Pteridines and mono-amines: relevance to neurological damage

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Summary: Patients with phenylalanine hydroxylase deficiency show increased concentrations of bipterins and neopterins, and reduced concentrations of serotonin and catecholamines, when phenylalanine concentrations are raised. The pterin rise reflects increased synthesis of dihydroneopterin and tetrahydrobiopterin, and the amine fall a reduction in amine synthesis due to inhibition by phenylalanine of tyrosine and tryptophan transport into neurones. The pterin and amine changes appear to be independent of each other and are present in the central nervous system as well as the periphery; they disappear when phenylalanine concentrations are reduced to normal. Patients with arginase deficiency show a similar amine disturbance but have normal pterin levels. The amine changes probably contribute neurological symptoms but pterin disturbance is not known to affect brain function.

Patients with defective bipterin metabolism exhibit severely impaired amine synthesis due to tetrahydrobiopterin deficiency. Pterin concentrations vary with the site of the defect. Symptoms include profound hypokinesia and other features of basal ganglia disease. Neither symptoms nor amine changes are relieved by controlling phenylalanine concentrations. Patients with dihydropteridine reductase (DHPR) deficiency accumulate dihydrobiopterins and develop secondary folate deficiency which resembles that occurring in patients with defective 5,10-methylene tetrahydrofolate reductase activity. The latter disorder is also associated with Parkinsonism and defective amine and pterin turnover in the central nervous system, and a demyelinating illness occurs in both disorders. In DHPR deficiency cerebral calcification may develop in a similar distribution to that seen in congenital folate malabsorption and methotrexate toxicity. Symptoms are ameliorated by therapy with 5-formyltetrahydrofolate but exacerbated by folic acid.

Introduction

Alterations in amine metabolism are a factor in the pathogenesis of many diseases. Two of the commonest neurological disorders, Parkinson's disease and Alzheimer's disease, are associated with a central deficiency of catecholamines and serotonin, many drugs in common use interfere with amine metabolism and it has been proposed that the major psychoses are due to defective control of amine turnover, although the mechanisms of amine disturbance are ill understood.

In the clinical literature relatively little attention has been paid to the possible role of the rate controlling reactions of amine synthesis although there are several clearly defined examples of disorders which lead to amine deficiency due to alterations at this site in amine metabolism. Patients with phenylketonuria for example have long been known to exhibit amine abnormalities due to this cause. The two hydroxylation reactions which control the rate of catecholamine and serotonin synthesis require the same cofactor, tetrahydrobiopterin (BH₄) which is a pteridine compound structurally related to folates, and 10 years ago the

first patients with the defective BH₄ metabolism were described (Smith, 1985). They exhibit profound amine deficiency and neurological symptoms consist initially of poor head control, floppiness, feeding problems, excessive drooling and sweating, blank facies, general immobility and lethargy usually appearing within a few weeks or months of birth. Later symptoms include profound truncal hypotonia contrasting with cog-wheel or lead-pipe rigidity of the limbs, choreiform movements, oculogyric crises, infantile spasms, swallowing difficulty, disturbance of temperature control and respiratory problems, progressive developmental regression and intractable epilepsy. Death in early childhood is common among patients recorded in the literature. Control of hyperphenylalaninaemia (HP) by means of a low phenylalanine diet fails to correct the amine disturbance or prevent the progression of clinical symptoms.

It is too soon to say how well patients with BH₄ deficiency will progress on long-term amine precursor therapy. Bearing in mind the effects of HP on brain protein and lipid metabolism and that L-dopa and 5-hydroxytryptophan enter the brain via the same

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transport process as the other neutral amino acids and will therefore compete for entry into neurones, one cannot be certain that such therapy will have long term success. The cause of the deterioration seen in patients with Parkinson's disease after a few years on L-dopa therapy is not understood but may be due, at least in part, to therapy.

These disorders have provided important insights into the mechanisms and consequences of altered amine metabolism *in vivo*. In addition, unexpected links between folate, bipterin and amine metabolism have emerged which throw new light upon an old problem, subacute combined degeneration of the cord. These recent discoveries are likely to add to understanding of the mechanisms of neurological disease, but, as yet, the findings are little known beyond the narrow field of metabolic disease and details of the biochemical background are not readily accessible to the general reader.

The aim here is to bring together the relevant biochemical information and illustrate this with some recent findings in children with phenylalanine hydroxylase deficiency, arginase deficiency, defects of bipterin metabolism and defects of folate metabolism. The relationships between neurological and biochemical abnormalities are discussed.

Pteridines

Natural pteridines contain a 2-amino,4-oxopteridine (or 'pterin') ring (Pfleiderer, 1982). Folic acid and bipterin contain a fully oxidized pterin ring (five double bonds) but *in vivo* the number and disposition of double bonds and protons are in the dihydro- or tetrahydro- form (Figure 1), enabling pteridines to act as proton donors and acceptors. Various substituent groups may be attached at position 6, bipterins having a dihydroxypropyl, and folates a methylene/*p*-

aminobenzoate/glutamate group. Folates may carry additional methyl (CH_3), formyl (CHO), or formimino (CHNH) groups at N5, CHO at N10 or methenyl (CH) or methylene (CH_2) groups linking N5 and N10, reflecting the role of folate in 1-carbon transfer. amethopterin (methotrexate) is a synthetic pteridine which differs from folic acid in having an amino- group instead of an oxo- group at position 4 and a methyl group at position 10. The molybdenum cofactor is also a pteridine (Coughlan, 1983) but will not be discussed here.

Metabolism of bipterins

5,6,7,8-tetrahydrobiopterin (BH_4)

BH_4 is the proton donor in the hydroxylation of phenylalanine to tyrosine, tyrosine to L-dopa and tryptophan to 5-hydroxytryptophan (Figure 2) (Kaufman, 1981). Phenylalanine hydroxylase, which is confined to liver, controls the rate of phenylalanine catabolism and deficiency of this enzyme, or of its cofactor BH_4 , leads to hyperphenylalaninaemia (Kaufman, 1976; Smith, 1985). Tyrosine and tryptophan hydroxylases control the rate of dopamine and serotonin synthesis respectively (Kaufman, 1981). 5-Hydroxytryptophan is also the precursor of melatonin in the pineal. BH_4 , or a similar tetrahydropterin, appears to be required for breakdown of the ether bond of plasmalogens (Tietz *et al.*, 1964) and the possibility of BH_4 being involved in other oxidation/reduction reactions cannot be excluded.

Synthesis and turnover of BH_4

BH_4 is synthesized (Figure 2) in liver and in amine producing cells from guanosine triphosphate (GTP) which is converted to 7,8-dihydroneopterin triphos-

Figure 1 (1) 5,6,7,8-tetrahydrofolate (THF); (2) 5,6,7,8-tetrahydrobiopterin (BH_4).

Figure 2 Requirement for tetrahydrobiopterin (BH₄) in the conversion of phenylalanine (Phe) to tyrosine (Tyr) and synthesis of BH₄ from guanosine triphosphate (GTP). The same mechanisms of BH₄ turnover are required in the hydroxylation of tyrosine to L-dopa and tryptophan to 5-hydroxytryptophan. PH = phenylalanine hydroxylase, DHPR = dihydropteridine reductase, 1 = GTP cyclohydrolase, 2 = 'dephosphorylating enzyme', 3 = sepiapterin reductase, NH₂P₃ = dihydroneopterin, NH₂P₃ = neopterin triphosphate, S = sepiapterins, BH₂ = 7,8-dihydrobiopterin, q-BH₂ = quinonoid-dihydrobiopterin.

phate (NH₂P₃) by GTP cyclohydrolase. NH₂P₃ is dephosphorylated and reduced to form BH₄ in a series of reactions requiring at least two enzymes ('phosphate eliminating enzyme' and sepiapterin reductase) and several tetrahydroketopterin intermediates (Curtius *et al.*, 1985; Kaufman, 1985). Organisms synthesizing folates do so from GTP via NH₂P₃.

BH₄ is oxidized during the hydroxylation of aromatic amino acids to quinonoid-dihydrobiopterin (q-BH₂) which is then reduced back to BH₄ by dihydropteridine reductase (DHPR) (Figure 2) (Kaufman, 1971). This re-cycling of biopterins is essential for maintenance of a normal rate of hydroxylation *in vivo*. q-BH₂ readily rearranges to 7,8-dihydrobiopterin (BH₂) which cannot then be reduced to BH₄ by DHPR.

7,8-dihydroneopterin (NH₂)

NH₂ is present in human tissue fluids (but not those of the rat). It has been assumed that NH₂ is a bi-product of BH₄ synthesis but with the possible exception of NH₂ in patients with defective conversion of NH₂P₃ to BH₄, it seems more likely that this pterin arises mainly in the reticulo-endothelial system. Macrophages, which do not synthesize biopterins, contain GTP cyclohydrolase and produce neopterins in response to activated T-cells. This is probably the origin of markedly elevated blood and urine neopterins in patients with viral infection, malignant disorders,

graft versus host disease and gluten enteropathy (Huber *et al.*, 1983).

GTP cyclohydrolase

This enzyme appears to control the rate of BH₄ and NH₂ synthesis. Incorporation of GTP into BH₄ in the rat is stimulated by hyperphenylalaninaemia (Milstien & Kaufman, 1983) which, in human subjects, leads to a rise in plasma and urine biopterins and neopterins (Leeming *et al.*, 1976a; Dhont *et al.*, 1981; Niederwieser *et al.*, 1980). Even when phenylalanine concentrations are within the normal range, plasma concentrations of biopterins change in parallel with phenylalanine suggesting that the latter exerts physiological control over BH₄ synthesis (Smith, 1985).

Measurement of pteridines in biological fluids

Reduced pteridines are readily oxidized and measurement of individual species presents many practical difficulties. Bio-assay of 'total folate' using *Lactobacillus casei* and 'total biopterin' using *Crithidia fasciculata* provide sensitive measures of the combined activity of reduced and oxidized species. Combined with measurement of DHPR activity bio-assay of biopterins in dried blood spots is used in the UK for routine testing for defective BH₄ metabolism in infants with hyperphenylalaninaemia (Leeming *et*

Figure 3 Relationship between plasma phenylalanine concentrations and plasma total biopterin activity in newborn infants with hyperphenylalaninaemia. Patient NF: DHPR deficiency. Patient AK: defective biopterin synthesis. (●) pre-diet; (○) on diet.

al., 1984). It is also useful for measuring the response of plasma biopterins to a phenylalanine load as a test of defective biopterin synthesis (Leeming *et al.*, 1976; Rey *et al.*, 1983). The assay provides no measure of neopterins and loss of BH₄ occurs during the procedure.

Chemical techniques for measurement of total biopterins and neopterins using high performance liquid chromatography (HPLC) with pre-column oxidation and fluorimetric detection (Nixon *et al.*, 1980) have been applied in the diagnosis of BH₄ deficiency (Niederwieser *et al.*, 1980; Niederwieser *et al.*, 1985). The technique will allow for an approximate estimate of the proportions of BH₄ in the original sample (Nixon *et al.*, 1980). By using electrochemical and fluorimetric detectors in series, BH₄, BH₂ and biopterin can be measured directly (Hyland, 1985; Hyland *et al.*, in press). Pre-column autoxidation is minimized by collection of specimens into tubes containing anti-oxidant (vitamin C, dithioerythritol and an iron chelator), protection from light and freezing to -70° at the bedside.

Pteridines in various forms of hyperphenylalaninaemia

Phenylalanine hydroxylase deficiency

As might be expected from the effects of phenylalanine on GTP cyclohydrolase, patients show a rise in plasma total biopterin in the presence of hyperphenylalaninaemia (Figure 3). Concentrations of biopterins and neopterins are increased in urine (Niederwieser *et al.*, 1980) and in blood (Dhondt *et al.*, 1981) most biopterins being in the tetrahydro-form (Nixon *et al.*, 1980; Dhondt *et al.*, 1981). These pterin changes disappear when phenylalanine levels are controlled by means of a low phenylalanine diet. Administration of BH₄ does not affect phenylalanine concentrations.

CSF biopterins and neopterins appear to be increased, reflecting peripheral pterins and again most of the biopterin activity is BH₄ (Table I). It is not known whether pterin changes have any effect on neurological status. Despite careful handling of specimens to prevent oxidation, significant amounts of BH₂ and dihydroxanthopterin, which are probably derived from oxidation of pteridines *in vitro*, could be detected in CSF.

Tetrahydrobiopterin deficiency

BH₄ deficiency may be due to deficiency of GTP cyclohydrolase, to defective conversion of NH₂P₃ to BH₄ or to DHPR deficiency (Niederwieser *et al.*, 1985) (Figure 2). Administration of BH₄ to these patients lowers plasma phenylalanine and raises tyrosine concentrations, although in DHPR deficiency amino acid changes are small or, in a few patients, undetectable. Plasma total biopterin activity is low in patients with defective biopterin synthesis (Leeming *et al.*, 1976b; Rey *et al.*, 1977; Niederwieser *et al.*, 1984) and the urine pterin pattern is characteristic of each defect. Patients with GTP cyclohydrolase deficiency have low concentrations of biopterins and neopterins and those with defective conversion of NH₂P₃ to BH₄ have high concentrations of neopterins and low biopterins (Niederwieser *et al.*, 1985). In DHPR deficiency plasma and urine biopterins are raised (Rey *et al.*, 1977; Smith *et al.*, 1985) whereas neopterins are normal (Dhondt *et al.*, 1981; Niederwieser *et al.*, 1980). Direct measurement of biopterins in the urine of two patients with DHPR deficiency has shown the main species to be BH₂ (Hyland, 1985), not biopterin as suggested previously (Milstien *et al.*, 1980).

CSF pterins in patients with defects of biopterin metabolism again reflect those in the periphery (Niederwieser *et al.*, 1984; McInnes *et al.*, 1984; Smith *et al.*, 1985; Hyland *et al.*, in press). In patients with

Table I Pterin species in CSF in patients with deficiency of phenylalanine hydroxylase or dihydropteridine reductase (DHPR)

Biopterin species (ng/ml)	Enzyme defect			Provisional normal range
	Phenylalanine hydroxylase		DHPR	
	1	2		
BH ₄	15.5	20.7	5.4	4-15
BH ₂	2.3	4.3	13.5	1- 3
B	none	none	1.0	none
XH ₂	7.1	11.3	7.7	1- 7
N*	5.2	36.8	9.0	2- 7
CSF Phe µmol/l	80	169	74	5-15

N = total neopterins, BH₂ = 7,8,dihydrobiopterin, BH₄ = 5,6,7,8,tetrahydrobiopterin, XH₂ = dihydroxanthopterin, Phe = plasma phenylalanine.

*Rises with viral illness to 50 ng/ml and above.

DHPR deficiency CSF bipterins are elevated (Smith *et al.*, 1985) and again most is BH₂ (Hyland *et al.*, in press). Recent direct measurement of BH₄ concentrations in CSF suggests, however, that some BH₄ is present (Table I) despite undetectable levels of DHPR in red cells (Leeming, personal communication). However, compared with CSF pterins in two patients with phenylalanine hydroxylase deficiency the proportion of total bipterin present as BH₄ was low.

It has been suggested previously (Smith *et al.*, 1985) that in patients with DHPR deficiency some turnover of BH₄ occurs in the liver via the folate enzymes 5,10CH₂FH₄ reductase and DHFR, and that this may explain the relatively modest phenylalanine accumulation and fall of phenylalanine levels in response to administration of BH₄. This hypothesis has yet to be confirmed but the CSF data support the idea that some turnover of BH₄ continues (this time in the CNS) in the absence of DHPR activity, although perhaps outside amine producing cells since patients show severe amine deficiency.

Neurotransmitter amines

Amine synthesis

Synthesis of catecholamines and serotonin requires adequate intra-cellular concentrations of tyrosine and tryptophan, BH₄ and the appropriate hydroxylase (Kaufman, 1981). The rate of amino acid transport across the blood-brain barrier is the main determinant of neuronal tyrosine and tryptophan concentrations (Pratt, 1981). The inter-play of these three factors is disrupted in all forms of hyperphenylalaninaemia with consequent disturbance of amine metabolism (Figures 4 and 5). Measurements of amines, and of amine metabolites such as homovanillic acid (HVA), the dopamine metabolite, and 5-hydroxyindoleacetic acid (5HIAA), the serotonin metabolite, have demonstrated a reduction in amine turnover in patients with phenylalanine hydroxylase deficiency (Lyman, 1961; McKean, 1972) and those with defective bipterin metabolism (Butler *et al.*, 1978; McInnes *et al.*, 1984; Niederwieser *et al.*, 1984; Smith *et al.*, 1985).

Phenylalanine hydroxylase deficiency

The central amine disturbance in patients with phenylalanine hydroxylase deficiency (Figures 4 and 5) is likely to be due to inhibition by phenylalanine of the transport of tyrosine and tryptophan from plasma into neurones (McKean, 1972; Pratt, 1981; Smith, 1985). Low concentrations of tyrosine and tryptophan, as well as of amines and their metabolites, are found in brain at post-mortem. The findings are consistent with the work of Wurtman & Fernstrom

Figure 4 CSF homovanillic acid (HVA) concentrations versus age in children with phenylalanine hydroxylase deficiency (○), arginase deficiency (▲) and DHPR deficiency (■) compared with concentrations in children with neurological symptoms but without movement disorder (●). The youngest patient with phenylalanine hydroxylase deficiency was on well controlled diet (patient 1, Table I, plasma phenylalanine 300 μmol/l) whereas the others were on a relaxed diet with blood phenylalanine concentrations between 900 and 1500 μmol/l.

Figure 5 CSF 5-hydroxyindoleacetic acid (5HIAA) (see Figure 4).

(1975) showing that the plasma amino acid pattern exerts physiological control over central amine metabolism.

Further evidence that amino acid competition is the cause of amine disturbance in phenylalanine hydroxylase deficiency comes from a recent study showing that a similar disturbance in CSF amine metabolites occurs (Figures 4 and 5) in arginase deficiency, a defect of the urea cycle causing hyperargininaemia but little or no hyperammonaemia (Hyland *et al.*, 1985). There are no detectable pterin changes in arginase deficiency and, as in phenylalanine hydroxylase deficiency, amine disturbance is reduced by dietary treatment (Table III). In both disorders the effects on serotonin appear to be more severe than the effects on dopamine which is compatible with the finding that tryptophan hydroxylase is only 50% saturated by its substrate even at physiological concentrations of tryptophan (Kaufman, 1981). In contrast to the amino acid competition due to increased phenylalanine or arginine concentrations, hyperammonaemia damages the selectivity of the blood-brain barrier for amino acids and increases the rate of tryptophan transport into neurones from plasma. As a result, patients with defects of the urea cycle causing hyperammonaemia have raised serotonin metabolite levels in CSF (Bachman & Colombo, 1983).

The neurological illness in patients with phenylalanine hydroxylase deficiency is dominated by mental retardation although other neurological abnormalities occur including myoclonic epilepsy, poor head control, hyperkinesia, increased limb tone, brisk tendon reflexes, ankle clonus and extensor plantar responses. Arginase deficiency is associated with similar symptoms. In older patients hyperactivity, fidgetiness, repetitive hand and facial movements and stereotyped

behaviour are prominent, and Parkinsonian features have been noted (Paine, 1957). Even early treated children may, as the control of phenylalanine concentrations relaxes, develop subtle behaviour changes, intellectual deterioration, brisk jerks, ankle clonus and intention tremor (Smith, 1985).

It is possible that imbalance of central amine turnover contributes to the behaviour changes, dopamine deficiency to the Parkinsonian features and serotonin deficiency to the myoclonic epilepsy. In addition, the increased latency and decreased amplitude of visually evoked responses occurring in the presence of hyperphenylalaninaemia revert to normal when balanced amounts of L-dopa and 5-hydroxytryptophan are given (McKean, 1972). Whether amine deficiency contributes to the more general neurological damage caused by hyperphenylalaninaemia is unknown.

Tetrahydrobiopterin deficiency

Patients with defective biopterin metabolism exhibit profound dopamine and serotonin deficiency (Figures 4 and 5) which is not corrected by control of phenylalanine accumulation. Such patients were first recognized (Smith *et al.*, 1975; Kaufman *et al.*, 1975; Rey *et al.*, 1977; Bartholome *et al.*, 1977) by the unusual and progressive character of the neurological illness and failure of the low phenylalanine diet to prevent symptoms. These include extreme hypokinesia and trunk hypotonia, swallowing difficulty, drooling, Parkinsonian facies, myoclonus, limb rigidity, oculogyric crises and recurrent hyperpyrexia. The movement disorder is much more prominent than in patients with phenylalanine hydroxylase deficiency and is consistent with the more profound alteration in dopamine metabolism. Serotonin deficiency could again contribute to the myoclonic epilepsy and explain the disturbance of temperature control.

Confirmation of the role of amine disturbance in the pathogenesis of symptoms comes from the dramatic improvement in some patients on treatment with L-dopa, 5-hydroxytryptophan and carbidopa (Bartholome *et al.*, 1977). Yet other factors undoubtedly make an important contribution to the neurological illness. Patients with defective biopterin synthesis have reduced birth weights (Smith & Dhondt, 1985) suggesting an effect of the disease *in utero*. This may explain why amine replacement therapy and control of phenylalanine concentrations, even from infancy, may not prevent mental retardation (Endres *et al.*, 1982). Patients with DHPR deficiency are of normal birth weight but develop severe folate deficiency (Butler *et al.*, 1978), which is associated with progressive neurological deterioration (Harpey, 1983; Smith *et al.*, 1985).

Table II Effects of diet therapy on CSF dopamine and serotonin metabolites; patients with deficiency of phenylalanine hydroxylase or arginase

Enzyme defect	Diet	HVA ng/ml	SHIAA ng/ml
Phenylalanine hydroxylase			
Patient 1	Off	38	6
	On	44	16
Patient 2	Off	27	0
	On	74	19
Arginase	Off	33	5
	On	60	16
Normal range*		50–150	20–120

HVA = homovanillic acid, SHIAA = 5-hydroxyindoleacetic acid.

*Dependent on age, see Figures 4 and 5.

Relationship between biopterins, folates and amines

Links in bipterin and folate metabolism

The metabolism of bipterins and folates appears to overlap at several points (Figures 2 and 6). DHFR reduces BH_2 to BH_4 *in vitro* (Nichol *et al.*, 1983) and probably *in vivo* (Niederweiser *et al.*, 1979). *In vitro* DHPR reduces quinonoid-dihydrofolates as well as q- BH_2 (Pollock & Kaufman, 1978) and N5,N10-methylene tetrahydrofolate (5,10 CH_2 THF) reductase, which normally converts 5,10 CH_2 THF to 5 CH_3 THF reduces q- BH_2 to BH_4 (Matthews & Kaufman, 1980). 5,6,7,8-tetrahydrofolate (THF) shows cofactor activity for phenylalanine hydroxylase *in vitro* (Kaufman, 1971). Findings in patients with defective bipterin synthesis, DHPR deficiency, 5,10 CH_2 THF re-

ductase deficiencies, congenital folate malabsorption and methotrexate toxicity also point to important links between folate and biopterin metabolism.

Hydroxylase cofactor activity and folate

Administration of folic acid to a patient with defective biopterin synthesis led to lowering of plasma phenylalanine and a rise in plasma tyrosine and serotonin (Hase *et al.*, 1982). Folate antagonists, such as amethopterin, block the conversion of phenylalanine to tyrosine *in vivo* (Goodfriend & Kaufman, 1961) as well as inhibiting DHFR and displacing folate in polyglutamates. The data suggest that folates may have hydroxylase cofactor activity *in vivo* as well as *in vitro* which might explain the near normal 'hydroxylase cofactor activity' in liver from a patient with

Figure 6 Simplified outline of folate turnover to illustrate conversion of 5,10-methylenetetrahydrofolate (5,10CH₂,THF) to N⁵-methyltetrahydrofolate(5CH₃,THF), and transfer of methyl groups (–CH₃) to homocysteine to form methionine. Formamino-group transfer is omitted. THF = 5,6,7,8-tetrahydrofolate (tetrahydropyteroylglutamic acid); 5,10CH₂THF = 5,10-methenylTHF; 10CH₀THF = 10-formylTHF; 5CH₀THF = 5-formylTHF (folinic acid); DHF = 7,8-dihydrofolate; FA = folic acid (pteroylglutamic acid); SAM = *s*-adenosylmethionine; SAH = *s*-adenosylhomocysteine; dUMP = deoxyuridine monophosphate; dTMP = deoxythymidine monophosphate. Enzymes: 1 = 5,10CH₂THF reductase, 2 = formyl,methenyl,methyleneTHF synthetase, 3 = 5CH₃,THF:homocysteine methyltransferase, 4 = DHF reductase.

defective bipterin synthesis (Bartholome *et al.*, 1977) despite low biological bipterin activity (Leeming & Smith, 1979).

Folate antagonists

It has been suggested that the effect of amethopterin on phenylalanine metabolism *in vivo* is due to inhibition of DHPR activity (Goodfriend & Kaufman, 1961) but there is no confirmation of this. Indeed, children receiving amethopterin for treatment of leukaemia do not show decreased central amine turnover but rather increased turnover of both bipterins and amines (Leeming *et al.*, 1976c; Pinkerton *et al.*, 1985). This observation is compatible with the effect of amethopterin on bipterin metabolism *in vitro* where the drug blocks conversion of exogenous BH₂ to BH₄ (due to inhibition of DHFR) but increases *de novo* BH₄ synthesis (Nichol *et al.*, 1983). The data suggest that folate may be a physiological inhibitor of bipterin synthesis and indeed folates reduce the activity of GTP cyclohydrolase *in vitro* (Kapatos & Kaufman, 1983).

Folate disturbance in DHPR deficiency

Patients with DHPR deficiency develop low concentrations of total folate in serum, red cells and CSF (Butler *et al.*, 1978; Tada *et al.*, 1980; Harpey, 1983; Smith *et al.*, 1985). As yet there are no published reports of investigations of folate metabolism in patients with defective bipterin synthesis. Neurological deterioration has been clearly linked to the folate disturbance in patients with DHPR de-

ficiency (Table III) (Harpey, 1983; Smith *et al.*, 1985) and development of folate deficiency may be accompanied by CT scan changes showing calcification in the lentiform nucleus extending into the periphery with attenuation in the white matter suggestive of demyelination (Tada *et al.*, 1980; Smith *et al.*, 1985). This same appearance has been described in patients with congenital folate malabsorption and in children with leukaemia who have received amethopterin (Smith *et al.*, 1985; Corbeel *et al.*, 1985). Examination of the brain in one patient (Tada *et al.*, 1980) showed diffuse demyelinating lesions with micro-calcification localized around small blood vessels, an appearance also reported in a patient with 5,10CH₂THF reductase deficiency (Wong *et al.*, 1977).

Despite low levels of folate in red cells and serum, patients with DHPR deficiency show no haematological or other peripheral signs of folate lack and in this they resemble patients with 5,10CH₂THF reductase deficiency (Wong *et al.*, 1977; Niederwieser, 1979). The latter disorder causes neurological symptoms which include progressive pyramidal, spino-cerebellar and dorsal column degeneration accompanied by developmental regression and Parkinsonism. A diagnosis of subacute combined degeneration of the cord with severe cerebral involvement due to folate lack has been confirmed at post-mortem in one patient (Clayton *et al.*, 1985). Following the development of symptoms of cord damage a clinical diagnosis of subacute combined degeneration was also made in a patient with DHPR deficiency (Smith *et al.*, 1985).

5,10CH₂THF reductase is required for normal turnover *in vivo* of 5CH₃THF (Figure 6). This is the

Table III Folate and amine disturbance in a patient with dihydropteridine reductase deficiency. Total folate by *Lactobacillus casei* assay

	Age in months	Red cell folate (ng/ml)	Serum folate (ng/ml)	Folate (ng/ml)	CSF HVA (ng/ml)	5HIAA (ng/ml)
<i>Diagnosis DHPR deficiency – hypotonia hypkinesia</i>						
Diet only	3	1210	34	36*	17	5
<i>Good progress – normal development</i>						
Diet + L-dopa, 50Htrypt	9	920	26	25*	102	38
<i>Deterioration begins</i>						
BH ₄ + L-dopa, 50Htrypt	29	not done	not done	6*	34	14
<i>Progressive neurological disease</i>						
L-dopa 50Htrypt	33	61	3	4	116	60
<i>Diagnosis of folate deficiency – given folic acid</i>						
Folic acid 2.5 mg/kg for 10 days	33	134	13	3	20	12
<i>Given folinic acid – improved + + but cord lesion persists</i>						
Folinic acid 3 mg/d for 4 months	37	> 1000	36	26	109	28

Normal values: red cell 150–300 ng/ml, serum 10–15 ng/ml, CSF 18–50 ng/ml.

*Measurements made at 33 months of age on stored CSF.

form of folate present in plasma and it is transported preferentially across the blood-brain barrier (Levitt *et al.*, 1971). 5CH₃THF is also donor of the methyl group for methyl-cobalamin, the derivative of vitamin B12 (Figure 6) required for conversion of homocysteine to methionine (Nixon & Bertino, 1970; Scott *et al.*, 1983). It is not surprising therefore that deficiency of 5,10CH₂FH4 reductase should cause the same lesions as vitamin B12 deficiency.

To try and explain the folate disturbance in DHPR deficiency it has been proposed that the dihydropyrimidines which accumulate in this disease interfere with folate metabolism, perhaps by competitively inhibiting 5,10CH₂THF reductase and DHFR (Smith *et al.*, 1985). The beneficial effects of 5-formyltetrahydrofolate (folinic acid) in patients with deficiency of DHPR (Harpey, 1983; Smith *et al.*, 1985), or 5,10CH₂THF reductase (Harpey *et al.*, 1981), could then be attributed to an increase in the concentrations of substrate for the folate reductases and increased synthesis of THF (Figure 6).

Folic acid exacerbates symptoms in patients with DHPR deficiency (Harpey, 1983; Smith *et al.*, 1985), and in some patients with deficiency of 5,10CH₂THF reductase (Clayton *et al.*, 1985). The harmful effect of folic acid on the neurological disease in B₁₂ deficient patients is well known. Administration of folic acid causes a rise in plasma concentrations of non-methyl folates (Ratanasthien *et al.*, 1977), and 10 days' folic acid therapy in a patient with DHPR deficiency failed to raise CSF folate levels (Table III). It is suggested that folic acid reduced transport of 5CH₃THF across the blood-brain barrier (Levitt *et al.*, 1971) and

competed with dihydrofolate for DHFR thus exacerbating neuronal deficiency of tetrahydrofolates.

Finally, patients with 5,10CH₂THF reductase deficiency exhibit not only Parkinsonism but also severely reduced CSF concentrations of HVA, 5HIAA, bipterins and neopterins (Harpey *et al.*, 1981; Clayton *et al.*, 1985) and patients with DHPR deficiency receiving amine replacement therapy also show exacerbation of amine deficiency when folate deficiency supervenes (Harpey, 1983; Smith *et al.*, 1985). Folic acid administration was accompanied by increased clinical and biochemical evidence of amine disturbance in a patient with DHPR deficiency (Table III) (Smith *et al.*, 1985) and another with 5,10CH₂THF reductase deficiency (Clayton *et al.*, 1985). Dietary folate deficiency, or loading with folic acid, are associated with reduced amine metabolites in CSF in the rat and in humans (Botez *et al.*, 1979).

To explain the amine disturbance it is suggested that methyl-folate deficiency inhibits release of amine neurotransmitters at nerve endings, although no information is available to confirm this. There seems little doubt, however, that the metabolism of folates, bipterins and amines is closely linked, although the mechanisms and functions remain to be elucidated.

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