Malignant Hyperphenylalaninemia—Clinical Features, Biochemical Findings, and Experience with Administration of Biopterins

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Summary

Four cases of malignant hyperphenylalaninemia (MHPA) are described. Pretreatment serum phenylalanine levels were 1.5, 3.0, 2.4, and 0.9 mmol/L. Dihydropteridine reductase (DHPR) deficiency was proven in one patient by assays on cultured fibroblastic cells and was presumed in her sibling and in another deceased patient whose parents' fibroblastic cells show approximately 50% of normal enzyme activity. DHPR and phenylalanine hydroxylase deficiency were excluded by assays on liver obtained at autopsy in the 4th patient. Parenteral administration of tetrahydrobiopterin (BH₄) corrected the hyperphenylalaninemia and increased the levels of catecholamines and 5-hydroxy-indoles in the one patient studied in life, but BH₄ did not reach the cerebrospinal fluid. A 3-wk course of BH₄ therapy had no clinical effect. Oral biopterin was absorbed and excreted in the urine, but did not alter the serum phenylalanine level. The frequency of MHPA in Australia was estimated as 7 in 258 patients with phenylketonuria.

Speculation

Response of serum phenylalanine to a single injection of BH₄ appears to be the most simple method of diagnosis of MHPA, applicable to all newly diagnosed cases of PKU, provided the pharmaceutical industry can be persuaded to make small quantities of this compound available.

The enzymic hydroxylation of phenylalanine (Phe) is believed to comprise a complex group of reactions.

The relevance of reaction (4) *in vivo* is particularly debatable and the instability of q-BH₂ makes complete delineation of the reactions very difficult.

Classical PKU involves a genetic defect of PH. Deficiency of DHPR has been defined recently (15, 18, 25). Some patients with PKU have proved to have normal levels of both DHPR and PH (3, 25, 26) and presumably have a defect in *de novo* synthesis of BH₄. These defects in BH₄ synthesis and metabolism have been found among patients with PKU who have shown progressive cerebral degeneration despite early initiation of dietary treatment. This clinical syndrome has been named malignant hyperphenylalaninemia (MHPA) (6). The neurologic disorder in these patients

has been attributed to defective production of neurotransmitters derived from hydroxylation of tyrosine and of tryptophan (3, 4). The results of treatment with L-dopa and 5-hydroxytryptophan support this contention (2, 3, 7).

Four patients with MHPA seen in Melbourne since 1963 are presented. One patient has been shown to have DHPR deficiency and her sister is presumed to have died of this defect. Both parents of another baby had DHPR levels in the heterozygote range suggesting DHPR deficiency as the cause of her death. The 4th baby had neither PH or DHPR deficiency and defective BH₄ synthesis is presumed.

Detailed metabolic studies, especially related to BH₄ are presented in the one DHPR deficient patient diagnosed in life. The lowering of serum phenylalanine achieved by BH₄ administration in this patient is proposed as a test for identification of young babies with MHPA.

Some of the findings have been published in preliminary form (8, 9).

CASE REPORTS

CASE 1

G. P., female, born at full term on October 4, 1963, weighing 2.3 kg was the 1st child of Italian parents who were first cousins. Frequent convulsions commenced at 3 months. Motor development was moderately delayed, but no other abnormality was apparent. Head circumference was 37.5 cm. Serum phenylalanine was 1.52 mmol/L. A low phenylalanine diet was started at 15 wk of age and serum phenylalanine levels were maintained between 0.1–0.6 mmol/L. However, the convulsions increased, the EEG showed hypsarrhythmic features and she began to lose the few skills present. Progressive deterioration led to death at the age of 4 yr, 3 months. No autopsy was performed.

CASE 2

K. N., female, born July 2, 1968 was the 1st child of first cousin parents of Australian (British) origin, delivered at 32–34 wk of gestation weighing 2.15 kg. Routine Guthrie test was not performed. At 14 wk of age, she was failing to gain weight satisfactorily, and showed spastic quadriplegia and microcephaly (head circumference 35.5 cm). The serum phenylalanine and tyrosine were 3.0 and 0.16 mmol/L, respectively. An EEG showed recurring abnormal sharp transient waves. She was investigated intensively for a second cause of mental retardation without success.

Low phenylalanine diet maintained serum levels between 0.1 and 0.6 mmol/L. However, she showed further gradual deterioration of neurologic function and finally died at the age of 3 yr, 3 months. Dietary treatment had been abandoned at 21 months.

Autopsy was performed and the only significant abnormalities were the extremely small size of the brain and extensive vacuolation within the white matter.

CASE 3

M. Z., male, born November 18, 1971 at full term weighing 3.43 kg was the 1st child of unrelated parents of Maltese origin. Detected by Guthrie test on day 11, the serum phenylalanine was 2.4 mmole/liter on day 16 when the low phenylalanine diet was commenced. Control was satisfactory with serum phenylalanine levels always between 0.1 and 0.5 mmole/liter. Brief flexor spasms and developmental delay became apparent at 5 months of age, but an EEG was normal. Intensive investigations failed to reveal a second cause of retardation, metabolic or infective. Unusual physical features suggested the mosaic trisomy 8 syndrome; however, the karyotype was normal in cultivated blood lymphocytes and skin fibroblasts.

He deteriorated progressively with repeated major convulsions and died at 3 yr, 4 months. No autopsy was performed.

CASE 4

S. P., a sister of case 1, was born on March 3, 1974 at term weighing 3.12 kg. One normal boy had been born since the birth of case 1.

Guthrie test performed on day 4 gave a result of 0.4 mmole/liter. Serum phenylalanine was 0.42 mmol/liter on day 16. Breast feeding was continued and the level rose gradually to 0.9 mmol/liter by 5 wk, when a low phenylalanine diet was introduced. Serum phenylalanine levels were maintained between 0.1–0.6 mmole/liter. Progress appeared within normal limits until 8 months of age, when she had six brief convulsions in 24 hr and an EEG showed frequent epileptic discharges. Regression in abilities began, despite the use of anticonvulsants. Investigations performed at 11 months showed gross disorganization of the EEG and cerebral atrophy in a pneumoencephalogram. Diet was discontinued because the serum phenylalanine rose to only 0.6 mmole/liter on normal diet.

At this stage, the idea of a fault in BH_4 supply was first entertained.

A series of trials of administration of BH_4 caused biochemical changes described later in this paper, but a 3-wk course of BH_4 and a similar length course of neurotransmitter replacement therapy produced no clinical improvement. Both treatments were given at a stage of very severe brain damage.

Her disease progressed relentlessly with gradual loss of all motor and social skills, development of mild muscle spasticity, and frequent minor motor and myoclonic seizures. She suffered repeated chest infections and died at home in one of these episodes at 2½ yr. No autopsy was allowed. The parents allowed only limited investigation in life and did not allow liver biopsy.

Her growth showed an unusual pattern. Her weight and length were initially a little below the 50th percentile, but she showed progressive acceleration in growth from 3 months of age to be 0.5 kg and 2 cm above the 97th percentiles at 2 yr. Her head circumference failed to increase in this way and fell from 50th percentile at 3 months to the 3rd percentile at 2½ yr.

MATERIALS

Some tetrahydrobiopterin was kindly donated by Roche Products. Subsequently, it was made from biopterin purchased from Regis Biochemical Company, IL by hydrogenation over platinum oxide catalyst in 0.01N HCl (17), the product being identified by high voltage electrophoresis (27) and by its spectrum (20). 2-amino-6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine hydrochloride (DMPH) was obtained from Aldrich. Horse radish peroxidase type VI and nicotinamide adenine dinucleotide reduced form were from Sigma Chemical Company.

All other chemicals were analytical reagent grade.

METHODS

ADMINISTRATION OF PTERINS

Iv injections of BH_4 were prepared within 1 hr before administration by dissolving the required quantity in 1 ml of distilled water and filtering twice through two sterilizing 0.25 μ millipore filters. The injection of BH_4 was followed by at least 10 ml of 0.9% saline.

Im injections were prepared in a similar manner so that BH_4 (2 mg/kg body wt) and ascorbic acid (half the mass of BH_4) were contained in a solution made up to 0.5 ml with 0.1M citric acid/sodium citrate buffer, pH 5. This solution was stable over a period of 3 wk storage at -20°C as evidenced by the fact that the UV spectrum (265 nm peak) remained essentially unchanged. Oxidation was indicated by appearance of a band at 230 nm. In practice, the longest storage was for 2 wk at -20°C .

Oral doses were mixed with small quantity of food and administered by spoon, or else were given via nasogastric tube.

All human doses were given only after informed consent of the parents.

FIBROBLASTIC CELL CULTURE

Fibroblastic cells were grown from skin biopsies in Eagle's basal medium supplemented with 10% foetal calf serum. Cells were harvested at confluency after their 6th passage and stored frozen at -70°C after washing and pelleting. Extracts were prepared by homogenization or freezing and thawing of approximately 8×10^6 cells in 0.2 ml of 0.15M KCl. Cell debris was removed by centrifugation at 12,000 g for 30 min and the protein concentration in the supernatant was measured using bovine serum albumin as a standard.

ANALYSES RELATED TO PTERINS

DHPR activity was determined by measuring the pterin dependent oxidation of $NADH_2$ at 340 nm and 37°C using an optimized assay (10) based on the method described by Nielsen *et al.* (19). Pterins and fluorescent compounds in urine were studied semi-quantitatively by high voltage electrophoresis and quantitated by TLC (27).

Crithidia active pterins in plasma, urine, and cerebrospinal fluid were measured by previously described methods (11).

NEUROTRANSMITTERS AND OTHER METABOLITES

Total 5-hydroxyindole excretion was measured by a slightly modified method of Udenfriend *et al.* (28). Catecholamines were measured by published methods (chromatographic and spectrophotometric) (30, 31).

Serum folate was measured by the *Lactobacillus casei* bioassay. Serum phenylalanine and tyrosine were measured colorimetrically. Complete amino acid analyses were performed on a Technicon TSM automatic analyser.

RESULTS

Except where specifically noted, all results relate to patient S.P. (case 4). Her investigation was carried out over many months during which she received no specific dietary treatment, consumed a soft infant mixed diet with standard vitamin additives, and received only anticonvulsant therapy (diphenyl hydantoin and nitrazepam).

LEVELS OF METABOLITES WITHOUT TREATMENT

Amino Acids. Plasma phenylalanine levels were estimated on many occasions during this period of study and ranged from 0.37–0.55 mmole/liter. Plasma tyrosine was 0.06 mmole/liter. A complete amino acid analysis on urine and serum on many occasions showed all amino acids except phenylalanine to be in the normal range.

Tryptophan and Tyrosine Metabolites. 5-hydroxyindoles were barely detectable in urine (< 0.001 mmole/m mole creatinine). Normal children and adults were found to have levels between 0.002–0.03 mmole/m mole creatinine. Five untreated PKU adults excreted < 0.001 –0.003 mmole/m mole creatinine, and results in 8 untreated PKU children ranged from < 0.001 –0.003 mmole/m mole creatinine, with an inverse relationship between serum phenylalanine and urinary hydroxyindoles. The hydroxyindole level in cerebrospinal fluid of S. P. was 91.6 μ g/ml.

Table 2. Response of S. P. serum phenylalanine to iv BH_4

Dose of BH_4 (mg-iv)	Serum phenylalanine (mmole/l)	
	Before dose	2 hr after dose
1	0.55	0.49
10 ¹	0.44	0.34
50 ¹		0.14
100 ¹		0.09

¹ Doses given on 3 successive mornings. Patient's weight was 10 kg at this time.

Fig. 1. Response of serum phenylalanine in S. P. to an iv dose of BH_4 (2 mg/kg). The arrow indicates the initial serum Phe levels. Axes for Figure 1: X: time after BH_4 (hr) 0, 20, 40, 60, 80. Y: plasma phenylalanine concentration (mmole/liter) 0, 0.1, 0.2, 0.3, 0.4, 0.5.

Dopamine, adrenaline, and nonadrenaline excretions were 2.1, 0.76, and 1.58 μ g/24 hr, respectively (normal adult averages for the laboratory 197, 1, and 57 μ g/24 hr, respectively). The cerebrospinal fluid homovanillic acid level was 29.2 μ g/ml.

Lack of age matched control data hinders interpretation of some of these results, but most are grossly below normal adult levels and all serve for comparison with results after BH_4 treatment (Table 1).

Pterins. Urinary hydroxylalkylpterins (HAP), assayed as Crithidia activity, were measured on 6 occasions and ranged from 1.5–3.2 μ g/ml (normal adults 2.1 μ g/ml). Expressed as excretion per 24 hr, the average was 0.7 mg (normal adults 1.5–2.4 mg/24 hr) (16).

Plasma levels ranged from 3.0–17.5 ng/ml (normal adults 1.8 ng/ml). In CSF, a single estimate was 0.79 ng/ml (normal range (age unspecified) 0.25–0.7 ng/ml) (1). Normal levels in infants are not known.

HVE of urine showed large amounts of 7,8-dihydroxanthopterin (XH_2), a compound present in no more than trace amounts in normal urine, but seen in urine of PKU patients with high serum phenylalanine levels (29). The XH_2 concentration (27) in her urine ranged from 5–9 μ mole/m mole creatinine on 5 occasions. Similar levels were found in PKU patients with serum phenylalanine levels over 2 mmole/liter, but PKU patients under dietary control had levels below 1.0 μ mole/m mole creatinine. (27)

Other metabolites. Organic acids were normal by GLC except for variable mild excretion of phenylketones. Serum folate values were 0, 4, and 6 ng/ml on 3 estimations (normal 15–21 ng/ml).

Some key steroids were measured and were not remarkably different from normal (Table 1). A GC-MS urinary steroid profile was unremarkable (21).

RESPONSE OF METABOLITES TO ADMINISTRATION OF BIOPTERINS

Parenteral tetrahydrobiopterin. Table 2 shows the results of iv administration of increasing doses on successive days. This led to the choice of 2 mg/kg for all further studies.

Measurement of serum phenylalanine at intervals after a single iv dose (2 mg/kg) revealed maximal lowering at 6 hr and a slow return towards the pretreatment level (Fig. 1). The level of serum phenylalanine and the pattern and duration of response to BH_4 were not significantly altered by pretreatment with ascorbic acid (250 mg 6 hourly for 2 days before, and on the day of BH_4 ,

Table 1. Levels of metabolites in S. P. before and after parenteral BH_4 treatment

Fluid	Metabolite	Level		Treatment ¹
		Before first dose	After last dose (time)	
Plasma	Phenylalanine (mmole/liter)	0.43	0.08 (6 hr)	2
		0.53	0.09 (1 day)	1
	Tyrosine (mmole/liter)	0.06	0.07 (6 hr)	2
	Pterins (HAP) (ng/ml)	8.04	11.7 (1 day)	1
	Progesterone ² (nmole/liter)	0.45	0.45 (120 min)	4
	17 α hydroxy-progesterone (nmole/liter)	1.5	7.1 (165 min)	3
	Cortisol (nmole/liter)	650	830 (5 days)	3
	Testosterone (nmole/liter)	0.5	0.7 (165 min)	3
Cerebrospinal fluid	Homovanillic acid (μ g/ml)	29.2	43.8 (1 day)	1
	Pterins (HAP) (ng/ml)	0.79	0.75 (1 day)	1
	Hydroxyindoles (ng/ml)	91.6	146.6 (1 day)	1
Urine	Pterins (HAP) (μ g/mg creatinine) (mg/24 hr)	9.69	13.2 (1 day)	1
		0.7	1.6 (1 day)	1
	Hydroxyindoles (mole/mole creatinine) (mg/24)	< 0.001	0.02 (1 day)	1
		< 0.06	4.2 (1 day)	1
	Dopamine (μ g/ml)	2.11	6.12 (1 day)	1
	Adrenaline (μ g/ml)	0.76	2.16 (1 day)	1
	Noradrenaline (μ g/ml)	1.58	2.18 (1 day)	1

¹ Treatment: 1) Daily dose of BH_4 , 30 mg iv, for 3 days; 2) Single iv dose of 25 mg; 3) Three successive doses illustrated in Table 1; 4) After the 50 mg dose in Table 1.

² Approximate. At lowest limit of assay. (Level comparable to follicular phase of normal adult female).

Table 3. Effect of iv BH₄ (2 mg/kg) on serum phenylalanine and total urinary hydroxyindoles in patients with PKU, tested before diet was commenced

Patient	Time after injection (hr)	Serum phenylalanine (mmole/liter)	Urinary hydroxyindoles
			(mmole/mmol creatinine)
A	Before	2.4	0.003
	6	2.7	0.004
B	Before	0.7	0.003
	6	1.5	
	14		0.003
C	Before	>3.0	0.001
	5	>3.0	0.006
D	Before	3.3	0.001
	3	3.4	0.004
E	Before	0.8	0.003
	3	1.0	0.005
F	Before	1.0	<0.001
	2	1.2	0.007
G	Before	2.8	0.001
	5	2.8	0.004
H	Before	2.3	<0.001
	12	2.4	0.005

administration). In another experiment, BH₄ stabilized with ascorbic acid (described previously) and administered by im injection produced effects identical to those obtained by iv administration.

The effect of iv BH₄ given on 3 successive days upon various related metabolites was measured (Table 1). The increase in urinary 5-hydroxyindoles was confirmed on many occasions, but the other neurotransmitters and their metabolites were measured only on this one occasion. Crithidia activity (HAP) increased in plasma and urine, but not in c.s.f., although hydroxyindoles did increase in c.s.f. The increase in HAP in plasma persisted for only a few hr, but increased urinary excretion persisted for more than 2 days.

Finally, a trial of more prolonged im administration of BH₄ (2 mg/kg/day for 3 successive days and then, on alternate days, for eight further doses) maintained the serum phenylalanine at normal levels (< 0.1 mmole/liter), but failed to produce any change in the clinical state of the child, as assessed by the parents, nurses, and doctors, by EEG, and by recording of her very frequent minor epileptic seizures. At this time, her brain damage was very profound. Toxic effects on neutrophils, platelets, and renal function were sought, but not found. No local reaction occurred at injection sites.

Urinary steroid excretion increased after iv BH₄ and this effect was particularly marked for testosterone which increased 50-fold after the dose (21).

The interpretation of these findings is supported by observations in six babies with classical PKU and two babies with hyperphenylalaninemia given BH₄ (2 mg/kg iv) to exclude the presence of MHPA during initial investigation of a positive Guthrie test. No significant change in serum phenylalanine level was observed in any of these patients. Some increase in urinary 5-hydroxyindoles was observed (Table 3).

Oral bipterin. Pure bipterin (45 mg = 3 mg/kg) was given orally. The plasma Crithidia activity doubled by 6 hr, but there was no change in the plasma phenylalanine or in the urinary 5-hydroxyindole excretion. The phenylalanine/tyrosine ratio in urine collected at intervals for 70 hr after this dose was analyzed

to look for any possible delayed effect, but no change was found. (By contrast, iv BH₄ changed this ratio from 5.04–1.99).

RESPONSE TO NEUROTRANSMITTER THERAPY

The regime of treatment with L-Dopa, Carbidopa, and 5-hydroxy-tryptophan described by Bartholomé and Byrd (2) was used for 3 wk and failed to cause any clinical response judged by the criteria listed in assessing clinical response to BH₄ treatment. No toxic effects were seen. Urinary 5-hydroxyindoles levels rose from undetectable to normal levels within 2½ hr of administration.

DHPR ACTIVITIES

Table 4 shows results obtained on fibroblastic cells in culture from S. P. and her parents, and from the parents of K. N. and of M. Z. These show severe DHPR deficiency in S. P. and levels anticipated in heterozygotes for this mutation in her parents. A previous statement (8) that S. P.'s fibroblastic cells showed 25% of normal activity referred to assays performed with conditions different from those later found to be optimal (10). The DHPR levels in the parents of M. Z. were strongly suggestive of heterozygote status, suggesting that he suffered homozygous DHPR deficiency.

The results in K. N. and her parents are not so easily interpreted. The activities in her parents' cells are between the lower limit of normal and the heterozygote levels. However, DHPR activity in her liver, stored for 7 yr at –20°C after a very prompt autopsy, were within the range of results in other livers stored for comparable periods. Phenylalanine hydroxylase activity was also present in this liver (5).

FREQUENCY OF MHPA

Through the cooperation of the doctors in charge of all the PKU Clinics in Australia, it was possible to assess the clinical response to dietary treatment in 258 PKU patients diagnosed between 1962 and 1975. Only three further patients had shown progressive clinical deterioration despite treatment, making a possible frequency of MHPA of 7 in 258 or about 3% of patients with PKU.

Table 4. DHPR activity in cultured fibroblasts and in liver obtained at autopsy

Cells or tissue	DHPR Activity ¹ nmole NADH oxidized/min/mg protein
Fibroblasts	
Normal (11 individuals)	49.6 ± 9.3 (37.4–60.5)
Phenylketonuric (12 individuals)	46.3 ± 6.9 (35.0–60.5)
Patient S. P.	None detected ² (4)
Mother P	12.8 (4)
Father P	20.5 (4)
Mother Z	19.1 (3)
Father Z	22.3 (3)
Mother N	33.9 (3)
Father N	28.3 (3)
Liver obtained at autopsy	
Controls ³ (16 patients)	370 ± 80 (230–560)
Patient K. N.	402

¹ Values reported as mean ± SD for groups, numbers in parentheses being the range of values observed. Results for individuals are means of the number of separate estimates indicated in parentheses, each estimate from a different cell passage.

² DHPR activity 1% of normal could be confidently determined by this assay procedure.

³ Control autopsy liver samples were from patients of similar age to K. N. dying of unrelated diseases. Thirteen samples had been stored for a similar period of time (about 6 yr) at –20°C. Three samples were obtained within 12 hr of death and were assayed immediately. The results in these two groups were similar.

DISCUSSION

The clinical course of the illness in these patients was similar to that described in other patients with MHPA (summarized previously (7)). Somatic overgrowth in S. P. was the only unusual feature. Hypersalivation and difficulty in swallowing were not remarkable.

S. P. and her sister G. P. clearly had DHPR deficiency. The levels of DHPR activity in the parents of M. Z. suggest that he also had this defect. This defect seems very unlikely in K. N. who presumably had a defect in BH₄ synthesis.

The occurrence of DHPR deficiency in siblings of consanguineous parents gives strong support to other published data indicating autosomal recessive inheritance of this condition (7). Previous cases with defective BH₄ synthesis provided no clues to the pattern of inheritance. The parental consanguinity in the present case (K. N.) suggest autosomal recessive inheritance. However, genetic heterogeneity is particularly likely because there must be many steps in the synthesis of BH₄. MHPA appears to be present in 1–3% of patients with PKU, suggesting an overall incidence of 1–3/1,000,000 live births.

The main interest in this study centers upon the studies of pteridines in S. P. who appears to be the first human to whom BH₄ and biopterin have been administered.

The results obtained show that BH₄ administered iv or im acted as cofactor to the hepatic PH reaction and suggest that it also allowed hydroxylation of tyrosine and tryptophan. Oral administration was not adequately tested because traditional beliefs suggested that BH₄ was unlikely to be absorbed. However, Schaub *et al.* (26) have recently shown the oral BH₄ was effective in a patient with defective BH₄ synthesis. Oral administration of biopterin had no effect on phenylalanine metabolism despite changes in blood and urine Crithidia activity that strongly suggest that the biopterin was absorbed. This indicates that humans cannot reduce biopterin to BH₄ to any functionally important degree.

The effect of BH₄ on PH function was expected, but the dosage required was considerably less than that calculated on theoretical grounds, including the assumption that there is no recycling of BH₄ in a DHPR deficient patient (13). This may indicate residual capacity to reform BH₄ from q-BH₂ because of residual DHPR activity or using other pathways. DHFR (reaction 4 previously) might be credited with this role (14). The effect was even greater and more prolonged in a patient with MHPA and normal DHPR (26) presumably because recycling of BH₄ is intact. BH₄ had no effect on serum phenylalanine levels in patients with classical PKU as expected in a genetic fault in PH itself.

The increase in catecholamines and 5-hydroxy indoles in S. P. after BH₄ administration indicates hydroxylation of tyrosine and tryptophan, but does not define the organ in which this is occurring. The aim was to stimulate hydroxylation in the brain, but BH₄ has been shown to enter the brain very poorly (22) and HAP did not increase in the c.s.f. after BH₄ administration. It seems likely that the effect was on extracranial sites of catecholamine and serotonin production. Better definition of this point may be of some importance because long-term oral administration of BH₄ would be a very logical and complete form of therapy of MHPA if the BH₄ could be delivered past the blood-brain barrier. Even if this is impossible, oral BH₄ plus neurotransmitter replacement would be a relatively simple form of therapy, especially in patients with normal DHPR who can recycle the BH₄.

The fact that neither BH₄ therapy or neurotransmitter replacement had any clinical effects, prevents any clinical interpretation of the 3-wk trial of BH₄. Other patients, treated at a less advanced stage, have responded very well to neurotransmitter replacement (7).

Measurement of serum phenylalanine before, and 6 hr after, administration of BH₄ (2 mg/kg iv or im) seems a very simple method of recognizing cases of MHPA among babies with a positive Guthrie test (7, 8) applicable in patients with DHPR deficiency and in those with defective BH₄ synthesis (26). The test requires no new laboratory methods, and a result is obtained within 1 day and before dietary treatment is started. It will allow

doctors to treat the 97–99% of these patients who do not have MHPA with the confidence that existed before 1974 and to proceed to definitive enzyme assays in patients whose phenylalanine levels fall after BH₄ administration.

MHPA should be excluded in every patient with a persistent elevation of serum phenylalanine and not only in those with levels high enough to need dietary treatment. The relatively low levels of serum phenylalanine in S. P. and G. P. illustrate this need.

Urinary XH₂ levels may also be useful in diagnosis, but pre-treatment levels alone may not distinguish DHPR deficiency from classical PKU with certainty. XH₂ levels fall to normal on dietary treatment in classical PKU and remain elevated in DHPR deficiency. This approach is discussed in more detail elsewhere (27).

The evidence for the identity of XH₂ has been published (29), but its origin is a matter of debate. Current experimental evidence in animals would predict that it arises by oxidation of BH₄ in the bladder (23) because XH₂ present in the circulation should be oxidized very rapidly to xanthopterin and, thus, to leucopterin by the action of xanthine oxidase (22). However, increased excretion of BH₄ in DHPR deficiency would seem very unlikely. BH₄ deficiency would be predicted and has been demonstrated in liver of a DHPR deficient patient (15). The response observed after BH₄ administration is in accord with the expected tissue deficiency of BH₄. Consequently, it seems necessary to postulate that XH₂ in the urine results from filtration of q-BH₂ or one of its oxidation products followed by further oxidation in the bladder.

Ascorbic acid had no effect upon the serum phenylalanine levels and no potentiating effect upon the action of BH₄, despite *in vitro* evidence of a chemical effect upon reduction of q-BH₂ to BH₄ (12).

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