bioassay

Crithidia fasciculata culture was maintained by Dr. R. Leeming of the Department of Haematology, The General Hospital, Birmingham and urine samples assayed for biopterin by him as described by Leeming et al, 1976).

Figure 2.5 HPLC separation of urine samples



2.3.2.3 Creatinine concentration of urine

Creatinine was either estimated using an automated method or a bench top diagnostic kit. The automated method used a Technicon AutoAnalyzer method number SF4-0011FFS in the Department of Clinical Chemistry at Birmingham General Hospital. The bench top method used a Sigma Diagnostic Kit number 555.

2.3.2.4 Urinary cortisol

Urinary free cortisol was measured by radioimmunoassay (CIS International) at the MRC Neuropsychiatry Research Laboratory, West Park Hospital, Epsom, Surrey.

2.3.3 Plasma assays

2.3.3.1 Biopterin and neopterin measurements by HPLC

These measurements were made by Dr. C. Hamon of Aston University as described by Hamon (1984).

2.3.3.2 Folate estimation

Plasma folate values were estimated using a Quanta Count Folate kit (Bio-Rad Laboratories, Watford, UK) at the MRC Neuropsychiatry Research Laboratory, West Park Hospital, Epsom, Surrey.

2.4 ANIMALS

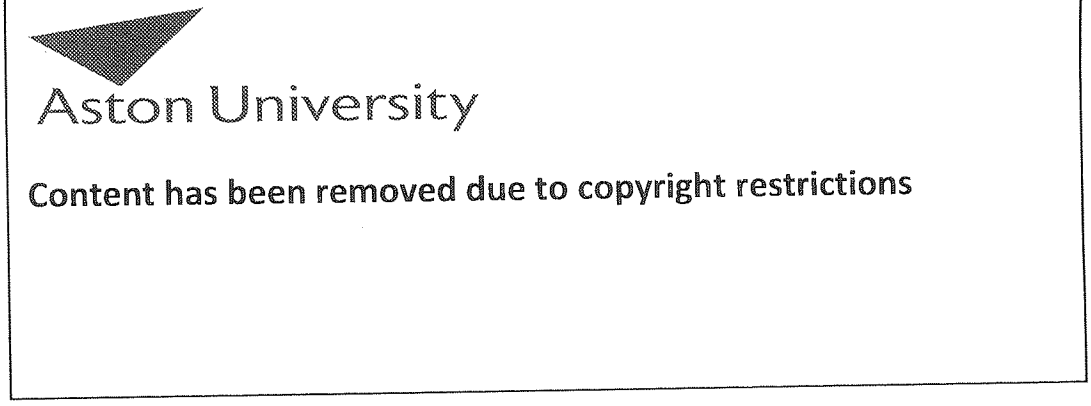
All animals used were male Wistar (outbred albino)

rats weighing between 120-180g, purchased from Bantin and Kingman Ltd., Grimston, Hull, England. Physiological data are shown in figure 2.6 and table 2.2. They were housed at 20°C in a 12-hour light/12-hour dark cycle with adequate access to food and water, except where indicated otherwise in the text. They were fed on Heygates Rat and Mouse Breeder Diet (Pilsbury Ltd.). Oral dosing was by bulbed syringe needles: solutions were delivered directly into the stomach. Where animals were starved overnight prior to dosing, they were kept on grids to prevent coprophagia. All animal experiments were performed in accordance with the guidelines laid down by the Home Office in the licence to experiment on living animals (ELA) and Certificate A.

Table 2.2 Physiological data on Wistar rats

Room temperature	21°C
Humidity	50± 5%
Diet-daily consumption	15 grammes
Water-daily consumption	35cm^3
Puberty	70-80 days
Weight at puberty	150-200 grammes
Oestrus cycle	4-5 days
Gestation period	21-22 days
Litter size	6-10 young
Weaning age	20-21 days
Weight at weaning	40-60 grammes
Rectal temperature	37°C

Figure 2.6 Growth chart for outbred albino Wistar rat



Graph supplied by Bantin and Kingman Ltd.

CHAPTER THREE:

TETRAHYDROBIOPTERIN METABOLISM IN DEPRESSION

3.1 INTRODUCTION

3.1.1 Cerebrospinal fluid studies of tetrahydrobiopterin metabolism in depression

One of the first studies connecting BH_4 cofactor activity and depressive illness was a report by Curtius et al (1983b). One female patient who had proved resistant to conventional tricyclic medication was given 1g of tetrahydrobiopterin orally for several days. She exhibited marked improvement of mood, and samples of CSF taken showed increasing concentrations of BH_4, 5HIAA and HVA. When the dose was lowered to 100mg per day (without the patient being told), her depressive symptoms returned, although the CSF values declined more slowly. In a later study, Kellner et al (1983) measured tetrahydrobiopterin in the CSF of 13 uni- and bipolar patients compared to 24 controls. They found that BH_4 was slightly, but not significantly, decreased in the depressed group. However, they noted that there was a significant negative correlation between CSF BH_4 and age in both groups; when the authors performed an age correction the CSF pteridine levels in patients and controls were shown to be very similar. They concluded that central tetrahydrobiopterin metabolism was not altered in depressive illness.

Levine and Lovenberg (1984) have criticised the study by Kellner et al (1983) for several reasons: information on patients was limited and there was some confusion as to the method used to determine cerebrospinal BH_4. They emphasised the need for a large, carefully conducted survey to establish the role of tetrahydrobiopterin in the neuropathology of depression.

3.1.2 Brain studies of tetrahydrobiopterin metabolism in depression

Blair et al (1984b) conducted the first study to measure

the capacity for tetrahydrobiopterin biosynthesis in post mortem brain samples from depressed patients. In an *in vitro* assay (Barford *et al*, 1984) temporal cortex samples from four depressed patients were found to have significantly lower BH_4 biosynthesis ($p < 0.01$, Wilcoxon's rank sum test) than three samples of control tissue: all samples were from the same Brodmann area (area 21) and were matched for age and post mortem delay. Further studies are awaited in this area after this interesting observation.

3.1.3 Urine studies of tetrahydrobiopterin metabolism in depression

Biopterin was first isolated from urine over thirty years ago (Patterson *et al*, 1955) but little clinical importance has been attached to this until recently (Al-Beir, 1982; Blair *et al*, 1984b). Biopterin is known to be actively synthesised by the kidney (Haberle *et al*, 1978) but its widespread occurrence in many tissues, especially in neuronal matter, implies that kidney biopterin is not the sole source of urinary biopterin. While acknowledging that CSF neopterin and biopterin are excellent markers for brain levels of these two pteridines (Kay *et al*, 1986), many authors believe that urinary pteridine levels are representative of central nervous system activity (Garbutt *et al*, 1985; Duch *et al*, 1984b; Blair *et al*, 1984b).

The results of three previous studies on urinary excretion of biopterin derivatives are discussed later in relation to the results of the survey performed here.

The present study on pteridine excretion in depressive illness is to investigate the pterins involved in tetrahydrobiopterin metabolism by making urinary measurements from a well-defined group of patients in order to elucidate the role of BH_4 in the pathogenesis of affective disorders.

3.2 EXPERIMENTAL SECTION

3.2.1 Patients and controls

76 outpatients (51 female, 25 male) attending a lithium clinic at West Park Hospital, Epsom, Surrey took part in this survey. They had been diagnosed as bipolar (n = 28) or unipolar (n = 48) affective disorder patients by two clinicians (working independently) according to the ICD Ninth Revision (WHO, 1978) and the Newcastle Diagnostic Scale (Gurney et al, 1972).

At the time of the study all patients were receiving lithium in the form of sustained-release tablets (Priadel, Delandale Laboratories, Canterbury) in the evening. Serum levels 12 hours later were within the range 0.32 to 0.87 mmol/l for female and 0.24 to 0.88 mmol/l for male patients. Patients had been receiving lithium for between one to 17 years. Some patients were receiving 200 μg/day folate supplementation, and some were receiving medication for somatic complaints; these are detailed in Appendix 1.

The patients were generally affectively well. Affective morbidity was measured objectively by a 'Doctors' rating' (DR) devised by Coppen et al (1973b) This four point scale ranged as follows:

0 = No conspicuous affective disturbance
1 = Mild depression or mania
2 = Moderate depression or mania
3 = Severe depression or mania

The maximum score per patient would therefore be 3.

The patients were also assessed subjectively with a self-rating scale, the Beck Depression Inventory, BDI (Beck et al, 1961; Appendix 2). This scale has 21 items with a score of 0 to 3 for each item, so that the maximum score per patient would therefore be 63.

Table 3.1 Distribution of affective disorder patients and controls

Diagnosis	Sex	n	Age (years) Mean (SD)	Range	BDI Mean (SD)	DR Mean (SD)
Unipolar	F	33	63.8 (9.0)*	42 - 83	5.6 (6.2)	0.08 (0.21)
	M	15	57.5 (9.1)	40 - 71	5.5 (6.4)	0.03 (0.07)
Bipolar	F	18	53.8 (13.7)	26 - 75	1.8 (2.4)	0.03 (0.12)
	M	10	50.8 (12.4)	32 - 64	2.0 (1.8)	0
Control	F	32	50.8 (14.7)	26 - 80	-	-
	M	28	53.5 (14.0)	23 - 75	-	-

Statistics: * Higher than male unipolars $p < 0.02$, female bipolars $p < 0.01$ and female controls $p < 0.001$

Spot ratings using the DR and BDI were obtained at the same time as the clinical samples (blood and urine) were taken.

Control subjects were 32 females and 28 males selected from healthy hospital staff and students. None showed any evidence of psychiatric illness. Any medication taken for somatic complaints is shown in Appendix 1.

The distribution of patients and controls, showing sex, age ranges and depression ratings is shown in table 3.1.

3.2.2 Collection and analysis of samples

Blood and urine samples were collected between 0900 and 1000 when the patients attended the clinic. Samples were taken from controls at the same time of day. Approximately 10mls of urine were collected. An aliquot was removed for cortisol estimation by radioimmunoassay at the MRC Neuropsychiatric laboratories, and the remainder kept frozen in plain plastic tubes in the dark at -20°C until assayed for pteridines and creatinine.

Blood was obtained by venepuncture and collected into calcium heparin tubes. Plasma folate estimations were obtained for 21 unipolar and 11 bipolar patients (10 male and 22 female). Plasma lithium was measured in all patients by atomic absorption spectrometry at the MRC Neuropsychiatric laboratories.

The urine samples for pteridine analysis were thawed in the dark and thoroughly mixed. An aliquot from each sample was removed for biopterin measurement by Dr. R. J. Leeming of the department of Haematology, the General Hospital, Birmingham using Crithidia fasciculata bioassay.

The samples for HPLC assay were each divided into two aliquots and two complete oxidation, filtration and column separations performed on each, as described

in chapter 2 (Materials and Methods), section 2.3.2.1. All samples were identifiable by initials on the tube only. Whether the initials belonged to patients or controls, and other information such as age, sex and diagnosis was not known until all duplicate analyses had been completed.

3.3 RESULTS

The results of the urine analyses are shown in tables 3.2 to 3.13. Summary tables only are shown. Raw data is contained in appendices 3 to 7. Statistical comparisons are by the Student's t test except where indicated.

3.3.1 Creatinine

Male bipolars excreted significantly less creatinine than male controls ($p < 0.01$, table 3.2). Male unipolars also excreted less but this difference did not reach significance (table 3.2). Both unipolar and bipolar female patients excreted less creatinine than female controls ($p < 0.025$, table 3.3). All patients ($n = 76$) excreted significantly less creatinine than the control ($n = 61$) group ($p < 0.005$, table 3.4). Table 3.4 shows that unipolars ($n = 48$) excreted less creatinine than controls ($p < 0.005$), as did all bipolars ($n = 28$) ($p < 0.001$).

Female controls excreted less creatinine than male controls but this difference did not reach significance (table 3.7). Female patients excreted less creatinine ($p < 0.05$) than male patients (table 3.8).

3.3.2 Cortisol

Male unipolars excreted less cortisol than male controls ($p < 0.025$, table 3.2). No significant differences

Table 3.2 Urinary creatinine and cortisol excretion in male subjects

Group	n	Creatinine mmol/l Mean (SD)	Cortisol μmol/mol creatinine Mean (SD)
Male controls	28	10.01 (4.20)	3.57 (1.85)
Male unipolars	15	8.09 (4.23)	** 2.18 (1.13)
Male bipolars	10	*** 6.69 (2.69)	2.60 (1.60)
All male patients	25	7.53 (3.83)	2.66 (1.15)

Table 3.3 Urinary creatinine and cortisol excretion in female subjects

Group	n	Creatinine mmol/l Mean (SD)	Cortisol μmol/mol creatinine Mean (SD)
Female controls	33	8.33 (6.52)	3.45 (1.32)
Female unipolars	33	5.77^{*} (3.88)	3.49 (1.46)
Female bipolars	18	5.09^{*} (4.15)	3.09 (1.38)
All female patients	51	5.40^{*} (4.03)	3.35 (1.46) (n = 50)

Statistics:

* Lower than female controls $p < 0.05$

** Lower than male controls $p < 0.025$

*** Lower than male controls $p < 0.01$

Table 3.4 Comparison between urinary creatinine and cortisol excretion in patients and controls

Group	n	Creatinine mmol/l Mean (SD)	Cortisol μmol/mol creatinine Mean (SD)
All controls	61	9.10 (5.64)	3.50 (1.59)
All patients	76	6.10* (4.04)	3.04 (1.46)
All unipolars	48	6.21* (4.26)	3.12 (1.46)
All bipolars	28	5.66** (3.77)	2.98 (1.39)

Statistics: * Lower than controls $p < 0.005$

** Lower than controls $p < 0.001$

Table 3.5 Biopterin, neopterin and pterin excretion in male subjects

Group	n	Biopterin μmol/mol creatinine Mean (SD)	Neopterin μmol/mol creatinine Mean (SD)	Pterin μmol/mol creatinine Mean (SD)	$N/_B$ Mean (SD)
Controls	28	360 (152)	600 (339)	46 (23)	1.69 (0.65)
Unipolars	15	324 (149)	740 (614)	82 (76)	3.49 (4.42)
Bipolars	10	325 (145)	668 (347)	72 (46)	2.24 (1.21)
All male patients	25	324 (144)	711 (516)	79 (64)	2.99 (3.51)

Table 3.6 Biopterin, neopterin and pterin excretion in female subjects

Group	n	Biopterin μmol/mol creatinine Mean (SD)	Neopterin μmol/mol creatinine Mean (SD)	Pterin μmol/mol creatinine Mean (SD)	$N/_B$ Mean (SD)
Controls	33	524 (240)	939 (545)	106 (55)	1.85 (0.86)
Unipolars	33	371* (155)	1079 (1207)	90 (106)	3.60 (5.37)
Bipolars	18	395* (184)	1039 (711)	96 (47)	2.97 (2.65)
All female patients	51	380* (163)	1064 (1051)	94 (88)	3.37** (4.58)

Statistics: * Lower than female controls, $p < 0.05$

** Higher than female controls, p 0.025

Table 3.7 Comparison between urinary excretion in male and female controls

Group	n	Biopterin μmol/mol creatinine Mean (SD)	Neopterin μmol/mol creatinine Mean (SD)	Pterin μmol/mol creatinine Mean (SD)	N/B Mean (SD)	Creatinine mmol/l Mean (SD)	Cortisol μmol/mol creatinine Mean (SD)
Male controls	28	360 (152)	600 (339)	46 (23)	1.69 (0.65)	10.01 (4.20)	3.57 (1.85)
Female controls	33	524* (240)	939* (545)	106** (55)	1.85 (0.86)	8.33 (6.52)	3.45 (1.32)

Statistics: * Higher than male controls $p < 0.005$

** Higher than male controls $p < 0.001$

Table 3.8 Comparison between urinary excretion in male and female patients

Group	n	Biopterin μmol/mol creatinine Mean (SD)	Neopterin μmol/mol creatinine Mean (SD)	Pterin μmol/mol creatinine Mean (SD)	$N/_B$ Mean (SD)	Creatinine mmol/l Mean (SD)	Cortisol μmol/mol creatinine Mean (SD)
Male patients	25	324 (144)	615 (494)	79 (64)	2.99 (3.51)	7.53 (3.83)	2.66 (1.15)
Female patients	51	380 (163)	1064†† (1051)	94 (88)	3.37 (4.58)	5.40 (4.03)*	3.35† (1.46)

Statistics:

* Lower than male patients $p < 0.05$

† Higher than male patients $p < 0.05$

†† Higher than male patients $p < 0.02$

Table 3.9 Corrected urinary biopterin excretion in patients and controls

Group	n	Corrected biopterin values (B + P) μmol/mol creatinine Mean (SD)
Female patients	51	470 (195) **
Female controls	33	630 (274)
Male patients	25	403 (154)
Male controls	28	406 (170)
All patients	76	448 (184) *
All controls	61	527 (256)

Statistics:

** Lower than female controls, $p < 0.01$

* Lower than all controls, $p < 0.05$

Table 3.10 Comparison between Crithidia and HPLC in measuring urinary biopterin

Group	n	Urinary Biopterin Crithidia μmol/mol creatinine Mean (SD)	Urinary Biopterin HPLC μmol/mol creatinine Mean (SD)	p t test	p paired t test	Correlation r
Patients	76	411 (239)	361† (157)	NS	0.001	+0.198 (NS)
Controls	61	475 (145)	449 (217)	NS	0.001	+0.223 (NS)
All determinations	137	440 (205)	400 (191)	NS	0.001	+0.220*

Statistics: * Significant correlation $p < 0.05$

† Lower than controls $p < 0.01$

Table 3.11 Plasma lithium in patient groups

Group	Sex	n	Lithium mmol/l Mean (SD)	Correlation of plasma lithium with urine biopterin
Bipolar	F	18	0.61* (0.13)	NS
	M	10	0.58 (0.14)	NS
Unipolar	F	32	0.53 (0.14)	NS
	M	15	0.54 (0.12)	NS

Statistics: * Female bipolar lithium higher than female unipolar lithium $p < 0.05$

Table 3.12 Plasma folate levels in patients in folate supplementation study

Patient group	Folate supplement (n)	Placebo (n)	Plasma folate ng/ml Mean (SD) Supplemented	Placebo
Male bipolars (n = 5)	1	4	6.8	3.9 (0.9)
Male unipolars (n = 5)	2	3	11.1 (3.3)	7.4 (1.6)
Female bipolars (n =6)	4	2	10.3 (2.8)*	4.7 (1.6)
Female unipolars (n=16)	8	8	10.1 (5.1)	7.8 (3.7)

Statistics: * Supplemented group higher than placebo $p < 0.02$

Table 3.13 Plasma folate in patients

Group	Sex	n	Folate ng/ml Mean (SD)	Correlation of plasma folate with urinary biopterin Mean (SD)
Bipolar	F	6	8.43* (3.34)	NS
	M	5	4.46 (1.36)	NS
Unipolar	F	15	9.34 (4.42)	r = +0.63†
	M	5	8.86** (2.54)	NS

Statistics:

† Significant correlation $p < 0.01$

* Female bipolar folate higher than male bipolar folate $p < 0.05$

** Male unipolar folate higher than male bipolar folate $p < 0.01$

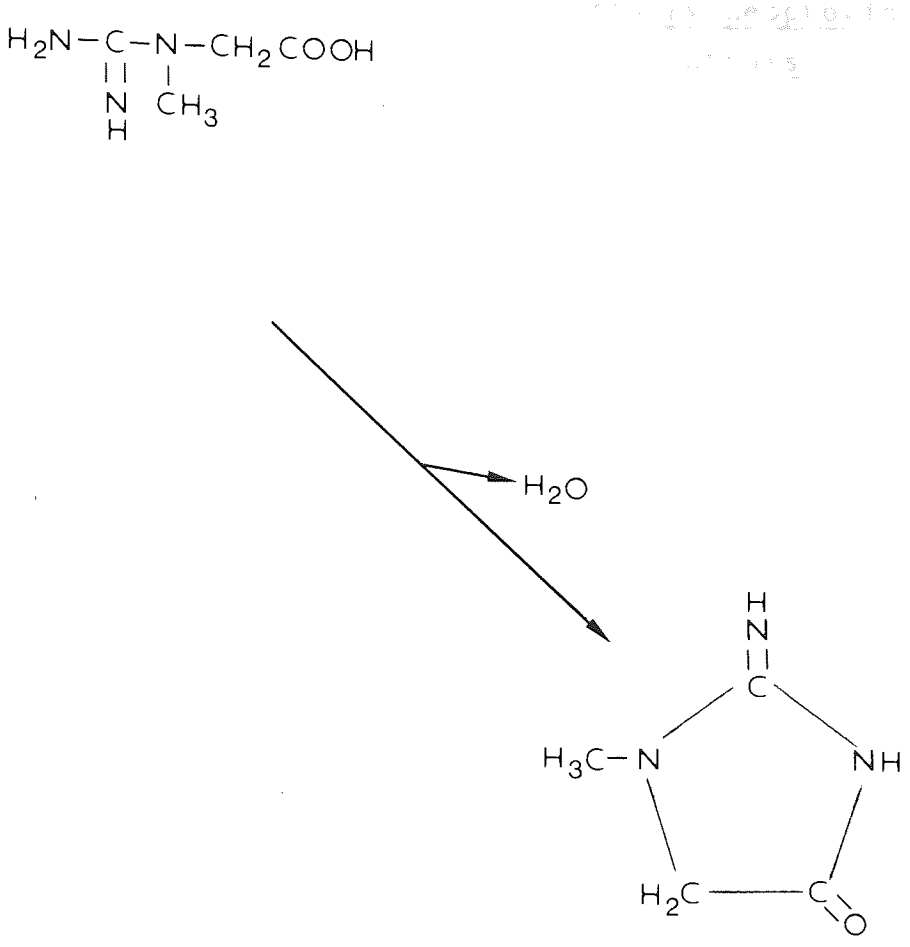


Figure 3.1 Catabolism of creatine to creatinine

Figure 3.2 Correlation between urinary neopterin and biopterin in female controls

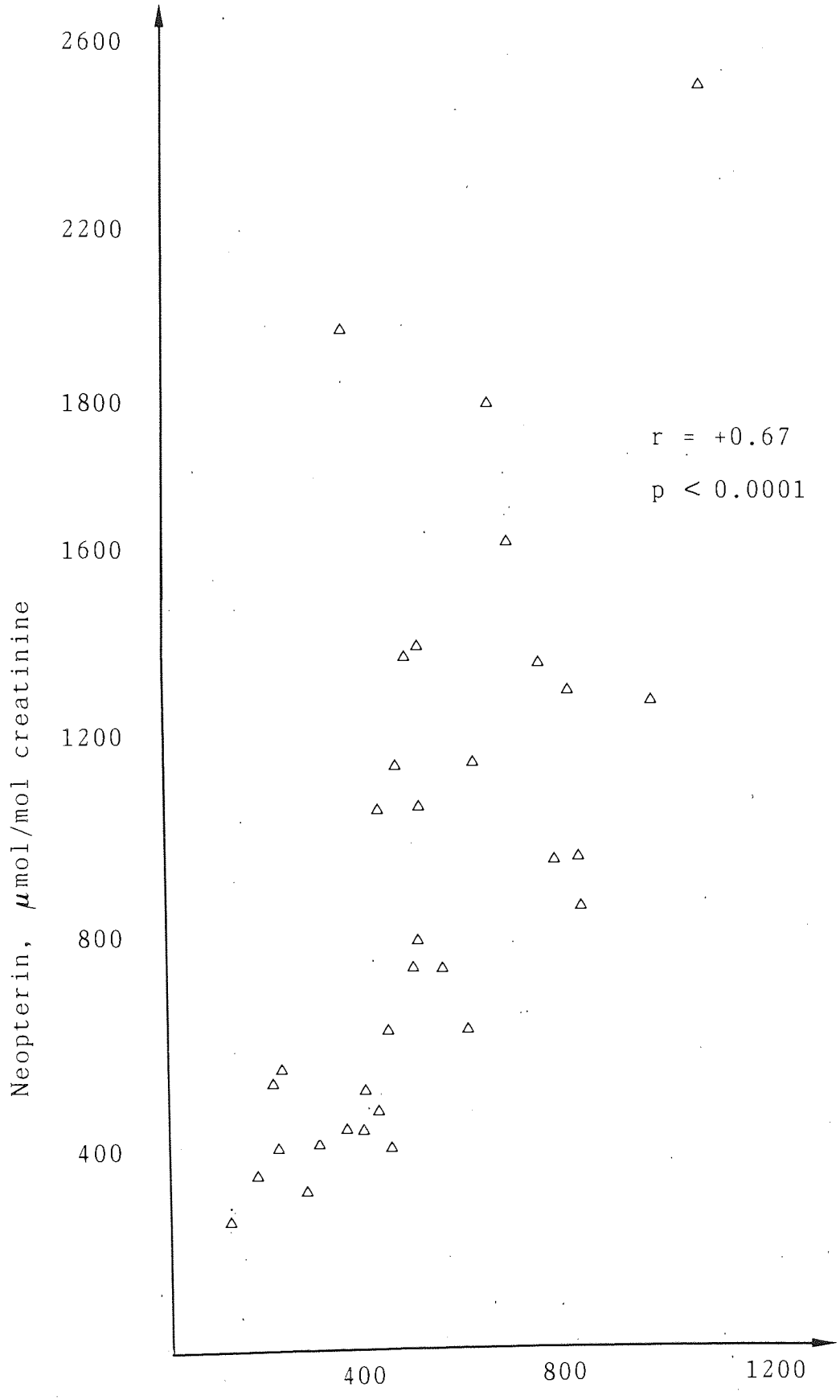


Figure 3.3 Correlation between urinary neopterin and biopterin in male controls

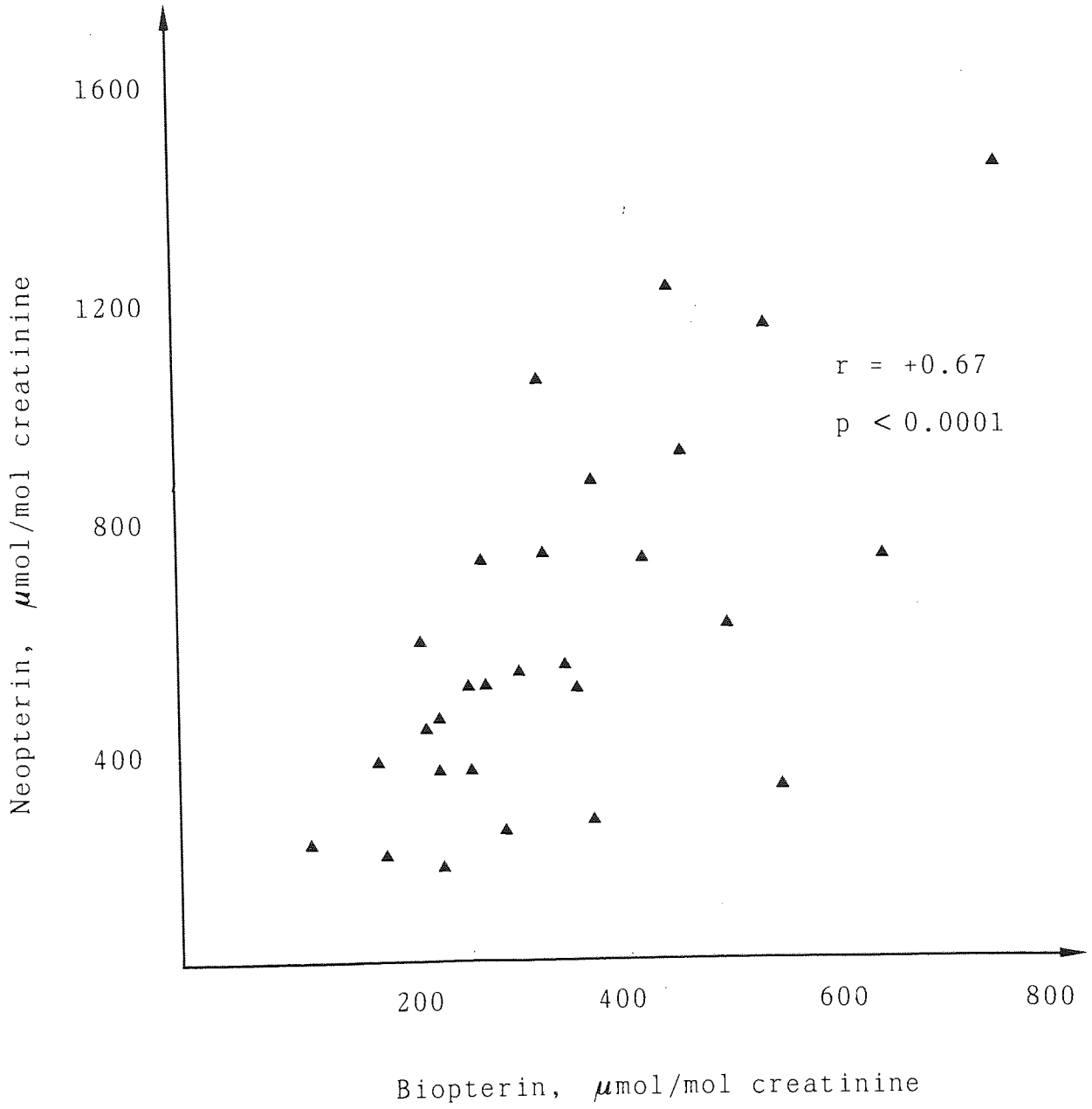


Figure 3.4 Correlation between urinary neopterin and biopterin in female patients

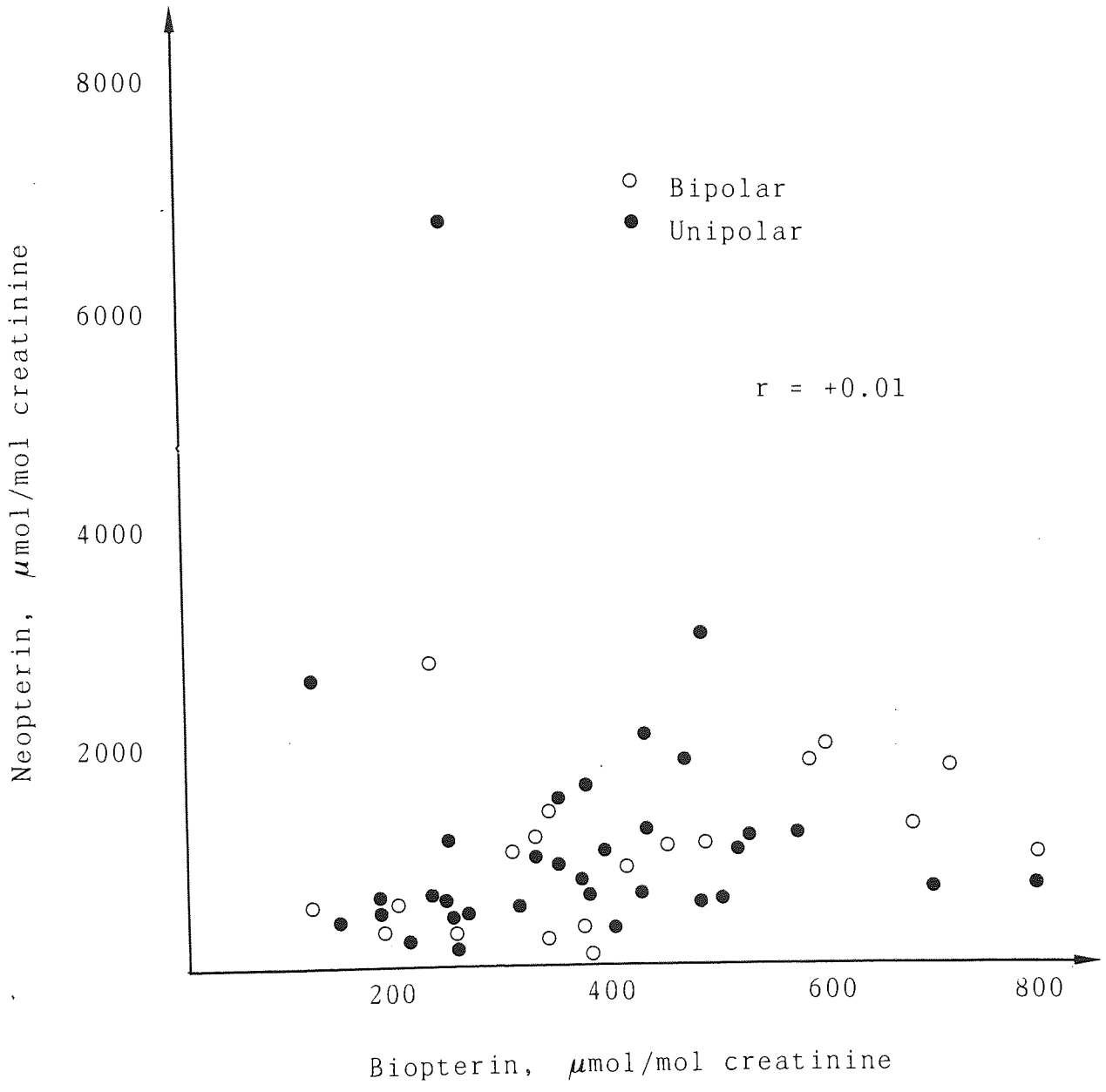


Figure 3.5 Correlation between urinary neopterin and biopterin in male patients

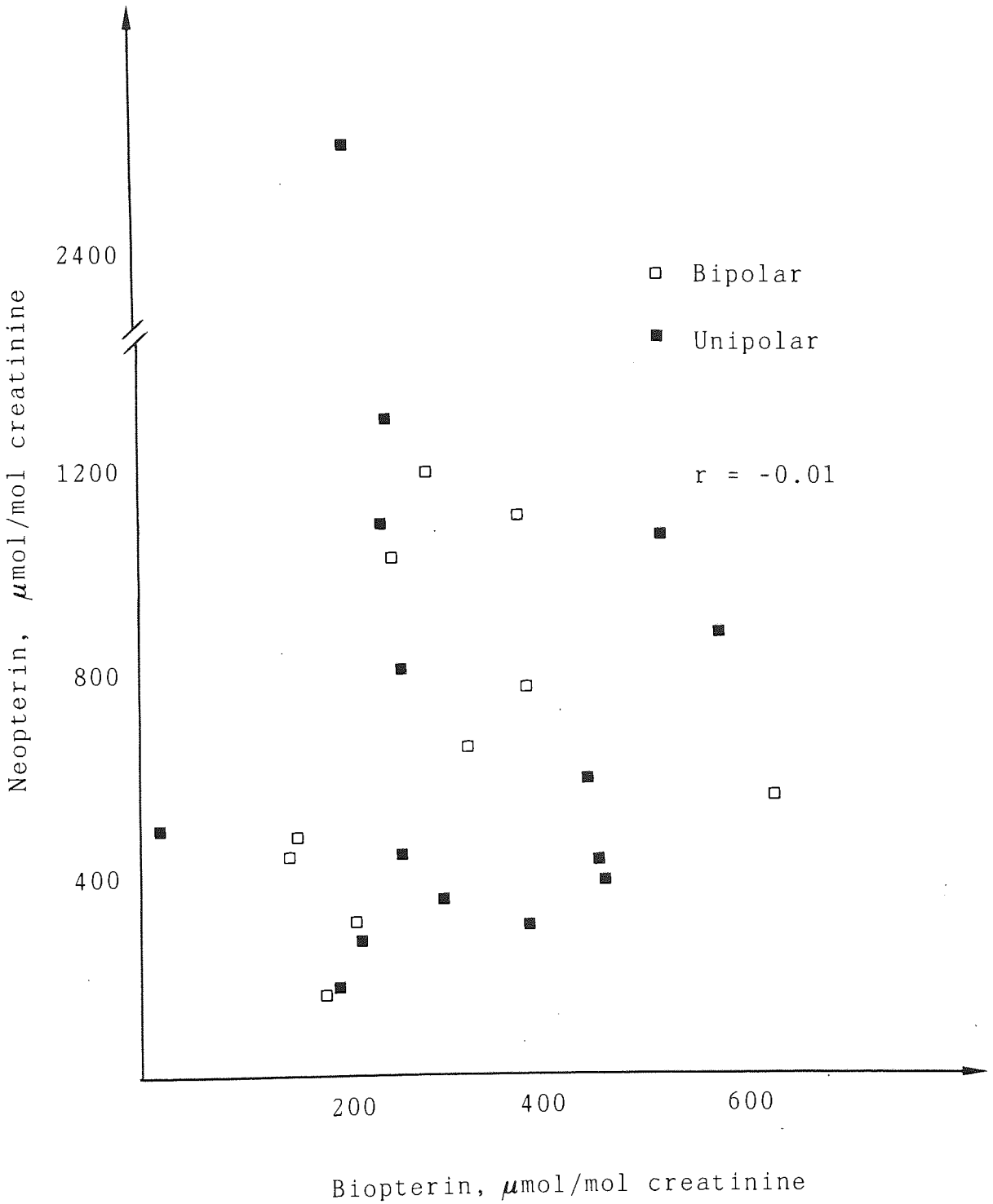
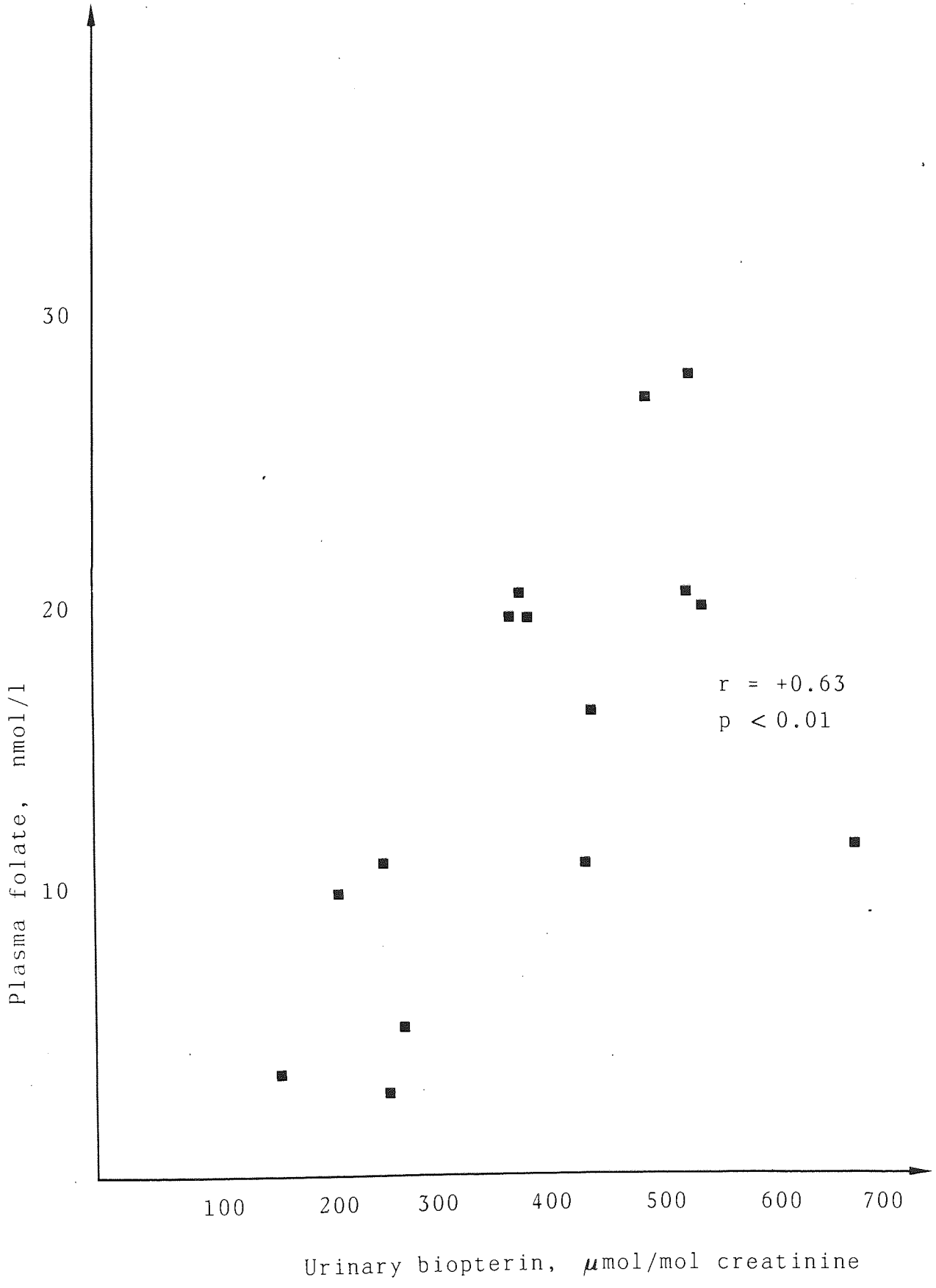


Figure 3.6 Correlation of plasma folate with urinary biopterin in female unipolars



were shown between female patients and controls (table 3.3), nor when unipolar and bipolar patients were compared to controls (table 3.4). Cortisol excretion was similar in male and female controls (table 3.7). However, female patients excreted more cortisol ($p < 0.05$) than male patients (table 3.8).

3.3.3 Biopterin, neopterin and pterin

No significant differences were shown between the excretion of biopterin, neopterin or pterin in male bipolar and unipolar patients and controls (table 3.5). In female patients, biopterin excretion was significantly reduced in unipolars ($p < 0.05$) and bipolars ($p < 0.05$) compared to controls (table 3.6).

Neopterin excretion was similar in male patients compared to controls and female patients compared to controls (tables 3.5 and 3.6).

Urinary neopterin correlated with urinary biopterin excretion in both male and female controls ($r = +0.67$, $p < 0.0001$). This correlation was absent in female patients ($r = +0.01$, NS) and male patients ($r = -0.01$, NS).

The ratio total oxidised neopterin total oxidised biopterin (N/B) was similar in male patients and controls (table 3.5), female controls and male controls (table 3.7) and female and male patients (table 3.8).

The ratio was raised in female patients compared to controls ($p < 0.025$; table 3.6). All patients (n=76) had a higher N/B ratio (3.24 (SD 4.24)) compared to all controls (n=61) (1.78 (SD 0.77)); $p < 0.005$.

Female controls excreted more biopterin and neopterin (both $p < 0.005$) and pterin ($p < 0.001$) than male controls (table 3.7). Female patients also excreted more neopterin ($p < 0.05$) than male patients (table 3.8).

A correction exercise was performed whereby 'corrected' total biopterin was considered to comprise [total biopterin + total pterin]. After correction, all patients were shown to excrete significantly less biopterin than controls ($p < 0.05$): female patients excreted less biopterin to a highly significant degree ($p < 0.01$) compared to controls (table 3.9).

3.3.4 Total biopterin estimation by HPLC compared to Crithidia fasciculata

When all determinations (n = 137) were compared by Crithidia and HPLC, the results were very similar (table 3.10). However, the results for the 76 patients were lower by HPLC ($p < 0.01$) than by Crithidia. There was a highly significant difference between the two assays by the paired t test ($p < 0.001$ in all cases). Total biopterin measurements by the two methods showed a weak positive correlation that reached significance when all 137 determinations were considered ($p < 0.05$).

3.3.5 Plasma lithium

Plasma lithium was significantly higher in female bipolars ($p < 0.05$) compared to controls (table 3.11). No differences were shown in male patients. Plasma lithium did not correlate with urinary biopterin in any group.

3.3.6 Plasma folate

Plasma folate was higher in a female bipolar group that was receiving folate supplementation compared to a placebo group ($p < 0.02$, table 3.12). Female bipolars had higher plasma folate than male bipolars ($p < 0.01$, table 3.13). Plasma folate correlated with urinary biopterin in female unipolars ($r = +0.63$, $p < 0.01$).

3.4 DISCUSSION

3.4.1 Creatinine

Creatinine is the anhydride of creatine (figure 3.1). Plasma creatine is not affected by diet, and circulating levels derive from metabolism of tissue creatine. In man it is excreted by the kidney tubule and not reabsorbed. The normal excretion of creatinine is 9 - 18 mmoles/24 hours in adults (Clinical Chemistry Department handbook, The General Hospital, Birmingham) and is estimated clinically as a measure of glomerular integrity. However, it can also be used as a reference standard for measuring parameters in urine when it is impractical to obtain 24-hour collections (Chalmers and Lawson, 1982). Table 3.2 shows that in this survey male bipolars excreted much less creatinine than male controls ($p < 0.01$), although the lowered excretion by unipolar patients did not reach significance. Table 3.3 shows that both unipolar and bipolar female patients excreted significantly smaller amounts ($p < 0.025$) than their control group. The lowered creatinine excretion in patients may have been due to polyuria, as lithium administration affects the vasopressin response of the kidney (Schou, 1968; MacNeil and Jenner, 1975). However, in the absence of 24 hour collections of urine or other information, this could not be proven or discounted. When all patients are compared with all controls, (table 3.4), there is a highly significant decrease in urinary creatinine excretion ($p < 0.005$). When all female patients are compared with all male patients (table 3.8) they are again seen to excrete less creatinine than males. However, females do have a lower fat free muscle mass than males (Van Pilsum and Selijeskog, 1958) and this is reflected in the urinary creatinine values for male and female controls (table 3.7). Female controls also excreted less creatinine than males, although the difference did not reach significance.

3.4.2 Cortisol

Cortisol is a steroid hormone secreted by the adrenal cortex in response to adrenocorticotrophic hormone from the anterior pituitary. It is responsible for stimulating gluconeogenesis and helps maintain blood pressure and renal water excretion. The secretion of cortisol is episodic and follows a circadian rhythm, with plasma cortisol at a peak between 0700 and 0900, and lowest between 2300 and 0400. Cortisol is bound by a plasma protein known as cortisol binding globulin, CBG, which is normally fully saturated. An increase in cortisol secretion increases the free fraction in plasma, and it is this non protein-bound cortisol that is filtered and excreted. Measurement of cortisol in urine (urinary free cortisol, UFC) is therefore a sensitive index of secretion, with the normal range for UFC being less than 30-350 nmoles/24 hours in adults (Clinical Chemistry Department handbook, The General Hospital, Birmingham). Stress overrides the controlling mechanisms of adrenocorticotrophic hormone secretion and leads to the loss of the normal circadian variation of plasma cortisol. UFC values may be markedly raised in some stressful situations such as relatively minor physical illnesses. The measurement of UFC has particularly been applied where Cushings syndrome is suspected, and also in psychiatric disturbance. Raised UFC has been applied in the diagnosis and monitoring of endogenous depression (Carroll *et al*, 1976; Rubinow *et al*, 1984).

As UFC values are quoted per 24 hour collection, no direct comparisons can be made between the values found in the random samples and standard reference range. However, as all the samples were taken at the same time of day, an intra-assay comparison is valid. Male unipolars, but not bipolars, had significantly ($p < 0.025$) lower cortisol values than male controls, table 3.2. The UFC values for female patients, both unipolar and bipolar, were very similar

to controls, table 3.3. When male and female patients were combined into unipolar and bipolar groups, no significant differences in UFC were observed, nor when all patients were compared to all controls, table 3.4. Table 3.1 showed that the patients had low mean ratings by the BDI and DR. As high urinary cortisol excretion is associated with depression, the moderate values shown by the patients in this study imply that the patients were not in any acute phase of illness and were affectively well.

3.4.3 Biopterin, neopterin and pterin

Biopterin, neopterin and pterin peaks could be discriminated in the HPLC traces of urines from both patients and controls, and quantified according to known standards.

Table 3.5 shows the results on male subjects. Biopterin excretion is slightly reduced in both unipolars and bipolars compared to controls, although not significantly. Neopterin excretion is raised in both affective disorder types but the rise did not reach a significant level. Similarly, urinary pterin is raised in patients but without reaching significance. There is great variation in the concentrations of these urinary pteridines as shown in appendices 4 to 7, and by the large standard variations from the mean values measured.

The $^{N}/_{B}$ ratio can be used as an index of tetrahydrobiopterin biosynthesis. Under the oxidising conditions of the assay, all reduction states of biopterin (dihydro- and tetrahydrobiopterin) are oxidised to the fluorescent biopterin species. Dihydroneopterin triphosphate, $NTPH_2$, is an intermediate in the synthesis of tetrahydrobiopterin. Under oxidising conditions, it is converted to fully oxidised, fluorescent neopterin. Thus the HPLC assay measures total biopterin and neopterin species. The lower the $^{N}/_{B}$ ratio, the more efficient the system has been, as there is less intermediate ($NTPH_2$) and more product (BH_4). Conversely,

an impaired biosynthetic system is represented by a high $^{N}/_{B}$ ratio. The lowered biopterin and raised neopterin levels mean that the $^{N}/_{B}$ ratio is also raised in male patients. However, a high degree of variation in $^{N}/_{B}$ results means that there are high SD values and correspondingly a lack of significant difference between patient and control data.

For female subjects, biopterin was significantly reduced in both unipolars and bipolars compared to controls (table 3.6). Neopterin excretion was raised in both groups but this did not reach significance. Pterin excretion was very similar in all groups examined. The $^{N}/_{B}$ ratio was again raised in patients compared to controls.

One very interesting observation was that urinary neopterin and biopterin correlated very well in controls. Figures 3.2 and 3.3 show that there are very strong, highly significant correlations in both female and male controls (both $r = +0.67$, $p < 0.0001$). This correlation was absent in the depressed patients, being slightly positive in females ($r = +0.01$) and slightly negative in males ($r = -0.01$), and not reaching significance in either case (figures 3.4 and 3.5). The absence of a correlation in the patients provides further evidence that there is diminished BH_4 synthesis in the patients, and that the lesion is between $NTPH_2$ and BH_4.

In table 3.7 it can be seen that female controls appear to excrete more biopterin and neopterin ($p < 0.005$ each), and more pterin ($p < 0.001$) than male controls. As females were also shown to excrete less creatinine than males, and the pteridines were related to the creatinine content of the sample, it may have been that the lower creatinine in the female samples caused the apparent rise in urinary pteridines. However, cortisol was also measured relative to a creatinine baseline, and cortisol was not raised in female controls compared to males. Higher pteridine excretion

by females may therefore be a valid physiological observation.

A comparison between male and female patients as in table 3.8 shows that creatinine excretion was significantly reduced in female patients compared to males ($p < 0.05$) and both cortisol ($p < 0.05$) and neopterin ($p < 0.02$) significantly raised. The apparent rise in cortisol may be attributed to the lowered level of creatinine. Biopterin and pterin were also raised in female patients compared to males, although the rise did not reach significance. The pattern of raised pteridine excretion in female patients reflects that shown by control females compared to control males.

As the urine samples were stored in plain tubes without an added antioxidant such as ascorbate, some breakdown of biopterin may have taken place. A recent report by Howells and Hyland (1987) found that under certain storage conditions, BH_4 could oxidise to compounds other than dihydrobiopterin and biopterin. In case BH_4 in the urine samples had autoxidised to pterin, with the loss of the side chain, the HPLC values for biopterin and pterin were summed to give 'corrected' biopterin values. Table 3.9 shows the result of this exercise. It is interesting to see that the female patients now have a highly significant ($p < 0.01$) reduction in biopterin values compared to their controls, while the male patients had very similar values to their controls. The group of 76 patients as a whole had a significant reduction ($p < 0.05$) in biopterin excretion. These corrected results reinforce the trends shown in the previous tables.

In the survey conducted by Duch et al (1984b), random urine samples were obtained from 28 control and 43 drug-free depressed patients. The general finding was that both neopterin and biopterin excretion was significantly raised in unipolars ($p < 0.05$ and $p < 0.01$ respectively) compared to controls and bipolars. When they divided the patients into groups of primary and secondary depression, they found that neopterin

and biopterin were raised in primary depression ($p < 0.05$) and biopterin alone in secondary depression ($p < 0.01$) when compared to controls. No differences were found in the neopterin and biopterin levels of a group of 21 schizophrenics sampled at the same time. However, a later study by the same group (Garbutt et al, 1985) found somewhat different results in that only urinary biopterin was found to be raised ($p < 0.02$) in a group of 9 patients with major depression compared to 28 controls. The authors had no explanation for the differences between the two reports. Neither study included details of patients (duration of illness, previous medication, current depression rating) other than the fact that they were not presently receiving medication.

The results of a study of urine analysis is depression by Blair et al (1984b) were quite different. Random morning urine samples were taken from nine female and eight male patients (13 unipolar and 4 bipolar) and from ten female and three male controls. The total urinary biopterins were measured on each sample and related to the creatinine content of each. When the whole group of patients was compared to the controls, urinary biopterin was seen to be lowered, but not significantly. However, when the patients were allocated according to unipolar or bipolar affective illness, the bipolar group exhibited significantly reduced biopterin excretion ($p < 0.05$, Wilcoxon's rank sum test) with respect to both controls and the unipolar depressives. Blair et al (1984b) found no significant differences in the excretion of total biopterins between males and females, either in the patients or the controls.

The results of the survey performed here mostly agree with the findings of Blair et al (1984b). When the pteridine values were converted to common units (μ mol/mol creatinine), Blair et al found that the patients ($n = 17$) had a mean urinary excretion

of 468 (SD 180). It was shown here that the patient group (n = 76) had a mean biopterin excretion of 448 (SD 184), which was very similar to the results of Blair's group. For the controls, Blair et al found a group of 13 had a mean urinary biopterin excretion of 559 (SD 260); here it was shown that the mean biopterin excretion for the control group (n = 61) was 529 (SD 256), again very close results to the earlier study.

However, these results differed from Blair's in some respects. In this study it was shown that control females (n = 33) excreted more ($p < 0.005$) biopterin than control males (n = 28). Blair et al found no difference between the two means, although they had only ten females and three males for comparison. In the study by Blair et al, biopterin excretion by unipolars was decreased but not significantly, compared to controls. Excretion by bipolar patients was similar to controls. In this study, it was seen that female unipolars and bipolars had significantly reduced biopterin excretion, but the decreases in male patients did not reach significance either for unipolars or bipolars.

As a corollary, the results of urinary biopterin excretion found here confirm and amplify the report by Blair et al (1984b) that BH_4 synthesis is diminished in depression.

3.4.4 HPLC and Crithidia measurements

For each patient and control group, biopterin measurements by HPLC were compared to Crithidia bioassay determinations (table 3.10). Here it can be seen that while the HPLC measurements showed a reduction in biopterin excretion in patients compared to controls ($p < 0.01$), the Crithidia measurements showed a reduction which did not reach significance. The difference between the means was less by Crithidia bioassay than by HPLC. When the mean results of all groups estimated

by Crithidia and HPLC were compared by the Student's t test, they did not differ significantly. However, when the paired t test was applied, the two sets of means varied a great deal to a highly significant degree ($p < 0.01$). There was a weak positive correlation between the results obtained by the two assays for controls and patients: the correlation reached significance ($p < 0.05$) when all determinations ($n = 137$) were considered.

The bioassay for biopterin using Crithidia fasciculata is based on the fact that it is an essential dietary requirement for this trypanosomid insect parasite (Baker et al, 1974). Previous studies have compared HPLC and Crithidia in assessing the concentration of biopterin in body fluids such as urine, serum and CSF (Blair et al, 1984a; Morar, 1984; Hamon, 1984). As shown here, differences in estimation have been observed. In cases where Crithidia biopterin values have been lower than those obtained by HPLC, the assay procedure of autoclaving and incubation at 29 - 32°C for 3 - 4 days has been implicated in the oxidation of biopterin to Crithidia-inactive substances (Milstein, 1983). Over-estimations of biopterin by Crithidia compared to HPLC have been attributed to other pteridines, for example sepiapterin, stimulating Crithidia growth and giving a falsely high value. HPLC is now the preferred method as it can distinguish between biopterin peaks and those of other species.

3.4.5 Plasma lithium

Plasma lithium values were obtained for 75 out of 76 patients (table 3.11). Female bipolar patients ($n = 18$) had higher plasma lithium than female unipolars ($n = 32$), $p < 0.05$. No significant differences were observed between any of the other groups with regard to lithium. As lithium has a postulated pathological effect on the kidney, correlations were measured between

plasma lithium and urinary biopterin in all groups. A negative correlation may have suggested that increasing lithium concentration was diminishing the excretion of urinary biopterin due to polyuric dilution. No such correlations were shown.

3.4.6 Plasma folate

Some of the patients that had blood samples taken for folate assay were taking part in a folate supplementation study. The patients were either given 200 μg folate or a placebo. The distribution of folate-supplemented and placebo groups is given in table 3.12 , with the results of the plasma folate assays. All supplemented groups had higher folate levels than placebo groups. The number of individuals measured in each group was very small and often too low to permit statistical analysis. However, supplemented female bipolars were shown to have significantly higher ($p < 0.02$) plasma folate levels than the placebo female bipolars. When all the folate estimations were combined, as in table 3.13, it was shown that female bipolars had higher plasma folate than male bipolars ($p < 0.05$) and male unipolars had even more significantly raised folate levels than male unipolars ($p < 0.01$). In contrast to the lithium results, there was a strong positive correlation between plasma folate and urinary biopterin in the largest group, the 15 female unipolars ($r = +0.63$, $p < 0.01$, figure 3.6). It has previously been shown that both lowered biopterin levels (Blair *et al*, 1984b, Morar *et al*, 1983) and lowered folates (Thornton and Thornton, 1978) are associated with depression. It could be suggested that one of the effects of folate supplementation was to restore urinary biopterin values to those of control subjects. This would imply that folate was linked with a stimulation of BH_4 biosynthesis *in vivo*.

CHAPTER FOUR:

TETRAHYDROBIOPTERIN METABOLISM IN SENILE DEMENTIA OF ALZHEIMER TYPE

4.1 INTRODUCTION

4.1.1 Post mortem brain studies of tetrahydrobiopterin metabolism in SDAT

Lowered brain biopterin in SDAT patients was first demonstrated by Nagatsu et al (1979) and has since been reported by numerous workers (Barford et al, 1984; Anderson et al, 1986; Sawada et al, 1987).

Sawada et al (1987) found tyrosine hydroxylase to be lower in 12 SDAT brain regions compared to controls, and significantly lowered in the substantia nigra ($p < 0.05$). Tryptophan hydroxylase was lower in 11 regions of SDAT brain, significantly so in the globus pallidus lateral segment, locus coeruleus and substantia nigra (all $p < 0.05$). Total biopterin was shown to be significantly lower in the putamen and substantia nigra (both $p < 0.05$) with neopterin content not changing significantly in any region of SDAT brain compared to controls.

Anderson et al (1986; 1987) in comprehensive studies of BH_4 metabolism in normal ageing and SDAT affected brain found some similarities with Sawada's group. BH_4 synthesis was shown to decline with age ($p < 0.05$) in the frontal and temporal cortex of normal (non-demented) subjects, and a decline in DHPR in both these brain regions with age was also demonstrated ($p < 0.01$). Sepiapterin reductase activity and GTP-cyclohydrolase showed no correlation with age in the brain regions studied. BH_4 biosynthesis was significantly reduced in the locus coeruleus of SDAT brain and was also reduced in the temporal cortex compared to controls. The reduction of BH_4 in the temporal cortex in the group of SDAT subjects studied by Anderson did not reach significance, although this had been shown to be substantially reduced in a group studied by Barford et al (1984). DHPR and sepiapterin reductase activity were similar in SDAT and matched control samples. Anderson et al (1986) also examined BH_4

metabolism in a group of Down's syndrome (DS) patients. DHPR activity was lower in DS brain than in controls ($p < 0.05$) and BH_4 biosynthesis values were also significantly lower than age-predicted controls and similar to patients with SDAT. The authors point out that similarities in behaviour and neurochemical aspects do exist between older DS patients and those with SDAT. They suggest that the salvage and biosynthetic pathway for BH_4 in ageing brain and the biosynthetic pathway alone are diminished in SDAT. For SDAT, the fact that neither GTP-cyclohydrolase, DHPR or sepiapterin reductase are significantly affected suggests that the lesion in biosynthesis is at the level of the phosphate eliminating enzyme.

An interesting *in vitro* study was performed by Blair *et al* (1984a). In brain preparations from SDAT patients in whom BH biosynthesis had been shown to be reduced, addition of 5-methyltetrahydrofolate could stimulate the post-mortem tetrahydrobiopterin biosynthesis in temporal cortex into the normal range for age-matched controls.

Another study on normal brain examines the interaction between tetrahydrobiopterin biosynthesis and the neurotoxin aluminium, which has been postulated as causative in Alzheimer's disease (discussed in chapter one). Cowburn and Blair (1987) found that the addition of 1 mMol aluminium acetate to temporal and frontal cortex preparations from non-demented patients significantly decreased ($p < 0.001$) BH_4 biosynthesis, compared to the same samples in the absence of aluminium. This concentration of aluminium was similar to levels found in the brain of patients who had died with SDAT. The authors postulated the inhibitory effect of aluminium to arise from competition with magnesium in the assay.

4.1.2 Cerebrospinal fluid studies of tetrahydrobiopterin metabolism in SDAT

LeWitt et al (1985) found that CSF total biopterin decreased with normal ageing, but that SDAT patients had much lower ($p < 0.001$) CSF total biopterin compared to age-matched controls. The serotoninergic and dopaminergic metabolites 5HIAA and HVA correlated strongly with tetrahydrobiopterin values. CSF neopterin was similar in SDAT and controls, indicating that the metabolic lesion in central BH_4 production was after the formation of dihydroneopterin triphosphate.

Morar et al (1983) reported an increased ratio of total neopterin to total biopterin in the ventricular CSF of SDAT patients, and reached a similar conclusion.

Kay et al (1986) examined CSF biopterin in a group of thirty SDAT patients and fourteen controls. Biopterin was found to be significantly lowered in the demented group compared to controls ($p < 0.01$). Kay et al were the first to demonstrate that CSF biopterin did not correlate with CSF volume as measured by computerised tomography. This indicated that the lowered biopterin levels found were not a function of dilution due to ventricular dilation.

Nakamura et al (1984) reported a decrease in CSF DHPR concentration that correlated with age ($p < 0.05$); a greater depression of DHPR activity was shown in SDAT subjects compared to controls. No significant decrease in CSF DHPR was shown by a similar group of MID patients.

4.1.3 Peripheral studies of tetrahydrobiopterin metabolism in SDAT

Most peripheral studies of BH_4 metabolism in SDAT have been in the blood, including plasma, serum and erythrocyte studies.

Leeming et al (1979) found that serum biopterin levels were reduced in SDAT, and that there was a raised phenylalanine to tyrosine ratio, indicating

a partial failure of the hydroxylating system.

Leeming and Blair (1980) in extensive studies of tetrahydrobiopterin and its derivatives in man, found that serum biopterin was lowered to a highly significant degree ($p < 0.0001$) in a group of 15 patients with SDAT compared to 21 age-matched controls.

Similarly, Aziz et al (1983) showed that serum biopterin was significantly lowered in SDAT patients compared to two separate groups of controls, the first group confused but not demented, and the second group showing no mental symptoms at all.

Young et al (1982) reported that plasma BH_4 was lower in SDAT patients compared to controls, and there was a significantly elevated neopterin to biopterin ratio in these patients.

A recent study by Altmann et al (1987) found that high levels of serum aluminium had an inhibitory effect on DHPR activity in erythrocytes: they speculated that this might be important in the pathology of aluminium encephalopathy which is found in some dialysis patients. They also related these findings to senile dementia of Alzheimer type, where high brain concentrations of aluminium have been measured (Perl and Brody, 1980).

4.1.4 Urine studies of tetrahydrobiopterin metabolism in SDAT

At the time the studies in this thesis were commenced (1984), there were no reports of urinary measurements of biopterin or related compounds in SDAT.

Collection of urine is non-invasive and can easily be performed by nursing staff. This is particularly important when dealing with elderly, demented patients who cannot be relied upon to cooperate in more complicated studies.

Studies were therefore made of pteridine excretion in SDAT to give insights into BH_4 metabolism in this condition.

4.2 EXPERIMENTAL SECTION

4.2.1 Urinary excretion of pteridines in SDAT: Study one

The first study involved ten hospitalised elderly females who had been diagnosed as suffering from SDAT by a consultant geriatrician. The degree of dementia was not recorded. The age range of nine individuals was 61 - 93 years (mean 81.0 (9.5 SD) years). The age of one patient was unknown. Medication was not recorded. The control group was selected from the female controls described in chapter three. Data from 24 control females aged over 40 years was used for comparison with the SDAT group. The age range of the control group was 40 - 80 years (mean 55.9 (SD 2.1) years).

4.2.1.1 Collection and analysis of samples

Urine samples were collected between 0900 and 1100 and decanted into plain plastic tubes. A minimum of 5mls were collected. Identification of samples was by initials only. The tubes were stored in the dark at -70°C in this laboratory until assay. The tubes were thawed and mixed, and an aliquot removed for creatinine assay by an automated method (section 2.3.2.3). The urines were processed for pteridine assay by HPLC as previously described (section 2.3.2.1). Two complete analyses were performed for each sample. The values obtained for the samples were assigned to SDAT and control groups once all assays had been completed.

4.2.2 Urinary excretion of pteridines in SDAT: Study two

4.2.2.1 Patients and controls

Thirty women who had been diagnosed as suffering from SDAT were admitted to this study. They were either hospitalised at West Park Hospital, Surrey or were attending the associated day clinic. Their ages ranged from 65 to 99 years, with a mean of 81 years (SD 8.8). The control subjects were 21 women aged over 65 years who were attending their general practioner for minor physical complaints or were living in warden-controlled accomodation for physical reasons. The age range of the controls was 65 to 90 years, with a mean of 77.4 years (SD 8.3).

In selecting the thirty SDAT patients, three psychometric tests were employed.

The patients were tested on the Geriatric Mental State Scale (GMS) (Copeland et al, 1976) which is one way of diagnosing SDAT in elderly patients, and can exclude patients suffering from similar symptoms due to depression. The scale is detailed in appendix 9. Patients who scored in subsections of the test dealing with affective disorder as well as cognitive functions were excluded from the survey.

The modified Hachinski index (Hachinski et al, 1975) was completed for each patient to exclude patients suffering from multi-infarct dementia. The index is detailed in appendix 10.

Cognitive impairment in the SDAT patients was assessed by the modified Kew Cognitive Map test (Hare, 1978). Patients had to achieve an error score of 3 or more on the memory sub-section, or be too impaired to attempt to answer it, to be included in the survey. The control subjects were also asked to complete the Kew test, and were excluded if they too obtained a score of 3 or more. The test is detailed in appendix 11.

The medical history of each SDAT and control subject was obtained from her general practioner. Appendices 12 and 13 detail the medical status and medication of controls and SDAT patients. The prevalence of diseases in the patients and controls was summarised in table 4.1 for comparison. The duration of dementia ranged from one to seventeen years since onset, with a mean length of 7.7 years (SD 4.3). 29 out of 30 patients were given arbitrary assessments as to degree of dementia: 26 were described as severely demented, and three as not severe (appendix 13).

4.2.2.2 Collection and analysis of samples

Approximately 10mls of urine were collected from each of the thirty SDAT patients between 0900 and 1000, and decanted into plain plastic tubes. Blood samples were taken from 26 SDAT patients at the same time as the urine. Blood was obtained by venepuncture and collected into lithium heparin tubes. The plasma was immediately separated. Plasma folate was assayed at the MRC Neuropsychiatry laboratories, West Park Hospital, Surrey. Plasma and urine sample tubes for pteridine analysis were identified by initials and kept frozen in the dark at -70°C until assay at Aston University. The diagnoses of SDAT or control subject were not known until all assays had been completed.

Thawed urine samples were mixed and an aliquot removed from each for creatinine assay by the discrete method (section 2.3.2.3) in this laboratory. The urines were oxidised by acid/iodine and filtered (as in section 2.3.2.1) and assayed for total biopterin and total neopterin by HPLC. All assays were performed in duplicate.

Plasma samples were thawed, mixed and assayed for neopterin and biopterin using HPLC by Dr. C. Hamon.

Table 4.1 Disease in SDAT group 2 and controls

Disease	SDAT (n)	Control (n)
Bacterial urine infection	10	-
Arthritis	4	5
Hypertension	6	3
Oedema	2	2
Cancer	2	2
Epilepsy	7	-
Heart failure	5	-
Ulcers	1	1
Depression	-	2
Anxiety	-	1
Psoriasis	1	-
Diverticulitis	-	1
Bronchitis	-	1
Anaemia	1	-
Bacterial diarrhoea	2	-
Eczema	-	1
Asthma	-	1
Vascular disease	1	-

4.3 RESULTS

The results for the urine and plasma analyses are shown in tables 4.2 to 4.7. Individual data values are listed in appendices 14 to 16.

The results of the two studies are presented together.

4.3.1 Age

The mean ages of the two groups of SDAT patients examined were very similar: 81.0 years (SD 9.5) for group one (table 4.2) and 81.0 years (SD 8.8) for group two (table 4.3). However, the ten patients in group one were not age matched with their 24 controls, and were older to a highly significant degree ($p < 0.0001$). In the second study, the mean age of the 21 control subjects (77.4 years, SD 8.3) did not differ significantly from the whole group of 30 patients.

4.3.2 Creatinine

Creatinine was measured in all the urines collected in the two surveys (tables 4.2 and 4.3). Group one SDAT patients were shown to excrete less creatinine than their controls, although the difference did not reach significance. Group two SDAT patients excreted slightly more creatinine than their controls, but this again did not reach significance.

4.3.3 Biopterin, neopterin and pterin in urine

Biopterin and neopterin were measured in the urine of SDAT groups one and two. Pterin was measured in the first SDAT study only.

In group one SDAT patients there was a slight non significant rise in biopterin excretion compared to the controls (table 4.2). The thirty group two SDAT patients showed a decrease in biopterin excretion

Table 4.2 Urinary excretion in SDAT group one patients

Group	n	Age (yrs) Mean (SD)	Biopterin μmol/mol creatinine Mean (SD)	Neopterin μmol/mol creatinine Mean (SD)	Pterin μmol/mol creatinine Mean (SD)	N/B Mean (SD)	$B/(B+N)$ Mean (SD)	Creatinine mmol/l Mean (SD)
Control	24	55.9 (2.1)	520.0 (244.9)	894.0 (534.0)	102.1 (56.8)	1.72 (0.59)	0.384 (0.079)	8.1 (6.3)
SDAT	10	***81.0 (9.5) (n=9)	563.5 (210.4)	**1586.0 (639.0)	75.5 (40.1)	*3.20 (1.75)	$0.279^{\dagger}$ (0.117)	7.4 (3.2)

Statistics:

***	Significantly	higher	than controls,	$p < 0.0001$
**	Significantly	higher	than controls,	$p < 0.01$
*	Significantly	higher	than controls,	$p < 0.02$
†	Significantly	lower	than controls,	$p < 0.02$

Table 4.3 Urinary excretion in SDAT group two

Group	n	Age (years) Mean (SD)	Creatinine mmol/l Mean (SD)	Biopterin μmol/mol creatinine Mean (SD)	Neopterin μmol/mol creatinine Mean (SD)
Controls	21	77.4 (8.3) (n = 20)	6.85 (4.63)	270 (206)	169 (125)
SDAT	30	81.0 (8.8)	7.77 (5.34)	180 (116)	226 (139)
SDAT	10	** 86.7 (7.7)	8.50 (4.48)	* 132 (99)	*** 323 (134)

Statistics:

* Lower than controls, $p < 0.05$
** Higher than controls, $p < 0.02$
*** Higher than controls, $p < 0.01$

which did not reach significance due to the large SD values of the patients and controls (table 4.3).

Neopterin excretion was highly significantly raised in both SDAT study groups compared to their controls, at $p < 0.01$ each (tables 4.2 and 4.3). The raised neopterin excretion meant that the ratio $^{N}/_{B}$, an index of biosynthesis, was significantly different between patients and controls in each study: it was raised at $p < 0.02$ significance in group one (table 4.2) and at $p < 0.005$ in group two (table 4.4) compared to controls. When the distribution of the urinary $^{N}/_{B}$ ratio in the 30 SDAT patients and 21 controls in the second study was examined, an interesting feature emerged (figure 4.1). While most of the $^{N}/_{B}$ ratio results were clustered about a value of 0.8, there was a subgroup of ten SDAT patients with markedly higher $^{N}/_{B}$ values than the other SDAT patients and the controls. The mean $^{N}/_{B}$ value of this subgroup of ten patients was very much higher than that of the controls ($p < 0.001$) and higher than the SDAT group as a whole ($p < 0.02$), table 4.4. It was noted that this group was significantly older than the controls ($p < 0.02$, table 4.3). It was also interesting to note that biopterin excretion was significantly reduced ($p < 0.05$) in this subgroup with the highest $^{N}/_{B}$ values.

The ratio $^{B}/_{B+N}$ was calculated for each subject in the two studies as an estimate of the percentage conversion of neopterin triphosphate to tetrahydrobiopterin. If all $NTPH_2$ had been converted to BH_4, $^{B}/_{B+N}$ would be equal to one. If only neopterin, and no biopterin, had been detected, $^{B}/_{B+N}$ would be equal to zero. The higher the fraction, the more efficient the conversion. A fraction such as $^{B}/_{B+N}$ equal to 0.279 (as shown by the ten SDAT patients in table 4.2) implies that conversion of NH_2TP to BH_4 is approximately 28%. The calculation of these fractions was used as a rough guide to biosynthesis. No attempt was made to correct for any possible breakdown of pteridines during collection, storage

Figure 4.1 Distribution of $^{N}/_{B}$ ratio in 30 SDAT patients and 21 controls

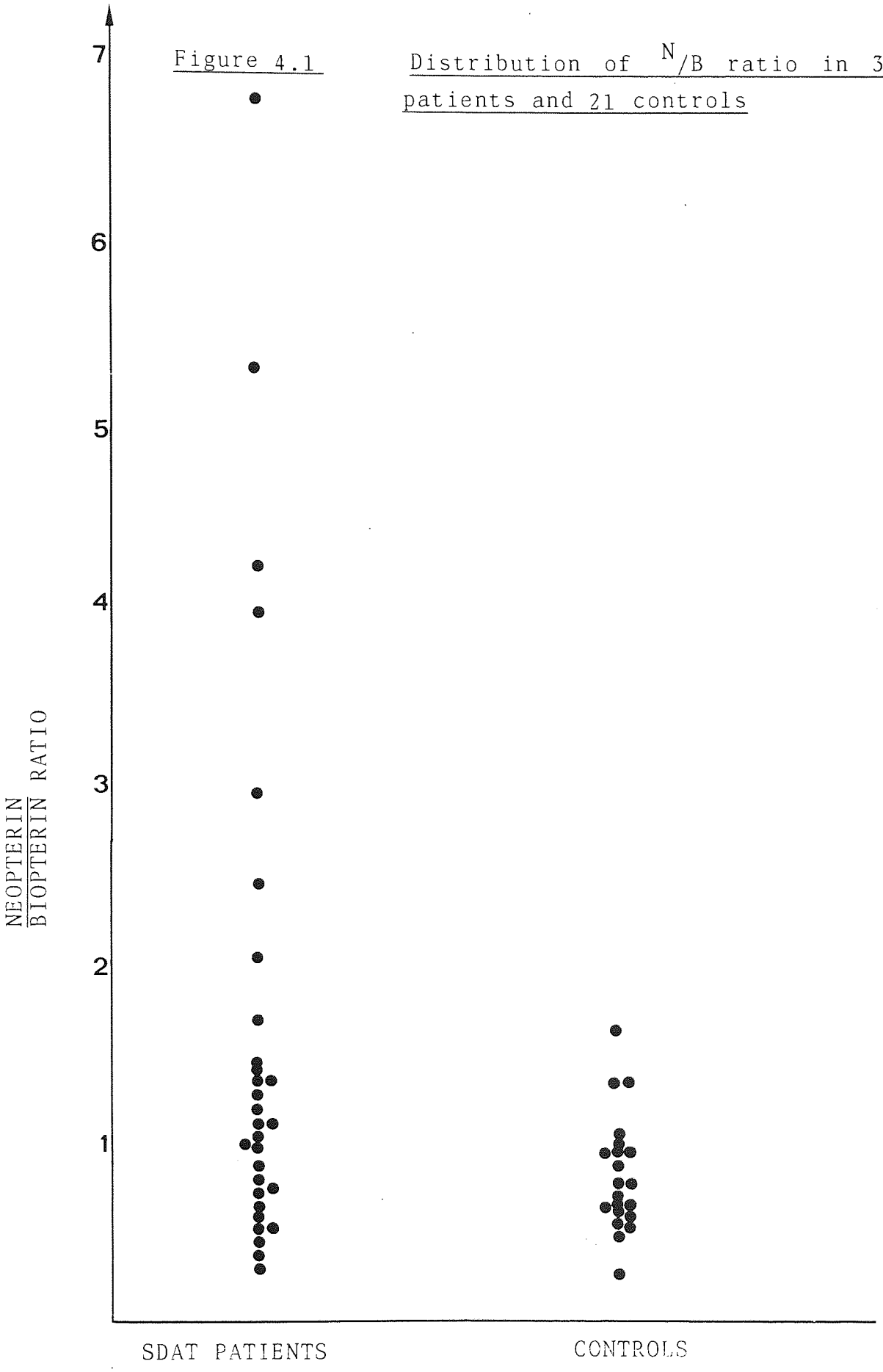


Table 4.4 Urinary pteridine ratios in SDAT group two patients and controls

Group	n	$^{N}/_{B}$ ratio Mean (SD)	$^{B}/_{B+N}$ ratio Mean (SD)
Control	21	0.80 (0.33)	0.537 (0.098)
SDAT	30	*1.67 (1.54)	†0.455 (0.164)
SDAT	10	**3.24 (1.81)	††0.270 (0.100)

Statistics:

* Higher than controls $p < 0.02$

** Higher than controls $p < 0.001$

† Lower than controls $p < 0.05$

†† Lower than controls $p < 0.001$

or assay. In the first survey it was seen that the ratio $^{B}/B+N$ was significantly lower for the ten SDAT patients than for the controls ($p < 0.02$). The same trend was shown in table 4.4 for the second study, where the $^{B}/B+N$ ratio was lower for the 30 SDAT patients ($p < 0.05$). The subgroup of ten SDAT patients with the highest $^{N}/B$ ratios naturally had a more pronounced decrease in $^{B}/B+N$ ratio ($p < 0.001$).

As the age range of the SDAT group two patients (65 - 99 years) and controls (65 - 90 years) were quite wide, correlations were calculated between the two indices of synthesis, the $^{N}/B$ ratio and the $^{B}/B+N$ ratio, and the age of the subject. Any decreasing capacity for synthesis in these elderly females as part of the normal ageing process would be demonstrated by a negative correlation of synthesis with age. There were no correlations between $^{N}/B$ or $^{B}/B+N$ ratio and age in the 21 control subjects in study two (table 4.5). However, there was a positive correlation between $^{N}/B$ and age in the 30 SDAT subjects which failed to reach significance (table 4.5). The significant negative correlation between $^{B}/B+N$ and age in the whole SDAT group ($r = -0.362$, $p < 0.05$) indicates that in the SDAT group alone, advanced age tends to support diminished BH_4 biosynthesis (table 4.5). Non significant correlations were obtained for $^{N}/B$ and $^{B}/B+N$ with age in the subgroup of SDAT patients, but this may have been due to the small number of pairs ($n = 10$) correlated.

In the second study, the excretion of urinary biopterin was correlated with that of neopterin. There was a very good positive correlation in the 21 control subjects ($r = +0.687$, $p < 0.001$) as shown in table 4.5 and figure 4.2. This positive correlation was again seen in the 30 SDAT subjects ($r = +0.529$, $p < 0.01$) as shown in table 4.5 and figure 4.3.

A summary table (table 4.6) compares the urinary excretion of pteridine in the two surveys. It can be seen that the trends were similar in both.

Table 4.5 Correlations between urinary biopterin and neopterin in SDAT group two

Group	n	$^{N}/_{B}$ correlation with age r	$^{B}/_{B+N}$ correlation with age r	Neopterin correlation with biopterin r
Control	21	-0.062 (NS) (n = 20)	-0.186 (NS)	$+0.687^{\dagger\dagger\dagger}$
SDAT	30	+0.221 (NS)	$-0.362^{\dagger}$	$+0.529^{\dagger\dagger}$
SDAT	10	-0.383 (NS)	+0.483 (NS)	+0.572 (NS)

Statistics:

† Significant correlation $p < 0.05$

†† Significant correlation $p < 0.01$

††† Significant correlation $p < 0.005$

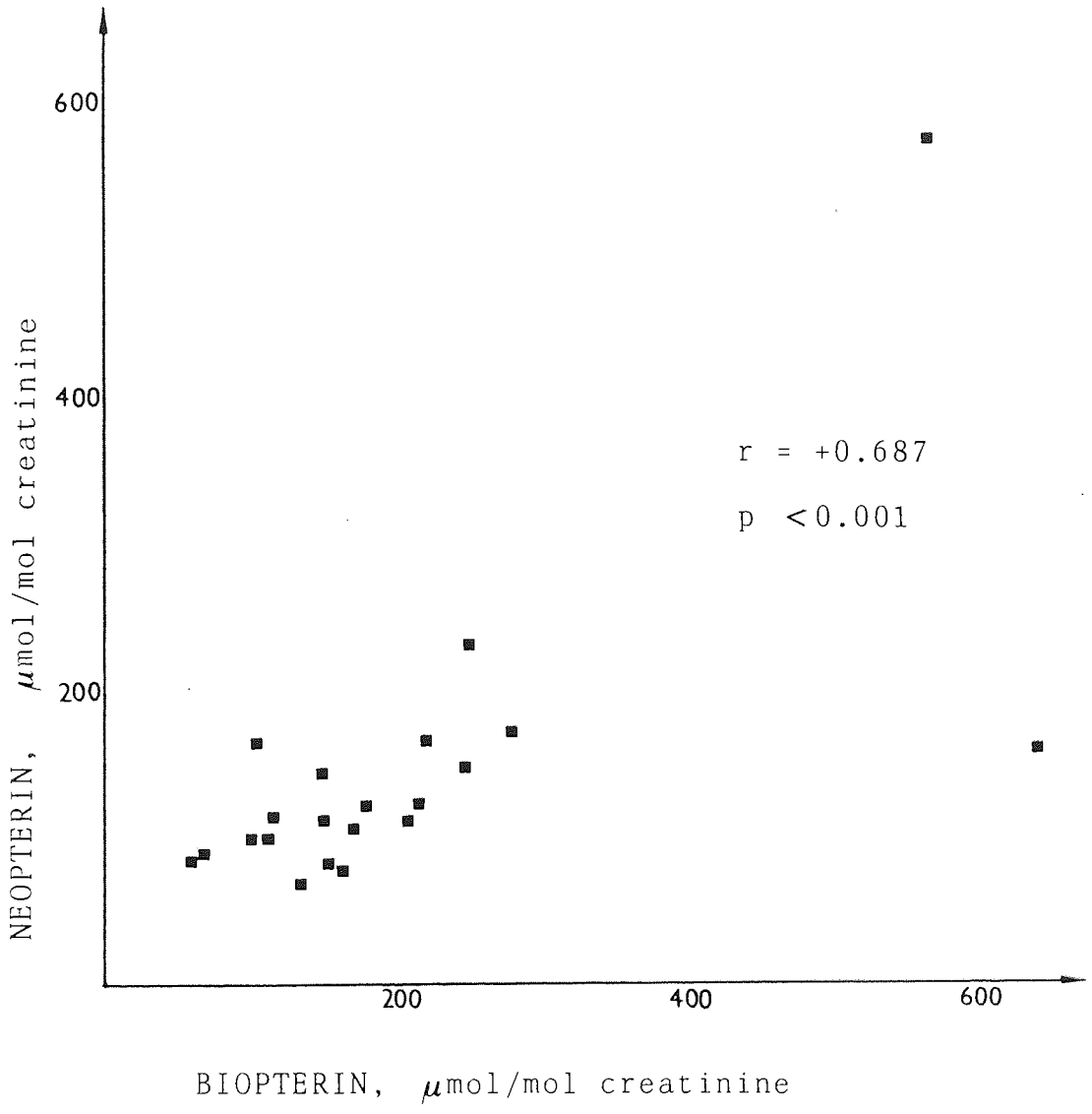


Figure 4.2 Correlation of urinary neopterin with urinary biopterin in 21 controls for SDAT group 2 patients

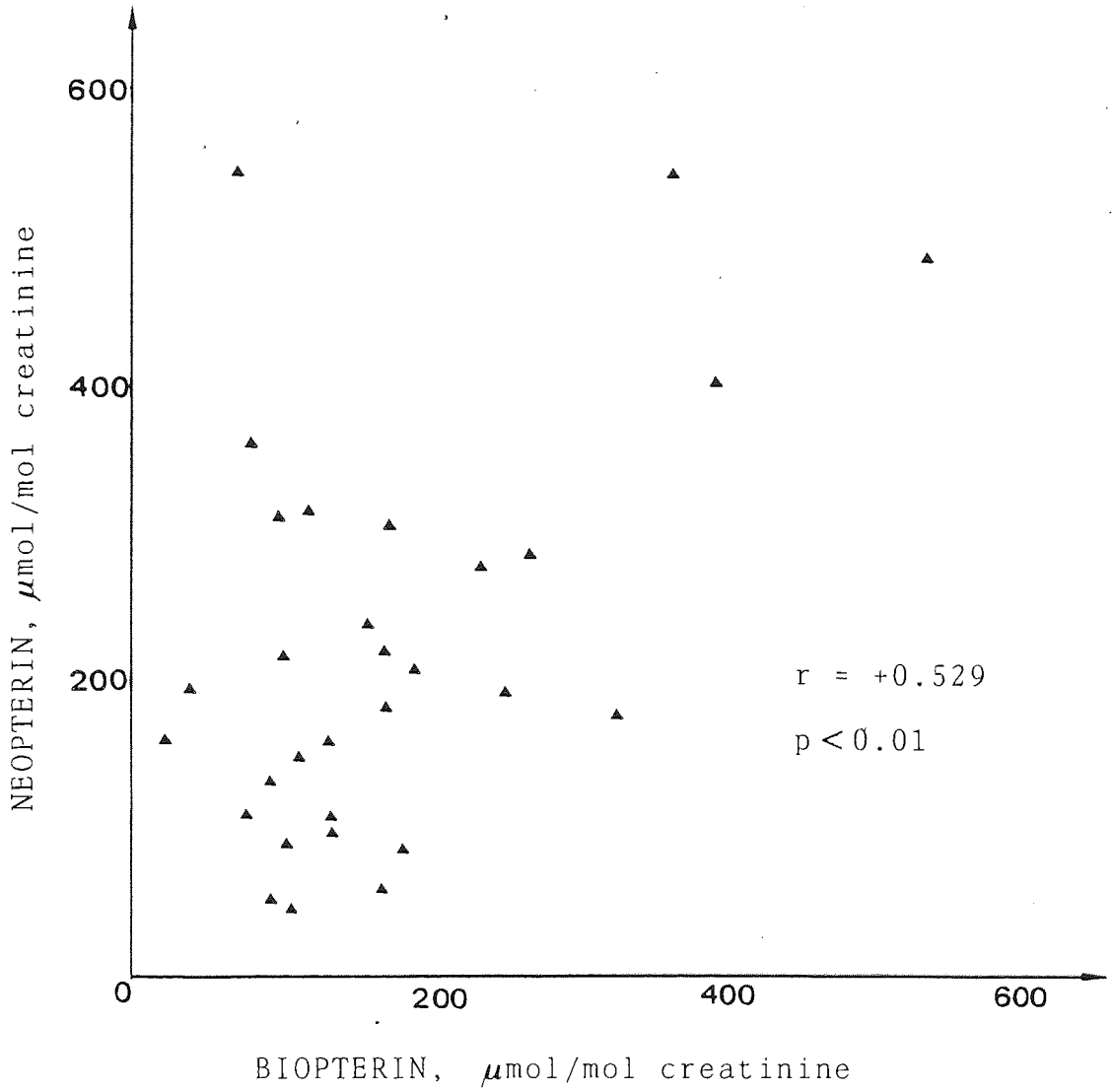


Figure 4.3 Correlation of urinary neopterin with urinary biopterin in 30 SDAT patients

Table 4.6 A comparison between the results of SDAT group one and SDAT group two

Parameter	SDAT group one versus controls	SDAT group two versus controls
Age	Higher $p < 0.0001$	Age matched
Biopterin	NS	Lower in sub-group of 10 $p < 0.05$
Neopterin	Higher $p < 0.01$	Higher $p < 0.01$
$B_{/B+N}$	Lower $p < 0.002$	Lower $p < 0.005$
$N_{/B}$	Higher $p < 0.002$	Higher $p < 0.01$

4.3.4 Plasma biopterin and neopterin

Biopterin and neopterin were measured in 23 plasma samples from SDAT patients. Individual values are given in appendix 16, and a summary in table 4.7. As plasma samples were not obtained for controls, comparisons were limited to an intragroup analysis only.

Plasma biopterin and neopterin values were similar and did not differ from each other. The mean plasma $^{N}/B$ ratio of 1.32 (SD 1.15) in 23 SDAT subjects was lower than the urinary value of $^{N}/B$ for 30 SDAT subjects (1.67, SD 1.54) but this difference did not reach significance. There was a positive correlation between plasma neopterin and biopterin, as shown in urine, but in this case it did not reach significance ($r = +0.292$). Plasma neopterin did not correlate with urinary neopterin ($r = +0.070$, NS) but there was a negative correlation between plasma and urinary biopterin ($r = -0.516$, $p < 0.05$).

4.3.5 Plasma folate

The mean plasma folate for 20 SDAT patients was 3.93 ng/ml (SD 2.90). Plasma folate did not correlate with either plasma neopterin or biopterin. The correlation between plasma folate and urinary biopterin was positive but weak, and failed to reach significance ($r = +0.115$). Plasma folate did not correlate with any other variable such as plasma or urine $^{N}/B$ ratio, plasma or urine $^{B}/B+N$ ratio, age, or years of onset of SDAT (table 4.7).

4.4 DISCUSSION

4.4.1 Urinary pteridine excretion in SDAT

A discussion of the urinary results of this chapter is necessarily limited by the fact that there has, until now, been little published work on urinary

Table 4.7 Plasma results of SDAT group two patients

Parameter	n	Mean (SD)
Biopterin (ng/ml)	23	2.08 (1.48)
Neopterin (ng/ml)	23	1.83 (1.43)
Folate (ng/ml)	20	3.93 (2.90)
$N/_{B}$ ratio	23	1.32 (1.15)
$B/_{B+N}$ ratio	23	0.499 (0.178)

pteridine excretion in dementia.

The results of the first study of biopterin and neopterin excretion in SDAT have been published elsewhere (Jones et al, 1987). The general conclusions of the study were that there was a raised neopterin level in SDAT compared to controls, and this in turn led to a raised neopterin to biopterin ratio (N/B) in the SDAT patients compared to controls.

In the first study, information as to patient medication, duration since onset of SDAT, degree of severity of dementia and other details were not known, and the study was open to criticism for two main reasons: the number of SDAT patients was small (n = 10), and the control group of twenty four individuals was significantly younger than the SDAT group.

The second survey was performed in response to the findings of the first. This study was more strictly controlled: patients and controls were selected according to recognised psychiatric and psychological criteria; the two groups of patients and controls were of similar size and were strictly age-matched; and details of medication and other somatic illnesses were recorded for comparison. In both studies, the identities of the urine samples were not known until all analyses had been completed, and the results were then allocated into control or patient groups. It is interesting to note that the second study confirms the results of the first in most respects, although they were conducted at different times and with different patients.

The most consistent finding is the increased oxidised neopterin to oxidised biopterin ratio (N/B) in the SDAT patients compared to controls. This result in the urine reflects previous observations on central N/B ratio findings. Other groups have studied this ratio in brain and CSF and in all cases the ratio has been shown to be elevated in SDAT patient samples (Hamon and Blair, 1987).

In the temporal cortex, Barford et al (1984) demonstrated a ratio of 5.35 in SDAT compared to control values of 0.55, and Morar et al (1983) showed the ventricular CSF ratio to be 1.10 in SDAT subjects and 0.90 in controls. Young et al (1982) found the plasma ratio to be 3.35 in SDAT and 2.50 in controls. The studies in this thesis represent the first finding of an elevated N/B ratio in SDAT urine samples, and indicate that urine values of pteridines may indeed reflect central tetrahydrobiopterin activity. The finding of an increased N/B ratio in all body fluids of SDAT patients examined so far provides substantial evidence for the metabolic lesion in BH_4 biosynthesis in SDAT coming between the conversion of dihydroneopterin triphosphate to tetrahydrobiopterin, at the level of the phosphate-eliminating enzyme as suggested by Anderson et al (1986).

A further application of the N/B ratio as an indicator of BH_4 metabolism has come from studies on the visual evoked potential (VEP) in man. Orwin et al (1986) found that a lag in the VEP in subjects with senile dementia was characteristic for this condition, and that the severity of SDAT correlated with the abnormal response in VEP. Hamon et al (1987) have since demonstrated that the abnormalities in VEP give an excellent correlation with increasing N/B ratio in SDAT. A combination of VEP and neopterin to biopterin ratio results could be useful in the diagnosis of SDAT in life.

Sawada et al (1986) examined neonatal urine and found that the N/B ratio here is much higher than that observed in adults: it is possible that these results indicate an incomplete or immature biosynthesis of tetrahydrobiopterin metabolism which compares with the impaired synthesis shown in SDAT.

4.4.2 Plasma results in SDAT

As plasma values of biopterin, neopterin and folate

were only obtained for the SDAT group and not controls, a discussion of the results is limited.

Of interest is the fact that plasma total neopterin and total biopterin showed a weak positive correlation which did not reach significance, compared to this correlation which was significant in the urine samples of both SDAT patients and controls. The mean plasma N/B ratio of 1.32 measured in 23 subjects was much lower than that found by Young et al (1982), who showed SDAT patients with a mean plasma N/B of 3.35. However, the plasma ratio shown in this study may still have been statistically different when compared to control samples assayed at the same time. The plasma ratio of N/B did not differ significantly from that shown in the urine of group two patients.

The significance of the negative correlation between urinary and plasma biopterin was not known.

The mean plasma folate for 20 patients was found to be 3.93 ng/ml which may be regarded as fairly low. An association between low plasma folates and SDAT has been shown by Abou-Saleh et al (1986), and it would have been interesting to compare the patient results with data from control subjects. The connection between low plasma folates and dementia is unclear: in common with other psychiatric states (such as affective disorder), folate deficiency may be a secondary phenomenon caused by poor nutrition as a result of lack of interest in food. However, dietary folate concentration should be adequate in institutionalised patients, and most of the patients in the study here were hospitalised or at least subject to monitoring and community care. Lowered folates in SDAT patients may be representative of the pathology of the disease. It was interesting to note the weak, non-significant correlation between plasma folate and urinary biopterin in these patients.

CHAPTER FIVE:

STUDIES ON TETRAHYDROBIOPTERIN METABOLISM IN THE RAT

5.1 INTRODUCTION

5.1.1 Tetrahydrobiopterin metabolism in the rat

Several groups have examined BH_4 metabolism in the rat. Fukushima and Nixon (1980) performed detailed analyses of tetrahydrobiopterin distribution in rat tissue using HPLC: they showed that the bulk of BH_4 was found in the pineal gland, followed by the liver, spleen, lung, ileum, kidney and brain, in decreasing order. The pineal gland had some 140-fold greater concentration than the brain. Duch et al (1984a) measured the activity of GTP cyclohydrolase in various tissues of the mouse as an indicator of tetrahydrobiopterin distribution, and showed the enzyme to be present in every tissue examined. Using selective oxidations and HPLC, they confirmed that 85 to 100% of total biopterin is in the tetrahydro- form. Duch et al (1984a) also measured neopterin levels in various species. While they found high levels in blood, CSF and urine from man and monkey, they could detect only small amounts of neopterin in dog urine and no neopterin at all in any tissue or fluid from the rat or mouse. It is interesting that there is a species specificity in neopterin distribution, being undetectable in the lowest mammals and present in ever increasing amounts in the highest mammals. A probable explanation for the lack of neopterin in the rat is that the intermediate $NTPH_2$ is rapidly metabolised to BH_4 and not oxidised to dihydroneopterin and then neopterin.

Kapatos et al (1982) established in vivo biosynthesis of BH_4 in rat brain using [^{14}C] guanosine. In the brain, the distribution is uneven, the highest levels contained in the hypothalamus and striatum, and the lowest in the cerebellum and hippocampus (Fukushima & Nixon, 1980; figure 1). Kato et al (1982) showed that levels of BH_4 in rat brain peak 12 days after birth, and then decline to an adult plateau. Different

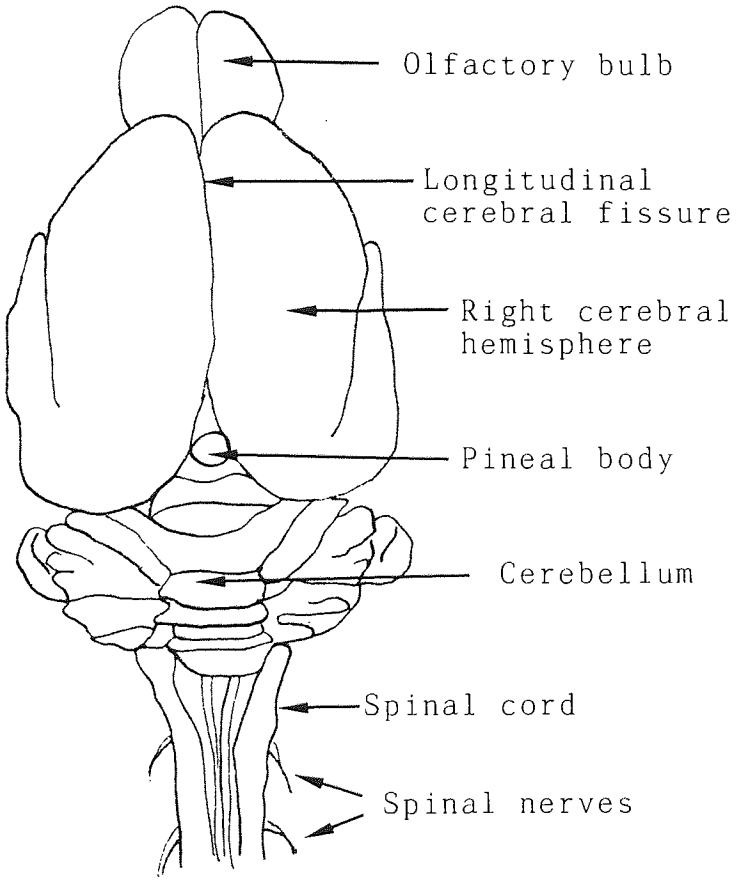


Figure 5.1 Dorsal view of rat brain

groups have reported different absolute values for brain total biopterin concentration: comparisons have been difficult due to a variety of baselines chosen to relate the tetrahydrobiopterin concentration to. However, Kapatos et al (1982) found brain BH_4 to be approximately 433 pmol/g tissue (SD 51) in a group of four adult rats. Mandell et al (1980) showed total biopterin to exhibit a diurnal pattern, and to vary up to two-fold around a mean value.

The Wistar rat is a convenient small mammal in which to study tetrahydrobiopterin metabolism: there have been many studies using a variety of agents on the rat in order to study putative changes that can be induced in BH_4 metabolism and which may offer insights into various drug action and disease states (Mandell et al, 1980; Abou-Donia et al, 1983; Hamon, 1984; Lee and Mandell, 1985).

5.1.2 Drugs, other agents and treatments used to examine aspects of BH_4 metabolism in the rat

The various drugs and other treatments applied to the rat in this chapter are discussed. The treatments all relate to treatment of depressive illness, or to making rat models of the syndromes of depression or senile dementia; rat tissues are examined for changes in tetrahydrobiopterin metabolism which may reflect the changes such treatments or states cause in BH_4 metabolism in man.

5.1.2.1 Lithium

5.1.2.1.1 Historical background

The first report of lithium being used as a drug was by Cade in 1949 in the treatment of mania. The efficacy

of treating mania with lithium salts was confirmed in a later study by Noack and Trautner (1951). One of the first controlled trials of lithium as an antidepressant was performed by Schou in 1968 who claimed that there was no improvement in twelve patients. However, later studies have confirmed the validity of lithium treatment and prophylaxis in depression.

5.1.2.1.2 Lithium as an antimanic and antidepressant drug

Lithium is not a drug of choice in acute mania: a neuroleptic such as chlorpromazine or fluphenazine has a more immediate effect in reducing psychosis. When bipolar patients are started on lithium therapy following a manic episode, mood stabilisation occurs after 6 - 10 days of administration (Birch, 1982). About 70 - 80% of manic patients show a good response to lithium (Schou, 1959).

The use of lithium as an antidepressant is not fully established for acute depression (Coppen and Abou-Saleh, 1983) but is recognised as effective in the treatment and prophylaxis of bipolar disorder. Patients given lithium have fewer depressive episodes than placebo groups (Coppen *et al*, 1971; Fieve *et al*, 1976); lithium potentiates the efficacy of tricyclic antidepressants (Heninger *et al*, 1983) and is considered to be an antidepressant in its own right (Prien *et al*, 1973).

The serum lithium levels of new patients are carefully monitored for the first few weeks of treatment until stabilisation is achieved. Lithium has a fairly narrow therapeutic window. The accepted therapeutic range of serum lithium concentrations is 0.8 to 1.2 mmol/l (Schou, 1968). Toxic symptoms are seen at about 2 mmol/l and include slurring of speech and tremor. Brown (1973) considers serum levels of 0.4 to 0.8 mmol/l to be most effective, while Hullin

(1980) found that levels below 0.4 mmol/l resulted in relapses.

5.1.2.1.3 Side effects of lithium therapy

The early side effects of lithium therapy (within the first six weeks) include nausea, loose stools, fatigue, hand tremor, and both polydipsia and polyuria. Once stabilisation has been achieved, continuing disturbances due to lithium include weight gain, mild memory lapses and tremor (Bech et al, 1976).

One of the major side effects of chronic lithium administration is partial nephrogenic diabetes insipidus (Cox and Singer, 1981; Kosten and Forrest, 1986). This diuretic effect is the result of several actions on the kidney. Lithium blunts the kidney response to antidiuretic hormone, ADH (Forrest et al, 1973). It also inhibits reabsorption of sodium in the distal tubule, possibly due to direct competition for transport or due to an interaction with receptors for ADH or aldosterone (Birch, 1982). Christensen et al (1982) demonstrated that rats administered chronically with lithium over a period of up to 21 weeks had impaired renal concentrating ability, but that this could be reversed on discontinuation of the drug. Other work has suggested that the harmful influence of lithium on the kidney is overstressed: Hullin et al (1979) found only 6 out of 106 long term patients had polyuria; Lokkegaard et al (1985) found that lithium had no effect on plasma creatinine concentration in 153 patients maintained on lithium for at least five years, and that the glomerular filtration rate decreased significantly only after 5 years; Birch (1982) suggests that the polyuria of patients maintained on lithium is mainly due to polydipsia. Rudorfer et al (1985) administered a therapeutic dose of lithium for seven days to twelve young 'healthy' hospitalised men and found no difference in urine volume. Certain

reported side effects of lithium therapy may actually be unresolved symptoms of the depressive illness itself (Schulterbrandt et al, 1974).

Lithium is also known to inhibit thyroid hormone release resulting in the development of goitre.

5.1.2.1.4 Biochemical action of lithium

Lithium is normally present in the body only in trace amounts, and the biochemical role of lithium in the treatment of mania and depression has yet to be established.

A functional excess of dopamine in mania (Messiha et al, 1970) suggests that the primary role of lithium may be as a dopamine antagonist. Antipsychotics such as chlorpromazine have the unwanted antidopaminergic side effect of inducing parkinsonism in manic patients. However, Staunton et al (1982) have shown that lithium has no effect on rat brain dopamine receptors, and Silverstone (1985) found no effect of lithium on human synthesis of this neurotransmitter. Also, an antidopaminergic effect does not explain the antidepressant effect of lithium; the traditional monoamine hypothesis of affective disorder postulates a functional deficit of biogenic amines in depression.

As brain and CSF studies have implicated a central deficit of BH_4 in depression (Curtius et al, 1983b; Blair et al, 1984b), lithium may exert an effect in tetrahydrobiopterin metabolism.

5.1.2.2 Imipramine

5.1.2.2.1 Use of imipramine

Imipramine is the original drug in a class of compounds known as the tricyclic antidepressants: it was introduced by Kuhn in 1958 and has the structure shown in

figure 5.2. Imipramine is widely prescribed for acute depression (Glassman and Perel, 1973), with an improvement rate of 60 - 70% for new patients (Klerman and Cole, 1965). Imipramine is more useful in endogenous rather than neurotic depression. Demethylated and halogenated derivatives of imipramine (desipramine and chlorimipramine) have also been synthesised and shown to have antidepressant qualities. Imipramine has a fairly narrow therapeutic range; patients are usually started on 20mg daily, with the dosage increased until depressive symptoms remit: the maximum dose is normally 70mg per day.

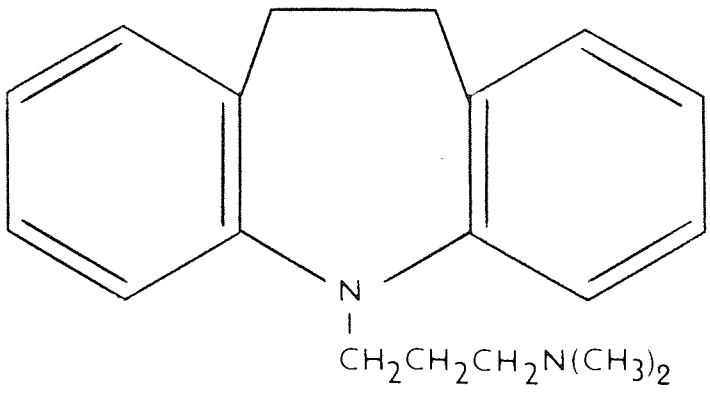
5.1.2.2.2 Side effects of imipramine

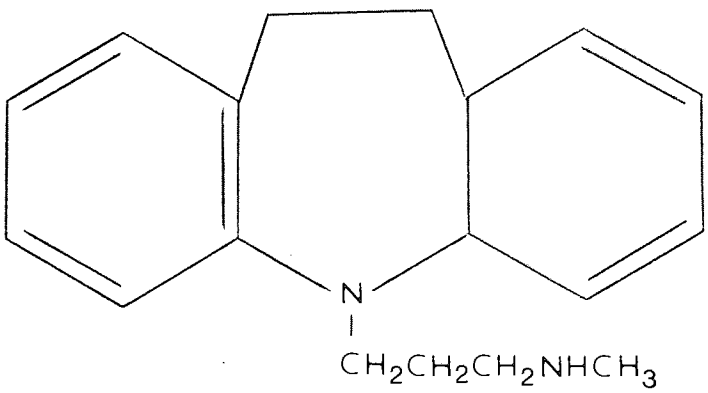
Imipramine and all tricyclic antidepressant drugs have side effects related to an anticholinergic action. These include blurred vision, memory impairment, dry mouth and constipation. Hypertension, palpitations, fine tremor and weight gain are also associated.

5.1.2.2.3 Biochemical action of imipramine

Imipramine does not inhibit the degradative enzymes MAO and COMT in the synaptic cleft (Klerman and Cole, 1965) and it was soon realised that the pharmacodynamic effect of imipramine and other tricyclics is monoamine uptake inhibition (Todrick and Tait, 1969). Imipramine inhibits presynaptic neuronal uptake of noradrenaline, thus increasing the concentration of neurotransmitter at the synaptic cleft (Langer, 1974). Derivatives of imipramine have been selected for more specific effects on uptake inhibition: desipramine is more specific for noradrenaline reuptake, and chlorimipramine for 5HT. As the best therapeutic response to tricyclics is achieved after two to three weeks administration (Oswald *et al*, 1972), a further role for imipramine

Imipramine (drug)





Desipramine (active metabolite)

Figure 5.2 Metabolism of imipramine

has been suggested by both pre- and postsynaptic receptor studies.

Langer (1978) suggests that chronic inhibition of neuronal uptake of noradrenaline by imipramine may lead to subsensitivity of presynaptic adrenoceptors. The time involved in the development of presynaptic subsensitivity could explain the lag period observed for the clinical aspects of tricyclic antidepressant drugs.

Another role for imipramine may be on postsynaptic receptors. Jones (1978) showed that tricyclics could enhance the postsynaptic noradrenaline-stimulated formation of cyclic adenosine monophosphate *in vitro*. A further study showed tricyclics to potentiate the stimulation *in vivo* (Jones and Roberts, 1978).

This thesis aims to assess the effect, if any, of imipramine, as a typical tricyclic antidepressant, on BH_4 metabolism *in vivo* in the rat.

5.1.2.3 Sodium valproate

5.1.2.3.1 Historical background

The sodium salt of valproic acid (sodium d-n-propylacetate) was originally synthesised by Burton in 1882 (figure 5.3). The first trials of its efficacy as a treatment for epilepsy were conducted by Carraz *et al* (1964).

5.1.2.3.2 Use of sodium valproate as an anticonvulsant

In general, phenytoin is the anticonvulsant drug of choice for epilepsy. However, valproate (Epilim in the UK) is useful in both grand and petit-mal forms of epilepsy, and controlled trials have reported valproate to have a very broad spectrum of anticonvulsant activity, probably greater than that of any other anti-

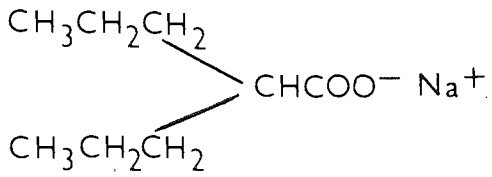


Figure 5.3 Structure of sodium valproate

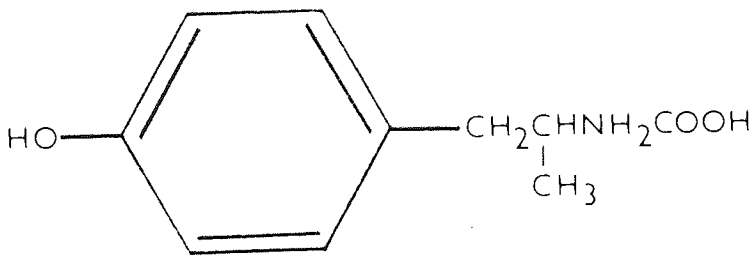


Figure 5.4 Structure of αmethylparatyrosine

epileptic in clinical use (review Chapman et al, 1982). The dosage of valproate as a monotherapy varies with the type of seizure (grand-mal, infantile spasms, photosensitive epilepsy) and the size of the patient (adult or child); the range is generally from 600mg to 3g per day.

5.1.2.3.4 Side effects of sodium valproate

As monotherapy, valproate produces little neurological toxicity. When valproate is given in conjunction with other convulsants such as hydantoin, pharmacokinetic competition for plasma protein binding can occur and side effects include ataxia, fine tremor and both drowsiness (Lance and Anthony, 1977) and irritability (Turnbull et al, 1982). Valproate has been reported as causing fatal hepatotoxicity due to inhibition of gluconeogenesis in the liver (Turnbull et al, 1986).

5.1.2.3.5 Biochemical action of sodium valproate as an anticonvulsant

The biochemical basis for the anticonvulsant actions of sodium valproate is thought to involve the gamma amino butyric acid (GABA) neurotransmitter system in the brain (Gram & Drachmann Bentsen, 1985). GABA is an inhibitory neutral amino acid in the brain, spinal cord and retina. Drugs that inhibit GABA synthesis or its receptors can induce seizures in animals. Valproate can inhibit GABA-transferase and succinic semialdehyde dehydrogenase, and thus enhance brain GABA concentration (Godin et al, 1969).

5.1.2.3.6 Use of sodium valproate in other disease states

Sodium valproate has been shown to have an effect on

other disease states which may have some implications in the pathology of depression.

Valproate shares many properties with benzodiazepines and has been shown to have antiaggressive and anxiolytic effects in animal studies (Simiand et al, 1983). A structurally related compound, valnoctamide (2-ethyl-3-methylpentanoicamide) is marketed as an anxiolytic in France (Davidou, 1966).

Emrich et al (1981) have suggested that valproate could be useful in the management of mania. They found up to 60% improvement in four out of five manic patients started on valproate therapy (900mg/day). One patient was non-responsive. They also gave valproate to seven outpatients that had not responded to lithium therapy for mania. They found that after 18 to 36 months administration, none of the patients had suffered a relapse: the predicted result was at least one relapse in the first year.

In an in vivo study in the rat, Crespi et al (1986) showed that valproate may reduce the release of dopamine and increase that of serotonin, having implications for the treatment of both mania and depression.

Sodium valproate also has an effect on the neuroendocrine system. Nelson's syndrome is characterised by massive hypersecretion of ACTH. Jones et al (1981) have shown that sodium valproate causes a fall in circulating ACTH concentration in this disease. More recently, Aggernaes et al (1988) showed that an intravenous injection of 800mg into normal subjects produced a significant suppression of serum cortisol, to less than 50% of basal levels. In depressed patients injected with valproate, only two out of seventeen had 50% suppression. Valproate, like dexamethasone, may therefore have a role in the diagnosis of the neuroendocrine abnormalities that are found in endogenous depression.

5.1.2.4 Folic acid

5.1.2.4.1 Historical background

Wills (1931) working in India described pernicious and other anaemias in poorly nourished women, and she showed that it could be cured by feeding with liver. Later, Mitchell et al (1941) extracted and characterised a compound from spinach ('foliage') which became known as folic acid. It was later proved that the factor in liver responsible for curing megaloblastic anaemia, and the folic acid isolated by Mitchell et al (1941) were identical, and there have been numerous studies since concerning the role of this compound in biochemistry.

5.1.2.4.2 Dietary requirements of folic acid

Folic acid is a vitamin. Man is completely dependent on dietary intake as his main source of folate, as gut flora are unable to prevent serum deficiency in man fed a low (5 μg/day) diet (Herbert, 1962). A normal mixed diet supplies between 200 to 400 μg folate daily. The World Health Organisation (1972) recommends the following minimum daily intakes: babies up to 6 months, 40 - 50 μg; between 7 months and one year, 120 μg; one to twelve years 200 μg; and 400 μg for those aged over 13 years. Pregnant women should receive at least 800 μg, reducing to 600 μg during lactation. A wide range of folate values has been quoted in various tables of food vitamin content. It is known that foods such as liver, beans and wholewheat flour are rich in folates, but other potentially excellent sources such as spinach and cabbage may lose up to 50% of their original folate content due to boiling and overcooking. Breast-fed babies receive adequate amounts in human milk, and most proprietary infant milk mixtures are supplemented with folate.

5.1.2.4.3 Roles of folate

It is beyond the scope of this thesis to detail the various reactions of folate in biochemistry in depth. The basic function of folate is as a coenzyme in the transfer of single atom carbon. The four most important pathways are in the synthesis of thymidine by addition of one carbon to deoxyuridine; synthesis of purine; formation of methionine from homocysteine; and conversion of histidine to glutamic acid.

5.1.2.4.4 Factors that lead to folate deficiency

Folic acid deficiency can be the result of one or a combination of factors.

Nutritional deficiency of folate is uncommon in Western societies and is generally confined to the lower socio-economic classes. The elderly are more prone to folate deficiency due to a lack of interest in food and an inadequate variety of diet.

Patients taking anticonvulsant drugs have reduced serum levels of folate (Flexner and Hartmann, 1960; Davis and Woodliff, 1971). The action of anti-convulsants on serum folates is not established: red cell folate is conserved.

In pregnancy, the increased metabolic demand for folate can lead to decreased serum folate values in the mother. As the foetus grows, there is a continual demand for folate for DNA synthesis in rapidly dividing cells. Mothers particularly at risk are those in developing countries with poor diets, and those taking antiepileptic drugs.

Alcoholics are often seen with symptoms of folate deficiency (megaloblastic anaemia) due to poor intake of folic acid and a metabolically impaired uptake of folate by the liver (Halsted, 1984).

Malabsorption due to a variety of causes can result in folate deficiency.

5.1.2.4.5 Haematological results of folate deficiency

Normal serum folate is 3 - 15ng/ μl and red cell folate 150 - 500ng/ μl packed cells (Chanarin et al, 1984). A patient with a serum level of less than 2.2 μg/l is described as folate deficient (Haematology department handbook, Queen Elizabeth Hospital, Birmingham UK). There is a well characterised series of haematological events that accompany folate deficiency. After the serum levels have fallen, many more neutrophils appear with five to six lobed nuclei. After the erythrocyte folate levels fall macrocytosis of these cells leads to the characteristic blood film appearance of megaloblastic anaemia.

5.1.2.4.5 Psychiatric illness associated with folate deficiency

Neuropathological signs have been associated with deficiencies of certain vitamins: beri-beri is caused by vitamin B1 (thiamine) deficiency, and severe B3 (niacin) depletion can result in agitated depression or schizophrenia.

Severe mental retardation is associated with low folate levels in the CSF (Haan et al, 1985) and there have been many reports of psychiatric illness associated with low serum folates (Manzoor and Runcie, 1976; Botez et al, 1978; Thornton and Thornton, 1978). In recent years, folate deficiency has come to be associated particularly with depression (review Abou-Saleh and Coppen, 1986) and dementia (Runcie, 1979; Abou-Saleh et al, 1986).

Carney (1967) found that 23% of 423 patients successively admitted to a general psychiatric hospital had folate deficiency: 30% of the depressed group of patients had serum folate values that put them into the deficient category. Reynolds et al (1970) examined 100 consecutive admissions for depression and found

folate deficiency in 24% of the cases. Shorvon et al (1980) found that 56% of 84 patients admitted with megaloblastic anaemia had affective disorder, while Coppen and Abou-Saleh (1982) in a study of 107 long-term lithium patients showed that those with the lowest plasma folate concentrations had the highest affective morbidity.

The importance of folate deficiency in dementia is less established. Spector et al (1977) claimed that idiopathic dementia was caused by regional vitamin deficiency states due to the failure of transport of water soluble vitamins, especially folates and ascorbate, into the brain. However Rawlinson and Eagles (1984) in a study of both MID and SDAT later claimed that dementia resulted in low vitamin B12 and folate values in the elderly, and not the other way around. Hullin (1984) in a study of 700 elderly patients, 290 of whom had SDAT, found the demented group to have significantly lower non-bound tryptophan and folic acid than non-demented controls, despite an adequate diet. Sneath et al (1973) showed that the mental assessment score of some demented patients positively correlated with their folate status, yet a more recent study by Abou-Saleh et al (1986) found that although folate deficiency was demonstrated in 35% of demented patients, there was no association between folate concentration and classification of dementia, degree of cognitive impairment or cortical dysfunction.

Folic acid has been proposed as a useful supplementation to usual antidepressant medication as the result of several recent reports (Botez et al, 1976; Reynolds et al, 1984; Coppen et al, 1986) and a study was performed here to examine an in vivo effect of a dose of folic acid on rat tetrahydrobiopterin metabolism.

5.1.2.5 α methylparatyrosine

5.1.2.5.1 Action of α methylparatyrosine

α methylparatyrosine, αMPT (figure 5.4) has no therapeutic actions. It is used experimentally as a specific inhibitor of both dopamine and noradrenaline synthesis both centrally and peripherally. It does this by a competitive inhibition of tyrosine hydroxylase (Spector et al, 1966).

5.1.2.5.2 Studies using αMPT

Bunney et al (1971) examined the antidopaminergic effect of αMPT as a treatment for mania. Eleven patients were diagnosed as manic (n = 7) or depressed (n = 4) and were given 3g of αMPT daily for 15 days. Urinary dopamine and the two metabolites of noradrenaline (VMA and MHPG) were decreased. A 50% reduction in CSF HVA concentration was shown, but CSF 5HIAA levels were unaffected. Clinically, five out of seven manic patients were considered improved by αMPT, and two out of seven became worse. Three out of the four depressed patients were markedly worse with αMPT, and two of these improved when αMPT was replaced by placebo. One depressed patient registered no change.

Bunney et al (1972) made a series of detailed behavioural and biochemical measurements on the mood swings in a group of patients with affective disorder and described a 'switch' process in the changing course of the illness. They found that the urinary excretion of noradrenaline increased as the patients switched from a depressed mood to mania, and that various pharmacological agents could provoke switching. αMPT was shown to switch patients out of a manic phase, which they related to its anticatecholaminergic action.

Shopsin et al (1975) treated five affective disorder patients (3 female unipolars and 2 male bi-

polars) with imipramine until a clinical response was achieved. They then added either α MPT or p-chlorophenylalanine (a specific inhibitor of 5HT synthesis) to the therapy. Three patients were given between 3.5 and 4g of α MPT, and two patients were given between 750 - 2500mg p-chlorophenylalanine. A return of depressive symptoms was shown in the p-chlorophenylalanine group within 72 hours, but no relapse in the α MPT group. Although the sample size was small, the authors concluded that serotoninergic mechanisms were more important in the biology of depression, and that α MPT was not effective at provoking depression.

In a study in monkeys, Kraemer and McKinney (1979) found that α MPT reduced motor activity and induced symptoms akin to depression in humans.

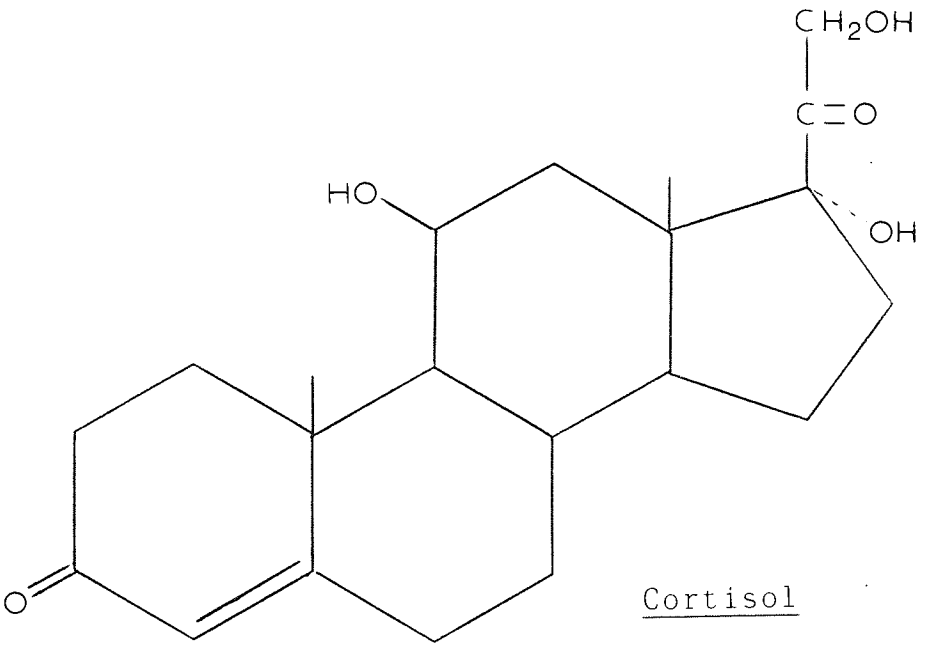
In this thesis, rats were dosed with α MPT to examine any possible secondary effects that this agent might induce in BH_4 metabolism, as well as the established effect on tyrosine hydroxylase activity.

5.1.2.6 Neuroendocrine studies on tetrahydrobiopterin metabolism in the rat

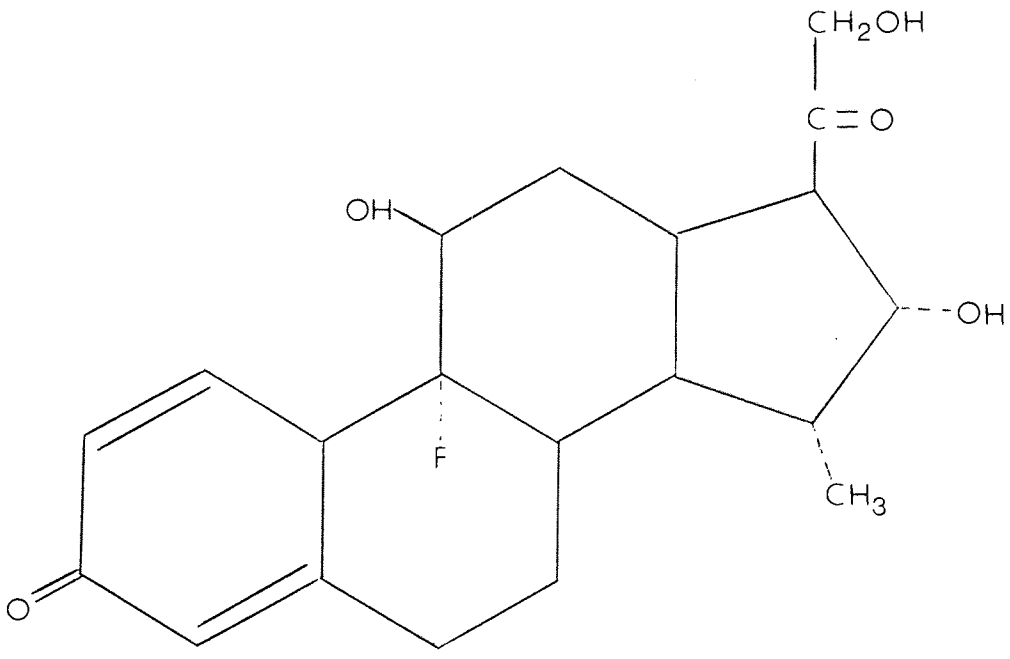
As discussed in chapter one, specific changes in the neuroendocrine system are associated with depression. Studies here aim to examine possible influences of the synthetic steroid dexamethasone and the induction of stress by physical restraint on BH_4 metabolism in the rat.

5.1.2.6.1 Dexamethasone

Dexamethasone is a synthetic structural analogue of the natural corticosteroid hormone cortisol (figure 5.5). Dexamethasone is used in a diagnostic test for Cushing's syndrome. In a standardised procedure (Carroll et al, 1981), baseline plasma and urinary



Cortisol



Dexamethasone

Figure 5.5 Structure of cortisol and dexamethasone

cortisol values are obtained prior to giving a 1mg oral dose of dexamethasone. The upper limit of baseline values prior to the test are plasma cortisol 20 μg/dl at 8am, 15 μg/dl at 4pm and 6 μg/dl at 12 midnight, with a urinary excretion of 100 μg cortisol per 24 hours. 24 hours after dexamethasone, plasma values of cortisol should be less than 6 μg/dl at anytime of the day, and the urinary output of free cortisol should be reduced to a maximum of 25 μg in 24 hours. Values higher than this indicate an abnormal response or early escape from suppression. An abnormal response may be due to various causes such as an adrenal tumour or ectopic ACTH secretion (Cushing's syndrome); severe alcohol abuse; obesity (especially when accompanied by hirsutism and hypertension); and stress (Zilva and Pannall, 1983). Stress can result from physical or psychiatric disease and as discussed in previous chapters, abnormal cortisol secretion is particularly associated with depressive illness (Carroll et al, 1976; Carroll et al, 1981; Cohen et al, 1984).

It is not clear how cortisol interacts with neurotransmitter function in the neuroendocrinological pathology of depression, but one report by Langlais et al (1984) found dexamethasone to have a direct effect in causing elevation of dopamine levels in both human plasma and rat brain.

A study was performed here to examine the effect of an oral dose of dexamethasone on in vivo aspects of tetrahydrobiopterin metabolism in the rat.

5.1.2.6.2 Stress by immobilisation

Placing laboratory animals in situations where they cannot escape the distressing conditions imposed upon them induces behaviour patterns that are recognised as mimicking human depression.

Weiss et al (1982) have developed an 'inescapable shock' model of depression in the rat. The signs and behaviour induced in their rat models correspond

to the DSM-III criteria for depression, including decreased food consumption, weight loss, poor performance in stress-coping tasks, insomnia and decreased grooming. There is diminished aggressiveness and competetiveness.

As cortisol excretion is raised in both stress and depressive illness, a study was performed here where rats were placed in a stressful situation (immobilisation), and possible changes in BH_4 metabolism were examined.

5.1.2.7 Scopolamine

5.1.2.7.1 Historical background

Scopolamine, also known as hyoscine, is the active poisoning agent in henbane, Hyoscyamus niger (figure 5.6). Poisoning with henbane leads to drowsiness and sedation; a highly toxic dose results in delerium and coma. Therapeutically, it was the earliest effective treatment for Parkinson's disease, and is still used in conjunction with L-dopa therapy, where it helps control tremor and rigidity.

5.1.2.7.2 Uses of scopolamine

Scopolamine blocks the effect of acetylcholine at both central and peripheral muscarinic receptors. Some of the effects of scopolamine include increasing heart rate and decreasing peristalsis of smooth heart muscle, salivary, bronchial and sweat gland secretion: these attributes make it useful as a preanaesthetic medicant. Other less desirable effects of scopolamine include blurred vision, constipation and memory impairment (Safer and Allen, 1971). The anticholinergic effect of scopolamine has been shown to produce a pattern of cognitive deficits in healthy young adults which resembles that seen in 'normal ageing', and, in exagg-

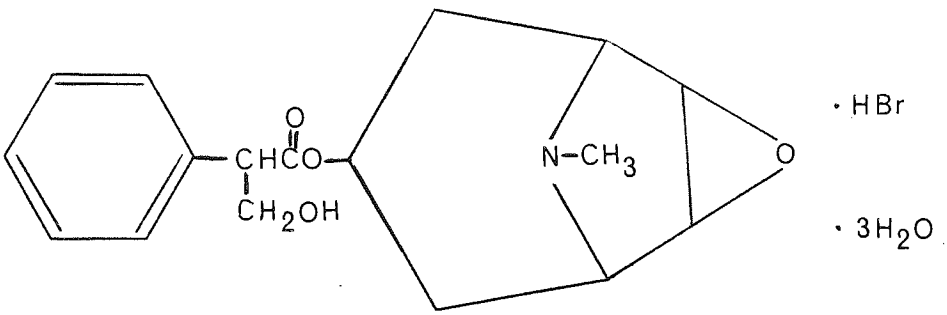


Figure 5.6 Structure of scopolamine hydrobromide

erated form, in dementia. Storage of new information in long term memory is more severely impaired than retrieval of recent or more remote events (Drachmann and Leavitt, 1974; Peterson, 1977).

Scopolamine-induced memory deficits may be reversed by enhancing the central cholinergic system. This has been shown by giving the anticholinesterase agent physostigmine, which provided clearing of scopolamine effects both in monkey (Bartus, 1978) and man (Drachmann, 1977).

In this thesis, an oral dose of scopolamine was given to the rat as a model system for dementia, and rat tissues were examined for any possible changes in tetrahydrobiopterin metabolism that that been induced.

5.2 EXPERIMENTAL SECTION

5.2.1 General procedures for animals

All rats to be dosed in acute experiments were starved overnight on grids to prevent coprophagia. All dosing was by oral administration using bulbed needles. In chronic experiments, rats were allowed access to food and water ad libitum. In general, the dose of drug or other agent to be given was calculated by the following formula:

$$\text{Human adult dose} \times \frac{1}{70} \times \text{rat weight in kg} = \text{g dose}$$

The drugs or other agents were dissolved or suspended in either water or corn oil. Control rats were dosed with an equivalent volume of the solvent or carrier alone.

Sacrifice was by cervical dislocation and thoracic opening following a light ether anaesthesia.

5.2.2 Total biopterin and DHPR in left and right side of rat brain

It was proposed to use only half brains in some studies so that both total biopterin and DHPR could be measured in one animal. A preliminary investigation was made to examine the distribution of total biopterin and DHPR in the brain of the rat.

Twelve rats were starved overnight on grids and sacrificed. Whole brains were dissected out onto a chilled clock glass and divided along the longitudinal fissure, also dividing the cerebellum (figure 5.1). Each half-brain from the first six rats killed was assayed for total biopterin, and the other six rat brains were compared for DHPR activity in left and right sides.

5.2.3 Proposed assay for 5-methyltetrahydrofolate by HPLC

It was proposed to perform a chemical conversion of 5-methyltetrahydrofolate to a fluorescent derivative that could be detected by the same HPLC procedure as pteridines. At room temperature, tetrahydrofolate is converted 100% to pterin (personal observation) in the presence of an acidic iodine/iodide mixture based on the original work of Fukushima and Nixon (1980). More drastic conditions (saturated iodine/iodide solutions and a boiling water bath) were used on a range of 5-methyltetrahydrofolate concentrations in an attempt to achieve maximum conversion of the reduced, non fluorescent folate to highly fluorescent pterin which could then be quantified using HPLC.

5.2.4 Studies on lithium

5.2.4.1 Uptake of lithium

Twelve rats weighing 150g were used. Six rats were

dosed with 6.4mg lithium carbonate in 0.3mls water. This dose was calculated from a maximal adult dose of lithium carbonate of 3.5g per day (Martindale's Pharmacopoeaia 28th Edition, 1982). The six controls were dosed with 0.3mls water. All animals were sacrificed four hours later. After thoracic opening, blood was immediately collected from the heart into sodium heparinised tubes. The tubes were spun, the packed cells discarded and the plasma frozen at -70°C until assayed. The brain and liver were removed and similarly frozen. Small samples of tissue or plasma were subject to an acid digest by Mr. R. Davie of Wolverhampton Polytechnic according to the method of Birch and Jenner (1973), and subsequently analysed by atomic absorption spectrometry (section 2.2.4).

5.2.4.2 Acute in vivo effect of lithium on biopterin

102 rats weighing 150g were used in this series of experiments. Three lithium carbonate solutions were made up to give final concentrations of 6.4, 32 and 64mg in 0.3mls (table 5.5). Over several days, rats were dosed with either one of the solutions or water, and sacrificed after four hours.

5.2.4.3 Acute in vivo effect of lithium on DHPR

16 rats weighing 165g were used in this series of experiments. Three lithium carbonate solutions were made up to give final concentrations of 7.04, 35.2 and 70.4mg in 0.3mls water. The rats were divided into four groups of four each, and assigned as control or one of three test groups to receive lithium (table 5.9).

5.2.4.3 Chronic in vivo effect of lithium on biopterin and DHPR

18 rats weighing 150g were orally dosed on day one with 6.4mg lithium carbonate dissolved in 0.3mls water, forming the test group. They were housed six to a cage and allowed access to food and water ad libitum throughout the study. 18 control rats were dosed with 0.3mls water on the same day and also housed six to a cage. The test animals were dosed each day with the lithium solution. The animals were individually weighed every three days, and the dose adjusted as the animals grew. Controls were dosed with water each day. After seven days of dosing, six rats from the test group and six control rats were sacrificed and the brain and liver removed and frozen at -70°C until assay. 12 rats were similarly selected and sacrificed after a further seven days, and the final animals after a total of twenty one days lithium or water dosing. Total biopterin and DHPR activity were measured in both brain and liver.

5.2.4.4 In vitro effect of lithium on DHPR activity

Supernatants suitable for DHPR assay were prepared from the brains of six rats weighing 165g. Lithium carbonate solutions were prepared to give final concentrations in the assay ranging from 3.5 to 70 mol/l (table 5.14). The assay was adapted so that the lithium solutions replaced the water in the assay.

5.2.4.5 In vitro effect of lithium on tetrahydrobiopterin biosynthesis

Supernatants suitable for in vitro tetrahydrobiopterin biosynthesis assay were prepared from the brains of six rats weighing 150g. The assay is dependent on the addition of 3mM magnesium (magnesium chloride). An experiment was performed to replace or supplement the exogenous magnesium with lithium. A lithium solution was prepared to give a final concentration

of 3mM in the assay. The assay was performed with four variations: with magnesium alone; without magnesium; with both magnesium and lithium; and with lithium alone.

5.2.5 Imipramine

5.2.5.1 Acute effect of imipramine on tetrahydrobiopterin and DHPR

Six rats weighing 165g were given 0.71mg imipramine dissolved in 0.1ml water. Six controls were given 0.1ml water. All animals were sacrificed after four hours. Total biopterin and DHPR activity were assayed in brain and liver.

5.2.5.2 Chronic effect of imipramine on tetrahydrobiopterin and DHPR

36 rats weighing 165g were used in this study. 18 rats were initially dosed with 0.71mg imipramine dissolved in 0.1ml water, controls being dosed with water alone. The rats were dosed each day for up to three weeks, the dosages adjusted as the animals increased in weight. Six animals from the test and control groups were sacrificed every seven days. The brains of all animals were assayed for total biopterin and DHPR activity.

5.2.6 Sodium valproate

5.2.6.1 Acute administration of sodium valproate

Six rats weighing 150g were dosed with 3.7mg sodium valproate dissolved in 0.4mls water. Controls were dosed with water. Sacrifice was after four hours. Total biopterin was assayed in brain and liver.

5.2.6.2 Acute administration of a high dose of sodium valproate

925mg of sodium valproate were suspended in 10mls of water. This solution contained 37mg in 0.4mls. Six rats weighing 150g were given 0.4mls of this solution, control rats were given water. All animals were sacrificed after four hours. Samples of rat brain and liver were assayed for total biopterin and DHPR activity.

5.2.7 Folic acid

0.8mg folic acid were made up to one litre with water. Four rats weighing 140g were dosed with 0.5mls of this solution, equivalent to a dose of 0.4 μg each. Four controls were dosed with 0.4mls water. Sacrifice was after four hours. Brain and liver total biopterin and DHPR were measured.

5.2.8 α methylparatyrosine

Six rats weighing 160g were orally dosed with 9.0mg αMPT in 0.5mls water, controls being dosed with water alone. Sacrifice was after four hours. Brain and liver total biopterin and DHPR activity were measured.

5.2.9 Dexamethasone

5.2.9.1 Acute administration of dexamethasone

A suspension of 5.36mg dexamethasone in 500mls corn oil was prepared. Six rats weighing 150g were orally dosed with 0.2mls of this solution, equivalent to 2.14 μg dexamethasone each. Controls were given 0.2mls corn oil. Dosed and control rats were housed separately and allowed free access to food and water until sacrifice 24 hours later. Total biopterin and DHPR activity were measured in brain and liver.

5.2.9.2 Acute administration of a high dose of dexamethasone

26.8mg dexamethasone were suspended in 250mls corn oil. Each of six rats were dosed with 0.2mls of this suspension. Controls were dosed with 0.2mls corn oil. After dosing, all animals were allowed free access to food and water. Sacrifice was 24 hours later. Total biopterin was measured in brain and liver.

5.2.10 Stress by immobilisation

5.2.10.1 Short duration of restraint

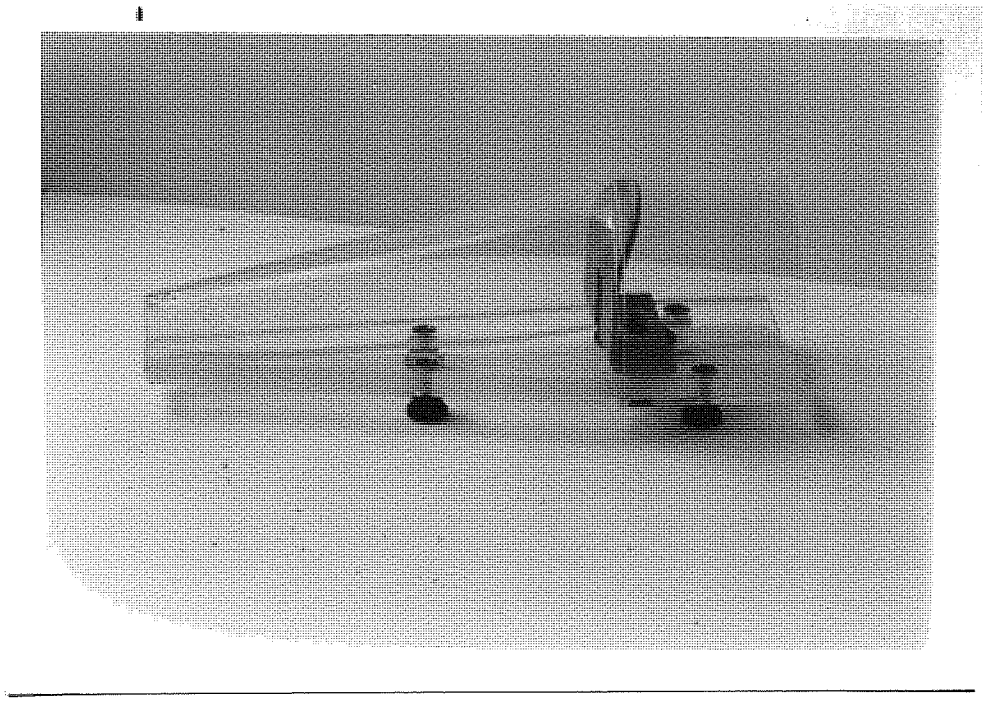
Six rats weighing 165g were subject to physical restraint for two and a half hours. The restraining devices, perspex and metal constraints, were made at Aston University for this purpose (figure 5.7). Rats were fed as normal until the restraint began, but had no access to food or water during the course of the experiment. Six controls were allowed normal freedom of movement within a cage, but no food or water. All animals were sacrificed after 2½ hours. Brains were assayed for total biopterin and DHPR.

5.2.10.2 Prolonged duration of restraint

Six rats were restrained as before for five hours, and six control rats were allowed freedom of movement within a cage. Neither group was allowed access to food or water. Brain and liver samples were assayed for total biopterin and DHPR.

5.2.11 Scopolamine

Six rats weighing 125g were given 22.7 μg scopolamine in 0.2mls water. This was equivalent to a 2mg dose for a 70kg adult. Controls were orally dosed with



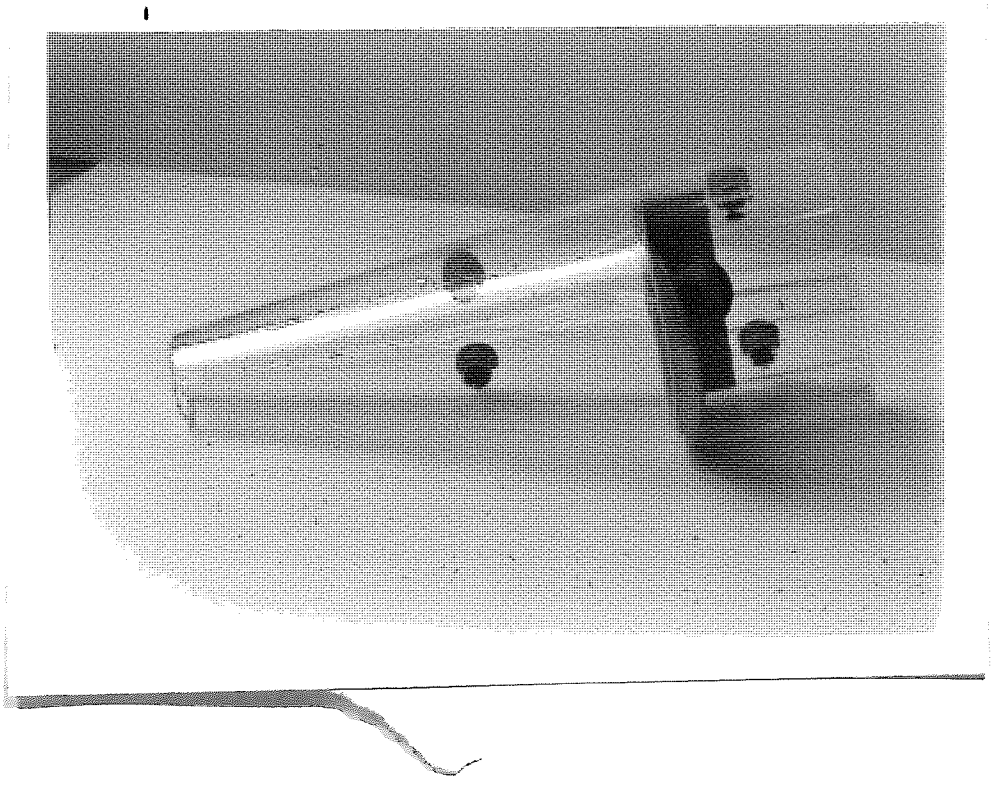


Figure 5.7 Restraining devices for rats

0.2mls water. Sacrifice was after six hours. Blood was drawn from the heart immediately after thoracic opening into heparinised tubes and spun. DHPR was assayed in brain and liver. Measurements of total biopterin in plasma, brain and liver, total pterin in brain and liver, and brain choline acetyltransferase activity were made by Dr. Paul Edwards of Aston University.

5.3 RESULTS

5.3.1 Total biopterin and DHPR activity in left and right side of rat brain

Table 5.1 shows that there was no significant difference (by paired t test analysis) between the two halves of rat brain in estimation of total biopterin. A difference was shown, however, in DHPR estimation (table 5.2). DHPR was significantly higher in right hand side of rat brain compared to left ($p < 0.02$); this difference was not due to a difference in the protein baseline of the samples.

5.3.2 Assay of 5-methyltetrahydrofolate by HPLC

The acid/iodine/iodide oxidations at 100°C gave inconsistent results (not shown). The conversion was attempted on at least twenty occasions using a range of 5-methyltetrahydrofolate concentrations. The molar conversion to pterin varied between a minimum of 12% and a maximum of 50%. Thin layer chromatography was performed as described in chapter two (2.2.1). Samples of the 5-methyltetrahydrofolate solutions before and after oxidation were compared to various pteridine standards. A fluorescent spot was detected in the oxidised sample that was identical to a standard sample of pterin. However, the bulk of the oxidised compound was seen (under UV light) to be near the origin and

Table 5.1 Total biopterin in left and right half-brain of the rat

Rat	Biopterin, pmol/g wet tissue	
	Left side	Right side
1	275.5	120.0
2	192.9	142.2
3	129.3	161.6
4	104.3	119.4
5	158.8	100.3
6	361.8	129.4

Table 5.2 DHPR and protein in left and right half-brain of the rat

Rat	DHPR, nmol NADH/min/mg protein		Protein, mg/ml	
	Left	Right*	Left	Right
7	206.6	239.8	7.1	5.7
8	214.3	214.4	7.5	7.5
9	198.6	139.9	8.5	7.9
10	171.9	190.4	7.6	7.6
11	137.2	160.0	6.3	6.3
12	244.5	212.6	6.0	6.0

Statistics: * Right side higher than left (by paired t test) $p < 0.02$

correspond to unconverted 5-methyltetrahydrofolate.

5.3.3 Lithium studies

5.3.3.1 Lithium uptake

Table 5.4 shows the distribution of lithium in rat plasma, brain and liver after administration of an oral dose of lithium carbonate for four hours. The highest concentration is found in the plasma, then brain and liver. Tissues of the dosed rats showed a highly significant increase in lithium ($p < 0.0001$) compared to undosed controls.

5.3.3.2 Acute dosing with lithium

Four hours after an acute dose of one of a range of lithium solutions administered there were no significant differences in brain total biopterin (tables 5.6, 5.7 and 5.8). Lithium at three dosages had no effect on *in vivo* DHPR activity after four hours administration (table 5.9).

5.3.3.3 Chronic dosing with lithium

No differences in brain total biopterin were shown between rats dosed for 7, 14 and 21 days, and controls dosed with water for an equivalent period (table 5.10). For the same rats, no differences were shown between the mean DHPR activity and protein baseline (table 5.11) or total biopterin in liver (table 5.12) or liver DHPR activity and protein baseline (table 5.13).

5.3.3.4 Lithium effects on in vitro assays

At a range of concentrations from 3.5 to 70 μmol/l, there was no effect of incorporation of lithium into *in vitro* assays of DHPR activity compared to controls (table 5.14).

Table 5.3 Lithium dosage in 4-hour uptake experiment

Rat group	n	Lithium carbonate (mg)	Water (ml)
Test	6	6.4	0.3
Control	6	-	0.3

Table 5.4 Distribution of lithium in the rat after 4 hours

Group	n	Lithium concentration Mean (SD)		
		Plasma μmol/l	Brain μmol/g	Liver μmol/g
Test	6	163.3* (16.4)	45.0* (4.7)	39.0* (2.0)
Control	6	3.7 (7.1)	0.0 (0.0)	0.0 (0.0)

Statistics: * Higher than control rats, $p < 0.0001$

Table 5.5 Range of lithium dosages for four hour acute dosing experiment

Group	Lithium carbonate (mg)	Water (ml)
Test group 1	6.4	0.3
Control group 1	-	0.3
Test group 2	32.0	0.3
Control group 2	-	0.3
Test group 3	64.0	0.3
Control group 3	-	0.3

Table 5.6 Brain total biopterin after a four hour dose of 6.4mg lithium carbonate

Group	n	Biopterin pmol/g wet tissue Mean (SD)
Test	23	392.3 (112.3)
Control	24	341.9 (60.3)

Table 5.7 Brain total biopterin after a four hour dose of 32mg lithium carbonate

Group	n	Biopterin pmol/g wet tissue Mean (SD)
Test	3	341.3 (58.4)
Control	3	306.6 (36.3)

Table 5.8 Brain total biopterin after a four hour dose with 64mg lithium carbonate

Group	n	Biopterin pmol/g wet tissue Mean (SD)
Test	24	351.3 (109.9)
Control	24	326.1 (83.0)

Table 5.9 Effect of three dosages of lithium carbonate on rat brain DHPR activity after four hours

Group	n	Lithium carbonate (mg)	DHPR, nmol NADH/ min/mg protein Mean (SD)
Control	4	0	126.0 (37.3)
Test group 1	4	7.04	137.9 (20.6)
Test group 2	4	35.2	110.5 (27.3)
Test group 3	4	70.4	142.8 (32.0)

Table 5.10 Rat brain total biopterin after chronic dosing with lithium carbonate

Duration of dosing	Biopterin pmol/g wet tissue Mean (SD)	
	Dosed (n = 6)	Control (n = 6)
7 Days	632.6 (84.8)	572.5 (128.9)
14 Days	546.9 (73.5)	521.3 (56.3)
21 Days	399.5 (31.1)	366.0 (34.4)

Table 5.11 Rat brain DHPR after chronic dosing with lithium carbonate

Duration of dosing	DHPR nmol NADH/min /mg protein Mean (SD)		Protein mg/ml Mean (SD)	
	Dosed (n=6)	Control (n=6)	Dosed (n=6)	Control (n=6)
7 Days	221.8 (56.3)	221.6 (45.2)	6.8 (0.9)	6.6 (0.9)
14 Days	198.4 (38.0)	183.3 (36.8)	6.8 (1.5)	6.8 (0.6)
21 Days	200.1 (25.9)	159.7 (41.0)	6.6 (0.8)	6.9 (0.7)

Table 5.12 Rat liver total biopterin after chronic dosing with lithium carbonate

Duration of dosing	Biopterin pmol/g wet tissue Mean (SD)	
	Dosed (n = 6)	Control (n = 6)
7 Days	2768.4 (425.7)	2522.5 (338.4)
14 Days	3163.4 (269.5)	3057.2 (267.8)
21 Days	2733.7 (188.4)	2799.3 (342.0)

Table 5.13 Rat liver DHPR and protein after chronic dosing with lithium carbonate

Duration of dosing	DHPR nmol NADH/min /mg protein Mean (SD)		Protein mg/ml Mean (SD)	
	Dosed (n=6)	Control (n=6)	Dosed (n=6)	Control (n=6)
7 Days	124.5 (14.8)	154.3 (39.6)	62.0 (13.0)	65.5 (7.8)
14 Days	266.2 (57.2)	251.5 (63.2)	78.6 (17.6)	68.0 (11.5)
21 Days	287.4 (75.4)	289.6 (54.7)	72.8 (10.0)	67.0 (10.7)

Table 5.14 Measurement of the in vitro effect of lithium on DHPR activity

Li^+ (aqueous solution) μ mol/l	DHPR activity nmol NADH/min/mg protein Mean (SD) n = 6
0	119.0 (25.5)
3.5	104.1 (13.3)
7.0	99.3 (19.0)
35.0	102.6 (24.4)
70.0	102.7 (27.9)

For the *in vitro* assay of BH_4 biosynthesis, there was no net effect of substituting or supplementing the assay magnesium with equimolar lithium (table 5.15).

5.3.4 Imipramine

5.3.4.1 Acute dosing

Administration of an acute dose of imipramine for four hours had no significant effects on total biopterin in rat brain and liver, and no effect on DHPR activity on the protein baseline of the samples (5.16).

5.3.4.2 Chronic dosing

Compared to water dosed controls, rats that had been dosed for 7, 14 or 21 days showed no significant differences in total brain biopterin (table 5.17) or brain DHPR activity and protein baseline (table 5.18).

5.3.5 Sodium valproate

Sodium valproate, at a concentration scaled down from a pharmacological dose for humans, had no effect on total biopterin in rat brain and liver after four hours (table 5.19). A ten-fold multiplication of the original dose had no significant effect on rat brain or liver total biopterin compared to water dosed controls (table 20); no effect on rat brain DHPR and protein (table 5.21); and no effect on rat liver DHPR and protein (table 5.22).

5.3.6 Folic acid

An acute dose of folic acid had no significant effects on total biopterin, DHPR or protein baseline in rat brain (table 5.23) or liver (table 5.24).

5.3.7 α methylparatyrosine

Table 5.15 Effect of substituting or supplementing magnesium with lithium in an in vitro assay for BH_4 biosynthesis in rat brain

Tissue	Biopterin pmol/hour/mg protein Mean (SD)			
	Magnesium present	Magnesium absent	Magnesium + lithium	Lithium only
Brain	0.35 (0.21) (n = 6)	0.49 (0.61) (n = 5)	0.41 (0.27) (n = 5)	0.29 (0.35) (n = 4)
Liver	0.45 (0.21) (n = 6)	0.57 (0.36) (n = 5)	0.93 (1.14) (n = 6)	0.69 (0.24) (n = 5)

Table 5.16 Effect of acute administration of imipramine on rat brain and liver total biopterin, DHPR and protein

Group	Tissue	Biopterin pmol/g wet tissue Mean (SD)	DHPR nmol NADH/min/ mg protein Mean (SD)	Protein mg/ml Mean (SD)
Control (n = 6)	Brain	258.5 (38.7)	163.6 (9.9)	7.7 (1.3)
	Liver	5665.0 (365.1)	86.4 (11.4)	152.0 (20.1)
Test (n = 6)	Brain	288.5 (22.0)	179.9 (31.1)	8.8 (1.0)
	Liver	5291.0 (525.5)	84.5 (4.9)	142.8 (17.5)

Table 5.17 Effect of chronic dosing with imipramine on rat brain total biopterin

Duration of dosing	Biopterin pmol/g wet tissue	
	Test (n=6) Mean (SD)	Control (n=6) Mean (SD)
7 days	263.0 (41.2)	212.4 (38.7)
14 days	220.1 (44.0)	197.3 (31.1)
21 days	262.4 (47.5)	271.6 (41.9)

Table 5.18 Effect of chronic dosing with imipramine on rat brain DHPR

Duration of dosing	DHPR nmol NADH/min/mg protein	
	Test (n=6) Mean (SD)	Control (n=6) Mean (SD)
7 days	141.6 (24.1)	161.4 (12.3)
14 days	150.0 (23.7)	178.6 (26.3)
21 days	139.7 (21.9)	151.4 (18.0)

Table 5.19 Effect of acute administration of 3.7mg sodium valproate for four hours on rat brain and liver total biopterin

Group	Biopterin pmol/g wet tissue	
	Brain Mean (SD)	Liver Mean (SD)
Control (n = 6)	338.9 (40.5)	1446.3 (231.0)
Test (n = 6)	367.9 (26.8)	1443.5 (215.9)

Table 5. 20 Effect of acute administration of 37mg sodium valproate for four hours on rat brain and liver total biopterin

Group	Biopterin pmol/g wet tissue	
	Brain Mean (SD)	Liver Mean (SD)
Control (n = 6)	190.0 (55.9)	1864.5 (471.7)
Test (n = 6)	201.8 (30.7)	1493.9 (329.1)

Table 5.21 Effect of acute administration of 37mg sodium valproate for four hours on rat brain DHPR and protein

Group	DHPR nmol NADH/min/ mg protein Mean (SD)	Protein mg/ml Mean (SD)
Controls (n = 6)	137.2 (20.3)	5.8 (1.5)
Test (n = 6)	137.7 (28.3)	6.3 (1.3)

Table 5.22 Effect of acute administration of 37mg sodium valproate for four hours on rat liver DHPR and protein

Group	DHPR nmol NADH/min/ mg protein Mean (SD)	Protein mg/ml Mean (SD)
Controls (n = 6)	322.0 (93.5)	46.0 (11.9)
Test (n = 6)	376.8 (46.8)	42.6 (5.7)

Table 5.23 Effect of an acute dose of folic acid on rat brain biopterin and DHPR

Rat group	Biopterin pmol/g wet tissue Mean (SD)	DHPR nmol NADH /min/mg protein Mean (SD)	Protein mg/ml Mean (SD)
Test (n = 4)	374.4 (65.8)	180.7 (65.0)	9.1 (1.0)
Control (n = 4)	404.3 (44.6)	143.5 (9.9)	7.7 (1.1)

Table 5.24 Effect of an acute dose of folic acid on rat liver biopterin and DHPR

Rat group	Biopterin pmol/g wet tissue Mean (SD)	DHPR nmol NADH /min/mg protein Mean (SD)	Protein mg/ml Mean (SD)
Test (n = 4)	2305.0 (538.5)	158.8 (23.0)	91.1 (31.6)
Control (n = 4)	2375.0 (457.3)	172.0 (19.7)	76.0 (10.1)

α MPT decreased both brain and liver total biopterin in the rat, but the difference did not reach significance (table 5.25). Similarly, both brain and liver DHPR activities were depressed (table 5.26) but not significantly. The protein baselines were very similar (table 5.26).

5.3.8 Dexamethasone

At a dose equivalent to that given to humans in a standardised dexamethasone suppression test, dexamethasone had no effect on brain total biopterin after twenty four hours. Liver total biopterin did not differ between test and control animals (table 5.27). However, a five-fold dose of dexamethasone caused a significant increase ($p < 0.025$) in rat liver, although not brain, total biopterin (table 5.27). The lower dose of dexamethasone caused no changes in brain or liver DHPR activity or protein baseline (table 5.28).

5.3.9 Stress by immobilisation

Two and a half hours restraint resulted in a significant increase in brain DHPR activity, although total brain biopterin was unaffected (table 5.29). When the experiment was repeated for five hours, DHPR activity was shown to be raised in both the brain and liver of stressed rats but the differences did not reach significance (table 5.30). Total biopterin was similar in both organs of stressed and control rats.

5.3.10 Scopolamine

Total biopterin did not differ significantly between test and control animals in plasma, brain, or liver. Total pterin was significantly lower in scopolamine dosed rats ($p < 0.01$) than control rats (table 5.31). No significant differences were found between dosed and control rat brain or liver DHPR or protein (table

Table 5.25 Effect of an acute dose of αmethylparatyrosine on rat brain and liver total biopterin

Group	Biopterin pmol/g wet tissue	
	Brain Mean (SD)	Liver Mean (SD)
Control (n = 6)	323.1 (50.4)	2983.5 (175.5)
Test (n = 6)	302.4 (17.7)	2743.0 (145.5)

Table 5.26 Effect of an acute dose of αmethylparatyrosine on rat brain and liver DHPR and protein

Group	DHPR nmol NADH/min/mg protein		Protein mg/ml	
	Brain Mean (SD)	Liver Mean (SD)	Brain Mean (SD)	Liver Mean (SD)
Control (n = 6)	285.7 (39.0)	298.4 (175.5)	6.5 (1.0)	67.3 (40.0)
Test (n = 6)	266.4 (32.0)	274.3 (145.5)	6.5 (0.8)	61.9 (50.2)

Table 5.27 Total biopterin in rat brain and liver at low and high doses of dexamethasone

Dose	Group (n=6 each)	Biopterin pmol/g wet tissue	
		Brain Mean (SD)	Liver Mean (SD)
Low	Control	73.0 (3.5)	1019.8 (184.3)
	Test	76.3 (8.4)	968.0 (156.0)
High	Control	49.4 (7.1)	797.1 (96.3)
	Test	54.1 (7.1)	914.8* (44.4)

Statistics: * Higher than controls $p < 0.025$

Table 5.28 DHPR and protein values for rat brain and liver at low dose dexamethasone

Group (n=6 each)	DHPR nmol NADH/min /mg protein		Protein mg/ml	
	Brain Mean (SD)	Liver Mean (SD)	Brain Mean (SD)	Liver Mean (SD)
Test	134.9 (50.2)	203.7 (36.3)	5.9 (2.0)	55.5 (9.7)
Control	140.4 (30.9)	216.7 (30.7)	5.3 (1.6)	43.0 (12.5)

Table 5.29 Effect of 2.5 hours immobilisation on rat brain total biopterin and DHPR

Group	n	Biopterin pmol/g wet tissue Mean (SD)	DHPR nmol NADH/ min/mg protein Mean (SD)
Control	6	517.6 (91.5)	101.8 (17.7)
Stressed	6	488.0 (39.9)	125.9* (10.8)

Statistics: * Higher than controls $p < 0.02$

Table 5.30 Effect of 5 hours immobilisation on rat brain and liver total biopterin and DHPR

Group	Tissue	Biopterin pmol/g wet tissue Mean (SD)	DHPR nmol NADH/min/mg protein Mean (SD)
Control (n = 6)	Brain	415.0 (53.8)	182.0 (40.4)
	Liver	2882.3 (668.6)	102.9 (48.1)
Stressed (n = 6)	Brain	418.5 (52.3)	211.3 (103.1)
	Liver	2873.2 (469.5)	125.9 (39.6)

Table 5.31 Effect of an acute dose of scopolamine on rat brain, liver and plasma biopterin and pterin

Group	Tissue	n	Biopterin pmol/g wet tissue Mean (SD)	Pterin pmol/g wet tissue Mean (SD)
Control	Brain	6	459 (103)	523 (36)
	Liver	6	4848 (101)	3406 (757)
	Plasma	6	121 (32)	ND
Dosed	Brain	6	448 (128)	375 (73)*
	Liver	6	4721 (135)	3179 (378)
	Plasma	6	136 (33)	ND

Statistics: * Lower than control brain, $p < 0.01$

Table 5.32 Effect of an acute dose of scopolamine on rat brain and liver DHPR, protein and choline acetyltransferease

Group	Tissue	n	Protein mg/ml Mean (SD)	DHPR nmol NADH/ min/mg protein Mean (SD)	CAT μmol ACh/ g wet tissue Mean (SD)
Control	Brain	6	5.9 (1.2)	169.5 (103.8)	9.46 (3.21)
	Liver	6	51.7 (8.3)	169.4 (101.2)	ND
Dosed	Brain	6	5.9 (0.6)	162.9 (73.0)	9.11 (2.68)
	Liver	6	60.0 (17.1)	218.3 (172.7)	ND

5.32). There was no difference shown in brain choline acetyltransferase activity between dosed and control rats.

5.4 DISCUSSION

5.4.1 Distribution of total biopterin and DHPR activity in rat brain

Regional differences have been reported in rat brain several times (Bullard et al, 1978; Fukushima & Nixon, 1980). Lee and Mandell (1985) measured left-right differences in rat brain total biopterin by HPLC in a similar fashion to the studies here. They showed that in six rats, four had higher total biopterin in the right hemisphere and two had the highest values in the left: when they summed all the highest-side values (4 right and 2 left) they found a 20% difference compared to the sum of the lowest-side values. In the studies here, it could be seen that there were some differences between left and right half-brain values in total biopterin, but this difference was not significant by a statistical evaluation. The paired t test did establish that DHPR activity was significantly higher in the right side compared to the left side of the brain in six rats. Lee and Mandell claimed that the hemispheric distribution of total biopterin that they found in rat brain could be related to the asymmetrical distribution of dopamine in rat striatum (Glick et al, 1974); noradrenaline (Oke et al, 1980) and serotonin (Knapp and Mandell, 1980). The functional value of increased DHPR activity in the right side of rat brain is unclear: increased DHPR activity implies increased conversion of inactive quinonoid BH_2 to the active cofactor BH_4 and may be related to increased consumption of tetrahydrobiopterin.

5.4.2 5-methyltetrahydrofolate

Despite numerous attempts, it was impossible within the confines of time to devise a simple chemical conversion of 5-methyltetrahydrofolate to a pterin derivative that could be assayed by HPLC and fluorescent detection. 5-methyltetrahydrofolate distribution in brain has been reported as being parallel to that of serotonin (Korevaar et al, 1973); co-analysis of this folate and total biopterin in rat brain might have provided an interesting insight into the connection between the two related compounds.

5.4.3 Studies on lithium

A single oral dose of lithium was found to be distributed in plasma, brain and liver after four hours. No evidence has been presented for active uptake of lithium and it is thought to be a passive process (Chung et al, 1973). Schou (1958) reported that the order of organ 'preference' for lithium after entry into the blood was kidney, liver, bone, muscle and brain. The slow entry into the brain is due to the low lipid solubility of the hydrated lithium ion. In contrast to earlier reports, the study here showed that after four hours, brain lithium concentrations were higher than liver in the rat.

There is some evidence for differential distribution of lithium in rat brain, although this was not examined here. Thellier et al (1980) found that in mouse brain, lithium accumulated in the basal ganglia, hippocampus, hypothalamus and cerebral cortex: the areas which achieved the highest concentrations of lithium were those which had integrated control over motricity, behaviour and emotional attitudes.

Lithium carbonate has been used in previous studies in the brains of animals in an attempt to find the role played by this compound in the treatment of

affective disorders. Bliss and Ailon (1970) found that lithium given chronically to rats over a period of 14 days resulted in no significant changes in brain concentrations of dopamine, noradrenaline or serotonin. However, Friedman and Gershon (1973) later found that dopamine transmission in the nigrostriatal pathway in rats was slowed by low-dose lithium administration.

Katz et al (1968) demonstrated that lithium had an in vivo and in vitro effect on noradrenaline in rat cerebral cortex. Cerebral slices incubated with tritium-labelled serotonin and noradrenaline were shown to take up the labelled compounds, releasing them on electrical stimulation. Rats dosed with lithium chloride prior to sacrifice showed significantly diminished efflux of both noradrenaline and 5HT compared to undosed controls. The efflux from undosed rats could be significantly inhibited in the presence of lithium ions. The effect was not repeated in the presence of other monovalent cations such as rubidium and caesium, showing a specificity for lithium in this inhibition.

This study showed that lithium had no effect on brain total biopterin in vivo, after acute doses at a range of concentrations. Brain DHPR activity was similarly unaffected. As the beneficial effects of lithium in humans are seen after a few weeks, chronic administration of a moderate dose of lithium was performed for up to 21 days. Again no significant differences were seen between the total biopterin values of dosed and undosed rats, either in the brain or the liver, as a reference organ. DHPR was not altered after chronic dosing with lithium in either organ.

The in vitro effect of lithium was also examined in the assay for DHPR: the activity was neither enhanced nor inhibited by the presence of lithium ions. In vitro BH_4 biosynthesis is dependent on magnesium ions, and magnesium and lithium have some chemical similarities that have been attributed to their relative

positions (a 'diagonal relationship') in the periodic table of the elements (Birch, 1970). The incorporation of lithium ions into the assay for tetrahydrobiopterin biosynthesis did not inhibit or supplement the action of exogenous magnesium.

5.4.4 Imipramine

Mandell et al (1980) performed several studies of the effect of various drugs on rat striatal BH_4 levels. Their detection system was different in that they used an enzymatic assay, but they found control brain values to vary two-fold around a mean of approximately 300pmol/gramme of tissue. In an acute study, they injected rats subcutaneously with a dose of imipramine equivalent to 25mg/kg, and sacrificed them after one hour. They found that the first experiment performed this way resulted in a significant ($p < 0.05$) drop in rat brain striatal BH_4 : however, in two successive repeat experiments this effect could not be reproduced.

In the study here, the dose of 0.71mg to a rat weighing 165g represents a dose of 4.3mg per kg. This is less than one-fifth the dose given by Mandell et al (1980), but as the maximal dose for an adult weighing 70kg would be about 70mg/day, it is closer to the therapeutic dose for humans than the dose of 25mg/kg.

The brain total biopterin values of the control rats in the acute study here (about 260 pmol/g) were similar to the control values shown by Mandell et al. However, the acute effect of imipramine was a slight non-significant rise in brain total biopterin and a non-significant fall in liver total biopterin. No effects were shown on DHPR activity. Similarly, no effects on total biopterin or DHPR activity were shown to be induced by chronic (up to 21 days) dosing with imipramine.

Imipramine is known to be an uptake inhibitor (Todrick and Tait, 1969). These results tend to confirm

that imipramine does not have a direct role in the synthesis of neurotransmitters.

5.5.5 Sodium valproate

Administration of an acute dose of sodium valproate had no effect on brain or liver total biopterin. Studies in animals have shown a species response to the anti-convulsant action of valproate (Chapman et al, 1982). Lower mammals such as rat, mouse and cat have required up to ten times the human dose of sodium valproate to inhibit artificially induced convulsions. However, after a ten-fold increase in valproate dose given to rats in the study here, no effects were seen in rat brain or liver total biopterin.

Besides its antiepileptic action, valproate has some effect on indoleamine metabolism. Horton et al (1977) showed that valproate increased the uptake of tryptophan into mouse brain. Previous studies have shown that the administration of tryptophan to depressives can have beneficial effects, presumably by stimulating central serotonin synthesis (Coppen et al, 1963; Moller et al, 1976). Of related interest is the fact that valproate has a stimulatory effect on serotonin release in the rat (Crespi et al, 1986) and also interacts with the neuroendocrine system in depressed humans (Aggernaes et al, 1988).

However, any role that sodium valproate may play in central indoleamine metabolism would appear, from the results of this study, not to involve the metabolism of the tetrahydrobiopterin cofactor.

5.5.6 Folic acid

The importance of folates in the biology of affective disorder is well established (Abou-Saleh and Coppen, 1986). Folate deficiency causes low CSF values of serotonin and 5HIAA (Botez et al, 1979) even when levels

of the precursor tryptophan are normal. From this, it has been postulated that folic acid supplementation would result in stimulation of serotonin synthesis and an improvement in symptoms of depression. Folate has indeed been claimed as an effective antidepressant by several workers (Botez et al, 1976; Reynolds et al, 1984; Coppen et al, 1986).

The study by Coppen et al (1986) was a double blind trial of either 200 μg folic acid or placebo given to 75 depressed patients maintained on lithium therapy. The results showed that patients who had the highest plasma folates had significantly decreased affective morbidity.

The relationship between folates and neurotransmitter synthesis is clearly complex; however, a connection between folate and tetrahydrobiopterin metabolism has been suggested by both human and animal studies.

The activity of the enzyme dihydrofolate reductase responsible for the reduction of dihydrofolate to tetrahydrofolate is low in human brain (Pollock and Kaufman, 1978) and tetrahydrofolate can alternatively be preserved by the BH_4 salvage enzyme DHPR (Lind, 1972). Evidence exists that brain with a total DHPR lesion has greatly reduced folate levels (Pollock and Kaufman, 1978) indicating a close functional relationship between the reduction of tetrahydrofolate and tetrahydrobiopterin in neural tissue.

Harpey et al (1984) found that tetrahydrofolate therapy improved the clinical status of a child with low BH_4 levels due to a tetrahydrobiopterin synthesis lesion. Hamon et al (1986) showed that an in vitro brain preparation from a child who died due to a methylenetetrahydrofolate deficiency had no detectable tetrahydrobiopterin biosynthesis. Hamon et al (1986) also showed that BH_4 generation could be produced on addition of 5-methyltetrahydrofolate.

The biosynthesis of BH_4 in rat brain prepar-

ations has been shown to be stimulated by tetrahydrofolate and vitamin B_{12} (Leeming et al, 1982) and 5-methyltetrahydrofolate (Hamon et al, 1986).

In the study here, administration of a dose of 0.4 μg to a rat weighing 140g was equivalent to giving 200 μg folic acid to a human weighing 70kg, which was the dose used by Coppen et al (1986). After four hours, it could be seen that this dose did not have any significant effect on in vivo rat brain or liver total biopterin levels or DHPR activity. Folic acid needs to be converted to 5-methyltetrahydrofolate before it crosses the blood-brain barrier (Spector and Lorenzo, 1975), and it is possible that these physiological changes had not taken place by the end of the acute dosing period. Further in vivo studies would be required to establish a role for folic acid in the in vivo interactions with tetrahydrobiopterin metabolism in rat tissues.

5.5.7 α methylparatyrosine

α MPT, as a structural analogue of the amino acid tyrosine, competetively inhibits tyrosine hydroxylase (Spector et al, 1965). From the results shown here, when acutely administered to the rat, αMPT decreased brain and liver total biopterin levels and DHPR activity in both brain and liver, but none of these decreases reached statistical significance. It must be concluded that this agent has no net effect on tetrahydrobiopterin biosynthesis in the rat.

α MPT was one of the psychoactive agents administered to the rat by Mandell et al (1980). They injected 100mg/kg αMPT subcutaneously into rats for one hour prior to sacrifice, and found no significant changes in either BH_4 or total biopterin concentration in rat striatum. The dose equivalent of αMPT used in the study here was 56.3mg/kg, which also produced no significant change in total biopterin. Mandell

et al also showed that the isomer αmethylmetatyrosine at a dose of 75mg/kg had no effect either under the same conditions. The study here goes further than the work of Mandell et al (1980) in that it establishes that BH_4 metabolism is not altered in a peripheral organ (the liver) by αMPT, nor does αMPT have any effect on DHPR, the enzyme responsible for maintenance of cellular fully-reduced tetrahydrobiopterin.

5.5.8 Dexamethasone and stress

The studies here were to examine any effect that changes in the neuroendocrine system may have on tetrahydrobiopterin metabolism. Dexamethasone acts in the physiological negative feedback loop of the hypothalamic-pituitary-adrenal axis, and its administration causes a fall in circulating cortisol levels (suppression). Physical restraint causes animals to become stressed and results in activation of the HPA axis and elevation of circulating cortisol levels.

Dexamethasone was given at two dose levels: one represented a scaled-down dose that would be given to humans in a standard dexamethasone suppression test, and the other was five times that amount.

Rat brain DHPR activity at the lower dose was unaffected in both brain and liver. Dexamethasone is lipid-soluble and would have permeated the lipid-rich blood-brain barrier after twenty four hours. Although there was a slight elevation in total biopterin in the brain at low and high dose dexamethasone, this did not reach significance. An interesting result was shown by the liver. There was no effect of dexamethasone at low dose, but a high dose resulted in a significant elevation of total biopterin after twenty four hours administration.

Langlais et al (1984) show dexamethasone has a stimulatory effect on dopamine synthesis in both rats and humans.

In the animal studies, ten rats were injected intraperitoneally with 20 μg dexamethasone and ten given saline as a placebo. Half of each group were sacrificed after one hour, and the remainder after four hours. Noradrenaline, dopamine and serotonin and their major metabolites were measured in samples of nucleus accumbens, striatum and frontal cortex by HPLC and electrochemical detection. Dopamine was increased by 100% in nucleus accumbens at one and four hours post-dexamethasone; dopamine and serotonin were significantly elevated in hypothalamus after four hours, and although dopamine was not significantly elevated in the striatum, there was evidence of reduced turnover.

In a human study, six men and six women were given 1mg of dexamethasone at 11pm on day 1. Blood samples were taken at 8am and 4pm on day 1, and at 8am and 4pm on day 2. Plasma samples were all analysed for noradrenaline, adrenaline and dopamine before and after dexamethasone. The only changes found were a highly significant ($p < 0.001$) increase in plasma free dopamine levels at both 8am and 4pm post-dexamethasone. Langlais *et al* also found that administration of dexamethasone caused significantly higher dopamine values in medication-free psychiatric patients with depression or personality disorders, although details of this study were not supplied.

Langlais *et al* (1984) speculate that the two mechanisms by which dexamethasone could induce increases in brain dopamine concentration would be increasing tyrosine hydroxylase activity and inhibition of monoamine oxidase activity. The study here, which showed that a dose of dexamethasone (albeit a high dose) caused a significant rise in total biopterin in the liver suggests a third mechanism, namely that tyrosine hydroxylase activity is stimulated by an increase in tetrahydrobiopterin activity. This interesting observation warrants further investigation.

The application of immobilisation as a means of inducing stress had equivocal results with regard to DHPR activity and total biopterin measured. It was shown that brain total biopterin was unaffected in a group of six rats that had been physically restrained for 2½ hours. After the same period, brain DHPR activity was significantly raised ($p<0.02$) compared to a group of rats allowed freedom of movement within a cage. The second part of the study doubled the length of time of incarceration, but did not show any significant results. Both brain and liver DHPR values did not differ significally from controls. Brain and liver total biopterin values were very similar in both controls and stressed animals. The fact that DHPR activity was not increased after extended stress, and the fact that total biopterin levels were preserved, implies that there is no net effect on BH_4 biosynthesis *de novo* in neural tissue in the rat.

Tetrahydrobiopterin is actively synthesised in the adrenal medulla as it is essential for the catecholaminergic hormone adrenaline secretion (Nagatsu *et al*, 1964). BH_4 is also present in the adrenal cortex (Blakley, 1969) although the role of tetrahydrobiopterin metabolism here is less clear.

Abou-Donia *et al* (1983) examined GTP-CH activity and BH_4 levels in the adrenal cortex following three stressful events: a single dose of reserpine (5 mg/kg); a four hour period of insulin-induced hypoglycaemia; and two and a half hours stress by immobilisation. All these treatments were known to stimulate the biosynthesis and release of adrenocortical steroids resulting in the secretion of ACTH. They found that all treatments resulted in a stimulation of GTP-CH activity (the enzyme responsible for *de novo* synthesis of BH_4), and tetrahydrobiopterin levels were increased by up to three-fold control values. The stimulated BH_4 production in the adrenal cortex was presumably a response to the increased metabolic demand for adrenaline in the 'fight or flight' response to stress.

The results shown in the study here are a clear indication that brain total biopterin levels, which are concerned with central neurotransmitter synthesis, are not raised in stress.

5.5.9 Scopolamine

Scopolamine is known to block central and peripheral muscarinic receptors. Experimentally, it has been used in both healthy humans (Safer and Allen, 1971) and in animals (Drachman and Leavitt, 1974) to induce the memory deficits seen in the condition of SDAT. Hence it has been proposed for use in inducing animal models of senile dementia. Assessment of the extent of memory deficits in laboratory animals can be performed by behavioural studies: these studies were beyond the scope of this thesis. The only measurements made in animals that had been given scopolamine were of biochemical parameters.

The acute dose of 22.7 μg scopolamine (equivalent to approximately 0.2mg/kg) for six hours had the effect of causing a significant drop in rat brain total pterin concentration ($p < 0.01$) compared to controls. In all other parameters measured, such as brain, liver and plasma total biopterin; liver total pterin; brain and liver DHPR activity and protein; and brain choline acetyltransferase activity, there were no differences observed between test and control animals.

In an earlier study, Mandell *et al* (1980) found that a subcutaneous injection of 1.0mg/kg scopolamine for 70 minutes had no effect on rat tetrahydrobiopterin levels in the brain. Scopolamine is an established anticholinergic agent, and physostigmine a cholinergic agent. Interestingly, a dose of 1.5mg/kg physostigmine administered for 15 minutes duration was initially found to cause a significant increase in rat striatal BH_4 levels ($p < 0.05$), but the change was not replicable in two further experiments.

Here, it was interesting to note that scopolamine caused a significant depression in brain pterin levels. This result has since been confirmed in repeat studies in the rat (J. Cox, Aston University, personal communication). As brain but not liver pterin was affected, scopolamine appeared to be selective for neural tissue. The pterin content of tissues can vary due to nutritional status: pterin can arise from folate metabolism *in vivo*, as it has been shown to be formed *in vitro* via the autoxidation of tetrahydrofolate (Pearson, 1974). How pterin and tetrahydrobiopterin interact in neural tissue is not known, but further studies in this area should prove interesting. Scopolamine effects are not confined to the cholinergic system: animal studies by Decker and Gallagher (1984) have shown that this drug may have an antiadrenergic effect, which may relate to a primary effect on tetrahydrobiopterin metabolism.

CHAPTER SIX:

GENERAL DISCUSSION AND IMPLICATIONS FOR FURTHER RESEARCH

6.1 Urinary measurements of pteridine levels

The basis for conducting surveys into the urinary excretion of pteridines in patients with neurological and psychiatric diseases and controls is that these levels accurately reflect central tetrahydrobiopterin metabolism: supporting evidence comes from previous reports (Duch et al, 1984b; Blair et al, 1984b; Garbutt et al, 1985).

Samples of urine have the advantage in that they are copiously produced and collection is non-invasive. The use of an internal reference standard such as creatinine enables random samples to be taken and pteridine excretion related to a creatinine baseline, thus avoiding the need for collection of the total volume of urine excreted over a twenty four hour period. Biopterin excretion has been shown to fluctuate over a twenty four hour period (Pheasant, 1986): to overcome this, all patient and control samples were taken within a narrow period of time. In contrast, the neopterin to creatinine ratio has been shown to remain very constant throughout a twenty four hour period (Hausen et al, 1982).

The results of urinary measurement in the large group of controls in chapter three are interesting in that they show a sex-related difference in pteridine excretion in urine. Female controls appeared to excrete more total biopterin, neopterin and pterin than male controls. Fluctuations in female serum biopterin are known and may be oestrogen-related: Morar (1984) showed a range of values throughout the pregnancy of one young woman, and Leeming and Blair (1980) showed a wide variation in serum biopterin values throughout the menstrual cycle of one young unmedicated female.

A complicating factor in the assessment of urinary levels of pteridines is the possible effect of any medication the patients or controls may be be receiving.

In the study of tetrahydrobiopterin metabolism in depression, all the patients were maintained on lithium as antidepressant medication, and had been receiving lithium for between one and seventeen years. A common side effect of lithium therapy is partial nephrogenic diabetes insipidus (Kosten and Forrest, 1986): dilution of urine due to both polydipsia and polyuria has been reported in patients on lithium. However, dilution of pteridines in watery urine is accompanied by diluted creatinine excretion, and as all pteridine measurements were related to this creatinine baseline, any dilution effects would be compensated.

The ideal situation, and one that could be aimed for in future studies, would be to collect urine samples before and after commencement of lithium therapy so that the effects of lithium on pteridine excretion could be directly examined. In a study similar to the one proposed here, Rudorfer et al (1985) gave therapeutic doses of lithium for seven days to 12 young men who had been hospitalised for reasons other than neurological or psychiatric disturbances. By taking measurements before and after lithium therapy, Rudorfer et al established that lithium induced no changes in cardiovascular function, blood pressure, pulse or plasma noradrenaline. In the measurement of urine parameters, no changes were found in urine volume, or concentration of serotonin or 5HIAA.

6.2 Tetrahydrobiopterin metabolism in depression

Significantly decreased total biopterin excretion was demonstrated in both bipolar and unipolar female patients; total biopterin excretion was decreased in male bipolars and unipolars, but the differences did not reach significance.

A difference in the urinary excretion of tetrahydrobiopterin related to the sex of the patient

is interesting in that there is some evidence for an increased prevalence of depression in women compared to men (Weissman and Klerman, 1977). Depression accompanies the premenstrual syndrome and is a side effect of oral contraceptive use.

When all the patient values were considered, there was clear evidence for a reduction in tetrahydrobiopterin metabolism compared to age matched controls.

The implied central deficit of BH_4 biosynthesis in depression found here is in good agreement with the results of Blair et al (1984b), who demonstrated reduced urinary excretion, and also diminished tetrahydrobiopterin levels in post mortem brain samples from patients who died with depression. However, these results are in conflict with those reported by Duch et al (1984b) and Garbutt et al (1985).

The patients in the study in this thesis were euthymic, in good health, had low objective and self-rated scores as to depressive symptoms, and had been maintained on prophylactic lithium therapy for a number of years. Spot cortisol samples showed no differences between patients and controls. All the evidence pointed towards reasonably stable patients in remission from the depressive episodes of either unipolar or bipolar depression. Studies in this thesis on the rat indicated that lithium had no acute or long term effect on central tetrahydrobiopterin metabolism, and it was assumed that the lowered urinary excretion of tetrahydrobiopterin was a valid reflection of central BH_4 activity.

The patients described by Duch et al (1984b) and Garbutt et al (1985) were all hospitalised in an acute phase of depressive illness: both studies report an increase in urinary tetrahydrobiopterin excretion in depressed patients.

The patients of Duch et al (1984b) were probably subject to HPA axis dysfunction as part of the acute phase of depression: activation of the adrenal

cortex is known to result in high circulating cortisol levels and an increase in urinary free-cortisol excretion. Along with cortisol production, tetrahydrobiopterin production is stimulated: this has been shown in studies on the rat by Abou-Donia et al (1983) where various forms of stress resulted in highly significant increases in adrenal BH_4 output. It is very interesting to note that the small number of depressed patients described as in remission by Duch et al actually had a lower urinary biopterin excretion than controls. These results imply that the acute phase of depression is accompanied by HPA activation and a corresponding increase in adrenal biopterin secretion which causes high urinary levels of biopterin to be excreted into the urine: this raised excretion serves to obscure (temporarily) the central deficit of tetrahydrobiopterin in depression.

The experiment in this thesis whereby rats were subjected to periods of immobilisation stress showed that brain production of BH_4 was not stimulated by stressful situations and activation of the HPA axis: any increase in tetrahydrobiopterin production in the acute phase of depressive illness was not of central origin.

The action of most antidepressant drugs is to increase the concentration of brain active amines at the synapse, either by inhibition of presynaptic uptake or inhibition of degradation in the synaptic cleft. Other means of enhancing neurotransmitter synthesis have been by loading with precursor amino acids such as tryptophan (Coppen et al, 1973a). If, as postulated, there is truly a central deficit in tetrahydrobiopterin metabolism in depression, supplementation of depressed patients with BH_4 may have a role as an antidepressant therapy.

Early studies on animals has shown that peripherally administered BH_4 could permeate the brain: Kapatos and Kaufman (1981) found that rats given 20mg/kg tetrahydrobiopterin intramuscularly had a two-fold

raised brain BH_4 level. Miwa et al (1985) showed that after four hours, rat brain DOPA was increased by 100%, and 5-hydroxytryptophan concentration by 70% by an injection of 500 μg tetrahydrobiopterin per rat (approximate weight 250g).

Tetrahydrobiopterin is not considered to be very toxic in animal studies. Lewandowski et al (1986) found that a single intraperitoneal dose of up to 1318 mg/kg had no effect on the morbidity or mortality of mice.

Evidence that tetrahydrobiopterin could cross the blood-brain barrier was provided by Kaufman et al in 1982. Kaufman et al (1983) showed that a tetrahydrobiopterin dose of about 20 mg/kg would increase plasma total biopterin from 1ng/ml to over 100 ng/ml, at the same time increasing CSF total biopterin by a factor of three.

Some studies have examined the efficacy of BH_4 as an antidepressant drug.

In an early study, Curtius et al (1982) found that a single dose of 1g BH_4 resulted in clinical improvement in two out of three depressed patients; this was repeated in a later study (Curtius et al, 1983b). Fleishhacker et al (1985) found that one out of eight depressed patients responded well to 1g doses of BH_4 administered randomly over five days. There was improvement on both self- and observer ratings. Four out of eight showed increased vigilance and drive as measured by electroencephalogram analyses. Other patients appeared not to be affected by tetrahydrobiopterin.

Woggon et al (1985) found that one out of five depressed patients improved in a similar study, but later Woggon et al (1984) reported that one patient who had successfully been treated with tetrahydrobiopterin on several occasions failed to respond to a similar dose of 1g per day in a further study.

Levine et al (1987) have proposed that very high brain concentrations of tetrahydrobiopterin are necessary to increase biogenic amine metabolism in human brain.

The finding that there are a few depressed patients who have failed to respond to traditional tricyclic and MAOI therapy, but will respond to tetrahydrobiopterin administration, is similar to the situation in atypical PKU. In atypical PKU, the failure of the tyrosine hydroxylase system is secondary to a metabolic lesion in BH_4 biosynthesis. The tradional monoamine theory of depression assumes that there is a functional deficit of biogenic amines in the brain, and drugs and other agents that enhance brain levels of serotonin and noradrenaline function as antidepressants because they restore normal levels of these neurotransmitters. It may be that there is a sub-group of patients with depression that have lowered central indoleamine and catecholamine neurotransmitters as a result of a primary decrease in tetrahydrobiopterin biosynthesis. These would be the patients who respond best to BH_4 supplementation as an antidepressant therapy.

The interaction between folate and tetrahydrpbiopterin remains intriguing. Administration of a single dose of folic acid to the rat results in no significant rise in brain or liver BH_4 metabolism after four hours: it may be assumed that there is no direct in vivo stimulation of synthesis. However, as the physiological form of folic acid is as tetrahydrofolate, and particularly as 5-methyl tetrahydrofolate, perhaps an acute dose of the parent compound did not allow reduction and uptake within the CNS after four hours. A future study might like to apply chronic dosing of folic acid, over a period of weeks if not months to examine the long-term effect of folic acid on tetrahydrobiopterin metabolism in mammals.

However, one very interesting observation was the correlation between plasma folate and urinary biopterin shown by the female unipolars. A useful study in the future would be to correlate plasma folate, urinary biopterin and a rating of depressive symptoms, the hypothesis being that a rise in urinary biopterin levels towards those found in controls is conducive to an improvement in the symptoms of affective disorder. A study that would be easy to perform would be to examine the influence of a folate supplement on the pteridine excretion of normal people: folic acid is part of the normal diet, and so increasing the intake would not lead to adverse effects in healthy adults.

This thesis established that lithium, imipramine, MPT and sodium valproate had no immediate effects on tetrahydrobiopterin metabolism in the rat brain or a peripheral organ, the liver. In addition, a chronic study conducted using lithium and imipramine was not able to induce any significant changes. This shows that classical antidepressant drugs do not have a simple effect in enhancing BH_4 biosynthesis.

6.3 Tetrahydrobiopterin metabolism in senile dementia of Alzheimer type

This thesis showed in two distinct studies that tetrahydrobiopterin metabolism in SDAT as measured by the urinary excretion of the pteridines neopterin and biopterin, was diminished.

The use of urinary measurements of neopterin and biopterin in SDAT may become more widely adopted in the future as a guide to the degree of dementia: the CDR and other rating scales were not recorded in this thesis, but it would be interesting to examine

any correlations between the N/B ratio and severity of dementia symptoms. A precedent for these type of comparisons has been shown by Hamon et al (1987), who showed that the characteristic lag in the VEP shown in SDAT correlates excellently with degree of dementia, and also with increasing N/B ratio. An important implication here is that SDAT are particularly difficult psychiatric patients to manage, and any test which can be performed non-invasively has an obvious advantage.

This thesis also showed that tetrahydrobiopterin metabolism is altered in depression, but there are some details that show important differences with regard to SDAT.

Although tetrahydrobiopterin (indicated as B/B+N) was shown to be decreased in SDAT, there was still a good correlation between the amounts of total neopterin and total biopterin in the urine. This correlation was only absent in the small group of ten patients in the second study who had very high values of N/B ratio. In contrast, this correlation was completely absent in both female and male depressed patients, although their control subjects showed very good correlations.

Another point is the connection between plasma folate and urinary biopterin. In female unipolars, there was shown to be a correlation between urinary biopterin and plasma folate. Although similar measurements were made for the larger group of SDAT patients, no such correlation was shown. A difference between the depressed patients and the SDAT groups was that some of the depressives were actually being supplemented with folate as part of their treatment: it would be interesting in a future study if SDAT patients, who tend to have low plasma folate values anyway, were to be supplemented with folate in order to examine an effect on urinary total biopterin: the inference

is that if folate can stimulate BH_4 biosynthesis, and BH biosynthesis is diminished in dementia of Alzheimer type, maybe some of the neurochemical and neurological deficiencies in this condition could be alleviated by this simple therapy.

APPENDICES

Appendix 1: Age and medical status of controls and affective disorder patients

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Appendix 1: (continued)

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Appendix 1: (continued)

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Appendix 2: The Beck Depression Inventory

1. Mood
2. Pessimism
3. Sense of failure
4. Lack of satisfaction
5. Guilty feeling
6. Sense of punishment
7. Self hate
8. Self accusations
9. Self-punitive wishes
10. Crying spells
11. Irritability
12. Social withdrawal
13. Indecisiveness
14. Body image
15. Work inhibition
16. Sleep disturbance
17. Fatigability
18. Loss of appetite
19. Weight loss
20. Somatic preoccupation
21. Loss of libido

Appendix 3:

Ratings according to BDI and DR and plasma values of male and female patients

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Appendix 3: (continued)

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Appendix 4: Urinary creatinine, cortisol and $^{N}/_{B}$ ratio in affective disorder controls

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Appendix 4: (continued)

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Appendix 5: Urinary creatinine, cortisol and $^{N}/_{B}$ ratio in affective disorder patients

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Appendix 5: (continued)

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Appendix 6: Urinary pteridine values of affective disorder controls

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Appendix 6: (continued)

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Appendix 7: Urinary pteridine values of affective disorder patients

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Appendix 7: (continued)

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Appendix 8: Urinary excretion in SDAT group 1 patients

Appendix 9:

Geriatric Mental State Scale (GMS)

Depression

Unpleasant worrying which returns repeatedly or which cannot be stopped
Overall rating of worrying
Looks or sounds tense or worried
Subjective fear or anxiety
Report of depressed mood
Future seems bleak or completely unbearable
Has felt that life was not worth living
Would like to be dead
Overall rating of depression
Looks sad; mournful or depressed
Eyes moist: tearful or crying
Sounds gloomy or mournful
Subjective memory impairment
Gets worn out or exhausted during daytime or evening
Difficulty in relaxing
Restlessness
Diminution of appetite
Loss of weight
Loss of weight of 10lb or more
Subjective feelings of sleep disturbance
Wakes up feeling tired
Difficulty in falling asleep
Sleep is interrupted
Overall rating of eating or sleeping difficulties
Subjective slowing in thinking
Feels indecisive
Brooding
Overall rating of subjective difficulties in thinking
Subjectively slowed in movements
Slowness in movements worse in last three months
Listlessness or subjective restriction of energy
Sits or lies around because of lack of energy
Overall rating of subjective slowness and lack of energy
Feels ill at ease with people
Wants to stay away from people
Self-depreciation
Overall rating of self-depreciation
Gets angry with self
Has less interest in things than is usual with him
Neglects his appearance
Almost nothing enjoyed doing recently
Spends little of his free time at recreational activity
Has lost interest in TV or radio
Overall rating of lack of interest

Appendix 9: (continued)

Depression

Difficulty in concentrating on entertainment
Difficulty in concentrating on reading
Overall rating of lack of concentration
Fleeting thoughts of suicide
Feels people avoid, reject or dislike him
Feels worthless
Errors in cognitive sections due to agitation, preoccupation, retardation, depression, disinterest or apathy

Appendix 10: The Hachinski Index

Table 1 Dementia score

Feature	Score
Changes in performance of everyday activities	
1. Inability to perform household tasks	1
2. Inability to cope with small sums of money	1
3. Inability to remember short lists of items eg shopping	1
4. Inability to find way about indoors	1
5. Inability to find way about familiar streets	1
6. Inability to interpret surroundings	1
7. Inability to recall recent events	1
8. Tendency to dwell in the past	1
Changes in habits	
9. Eating	
Messily with spoon only	1
Simple solids eg biscuits	2
Has to be fed	3
10. Dressing	
Occasionally misplaced buttons etc	1
Wrong sequence, commonly forgetting items	2
Unable to dress	3
11. Sphincter control	
Occasional wet beds	1
Frequent wet beds	2
Doubly incontinent	3
12. Increased rigidity	1
13. Increased egocentricity	1
14. Impairment of regard for feelings of others	1
15. Coarsening of affect	1
16. Impairment of emotional control	1
17. Hilarity in inappropriate situations	1
18. Diminished emotional responsiveness	1
19. Sexual misdemeanour (appearing *de novo* in old age)	1
20. Hobbies relinquinshed	1
21. Diminished initiative or growing apathy	1
22. Purposeless hyperactivity	1

Appendix 10: (continued)

Table 2 Information-memory-concentration test

Feature	Score
Information	
Name	1
Time (hour)	1
Time of day	1
Day of week	1
Date	1
Month	1
Season	1
Year	1
Place	1
Name	1
Street	1
Town	1
Type of place (eg home, hospital)	1
Recognition of persons (eg cleaner, physician, nurse, patient, relative)	2
Memory	
Personal	
Date of birth	1
Place of birth	1
School attended	1
Occupation	1
Name of siblings	1
Name of wife	1
Name of any town where patient worked	1
Name of employers	1
Non personal	
Date of world war I	1
Date of world war II	1
Name of monarch	1
Name of Prime Minister	1
Name and address (5 minute recall:	5
Concentration	
Months of year backwards	2
Counting from 1 to 20	2
Counting from 20 to 1	2

Appendix 10: (continued)

Table 3 Ischaemic score

Feature	Score
Abrupt onset	2
Stepwise deterioration	1
Fluctuating course	2
Nocturnal confusion	1
Relative preservation of personality	1
Depression	1
Somatic complaints	1
Emotional incontinence	1
History of hypertension	1
History of strokes	2
Evidence of associated atherosclerosis	1
Focal neurological symptoms	2
Focal neurological signs	2

Appendix 11:

Modified Kew Cognitive Test

1. MEMORY

What year are we in?

What month is it?

Can you tell me two countries we fought in the second world war?

What year were you born?

What is the capital city of England?

2. APHASIA

What do you call this (a watch)?

What do you call this (a wrist-strap or band)?

What do you call this (a buckle or clasp)?

What is a refrigerator for?

What is a thermometer for?

What is a barometer for?

3. PARIETAL SIGNS

Show me your left hand.

Touch your left ear with your right hand.

Name the coin in hand named (as 10p or two shillings).

No tactile inattention present.

Normal two point discrimination.

Draw a square.

Appendix 12: Medical status of controls for SDAT study two

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Appendix 13: Medical status of SDAT group two patients

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Appendix 13: (continued)

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Appendix 13: (continued)

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Appendix 13: (continued)

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Appendix 14: Urinary excretion in SDAT group two controls

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Appendix 15: Urinary excretion in SDAT group two patients

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Appendix 16:

Plasma results for group 2 SDAT patients

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