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Early Diagnosis of 6-Pyruvoyl-tetrahydropterin Synthase Deficiency

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Summary

6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency, which used to be called dihydrobiopterin synthase deficiency, is the most common kind of tetrahydrobiopterin deficiency. Early treatment by administration of tetrahydrobiopterin and neurotransmitter precursors helps to prevent neurological injury, so prompt diagnosis of neonates with hyperphenylalaninemia discovered by screening for phenylketonuria is necessary. Three patients with PTPS deficiency were diagnosed by pteridine analysis. All patients had low biopterin and high neopterin levels in the urine, resulting in a neopterin to biopterin ratio (N/B) much higher than that of age-matched controls. The mean N/B in the parents of these patients was twice that of healthy unrelated adults. PTPS activity was measured in one of these patients with PTPS deficiency and in his family members; the patient was homozygous and his parents were heterozygous for PTPS deficiency. This result meant that N/B could be used as an index of PTPS activity. In healthy subjects studied cross-sectionally, urinary levels of pteridine decreased in groups of increasing age, and the same change was found in subjects with hyperphenylalaninemia studied cross-sectionally. Thus, pteridine values of patients can be compared meaningfully only with age-matched controls. The urinary N/B is useful for the diagnosis of homozygotes and heterozygotes for PTPS deficiency.

Key words: Phenylketonuria, Tetrahydrobiopterin, Malignant hyperphenylalaninemia, 6-pyruvoyl-tetrahydropterin synthase deficiency

Abbreviations
PKU: phenylketonuria
BH_4: tetrahydrobiopterin
DHPR: dihydropteridine reductase
L-DOPA: L-3,4-dihydroxyphenylalanine
5-HTP: 5-hydroxytryptophan
BH_2: dihydrobiopterin
NH_2P_3: dihydroneopterin triphosphate
PTPS: 6-pyruvoyl-tetrahydropterin synthase
N: neopterin
B: biopterin
EEG: electroencephalogram
SD: standard deviation
CT: computerized tomography
HPLC: high-pressure liquid chromatography
Hb: hemoglobin

Introduction

Phenylketonuria (PKU) is an inherited metabolic disease once thought to be caused only by a defect of phenylalanine hydroxylase (phenylalanine 4-monooxygenase; EC 1.14.16.1), as described by Jervis (1) in 1953. Patients with PKU were at first thought to develop normally if they are kept from early infancy on a low-phenylalanine diet, as Bickel *et al.* (2) proposed in 1953. In 1975, Smith *et al.* (3) reported three patients with progressive neurological illness that was unlike classical PKU and did not respond to a low-phenylalanine diet. Since then many such patients have been identified, and the phenylalanine hydroxylase system in patients with PKU has been reevaluated.

Kaufman (4) reported in 1963 that tetrahydrobiopterin (BH_4) is a cofactor for phenylalanine hydrox-

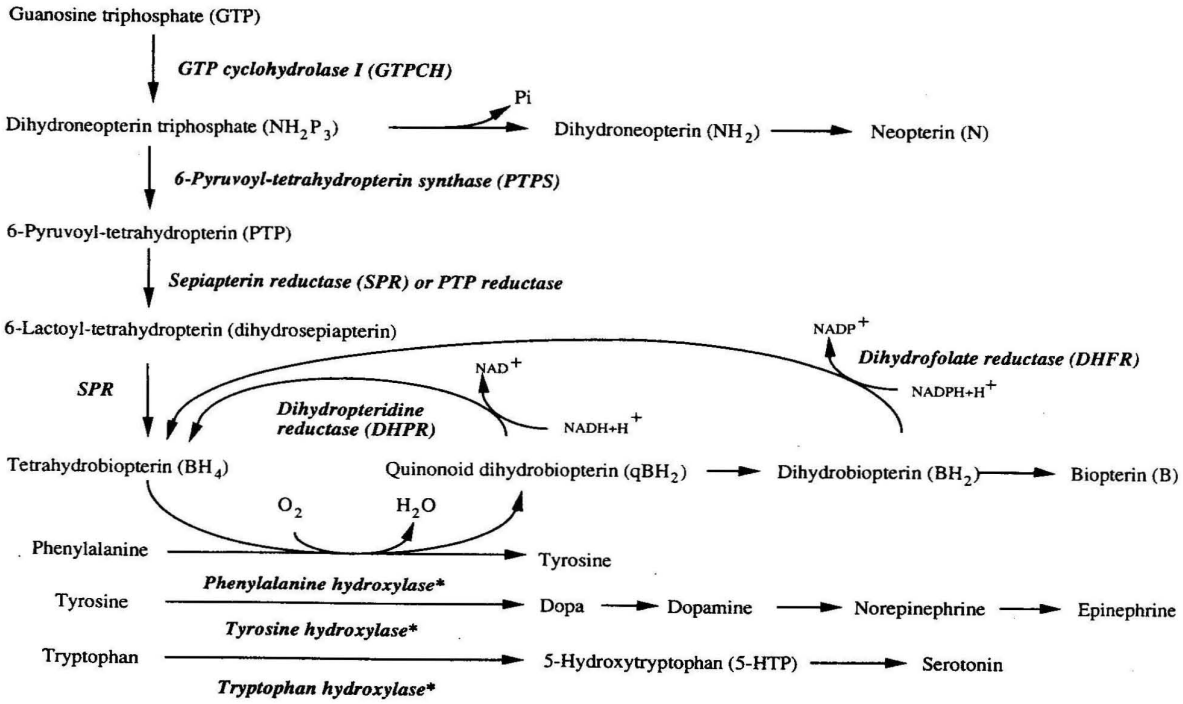

Figure 1. Tetrahydrobiopterin (BH_4) biosynthetic pathway and aromatic amino acid hydroxylase system.

ylase (3), and later it was found that BH_4 is a cofactor of tyrosine 3-hydroxylase (EC 1.14.16.2) and tryptophan 5-hydroxylase (EC 1.14.16.4), as well (5, 6). (Fig. 1).

Kaufman *et al.* recommended that patients with PKU resembling that reported by Smith *et al.* be examined not only for phenylalanine hydroxylase but also for BH_4 and dihydropteridine reductase (DHPR; EC 1.6.99.7). In one of the patients reported by Smith *et al.* (3), phenylalanine hydroxylase was normal but DHPR was deficient, so no BH_4 was detected in the liver (7). In DHPR deficiency, biopterin levels are higher than normal, but BH_4, the active form of biopterin, is deficient, so serum phenylalanine increases. In 1978, Kaufman *et al.* (8) reported that a child with PKU who had neurological impairment despite early dietary control of elevated blood phenylalanine levels had normal levels of phenylalanine hydroxylase and DHPR activity, but a very low level of BH_4, which pattern suggested a deficiency of biopterin biosynthesis. Seventeen patients who developed neurological illness in spite of a low-phenylalanine diet were reported at a workshop in Lausanne in 1977 (9), and their disorder was named "malignant hyperphenylalaninemia". There were nine patients with DHPR deficiency, two patients with normal DHPR but BH_4 deficiency, and six patients with an unclear diagnosis. Eleven of the seventeen patients had already died. Of the six survivors, three were younger than 1 year old and the oldest was 6 years old. The miserable clinical outcomes arose because the BH_4 deficiency led to dysfunction of the aromatic amino acid hydroxylase system, which produces neurotransmitters from tyrosine and tryptophan, so that serotonin and catecholamine are not synthesized. Bartholomé *et al.* (10) reported that neurological signs in these patients are treatable by the oral administration of the neurotransmitter precursors 3,4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP), which both pass the blood-brain barrier, and Curtius *et al.* (11) recommend that this treatment be started within 1 month after birth to help prevent neurological damage. Therefore, a noninvasive diagnostic method for malignant hyperphenylalaninemia for use during neonatal mass-screening for PKU is needed.

Before 1985, it was believed that dihydrofolate reductase produced BH_4 from dihydrobiopterin, which was thought to be synthesized from dihydroneopterin triphosphate (NH_2P_3) (11). For that reason, patients with BH_4-defective biosynthesis from NH_2P_3 were said to have "dihydrobiopterin synthetase deficiency." The BH_4 biosynthetic pathway as it is currently understood is shown in Fig. 1. The deficiency is generally believed to involve a defect in 6-pyru-

voyl-tetrahydropterin synthase (PTPS) (12). Therefore, the expression "BH_4 deficiency" should be used rather than the terms "malignant PKU" or "malignant hyperphenylalaninemia" (13).

I have seen three patients with PTPS deficiency, including the first patient to be diagnosed in Japan, and diagnosed these patients by BH_4 loading tests, phenylalanine loading tests, and assays of urinary pteridines. The results of assays of urinary pteridines showed that the neopterin to biopterin ratio (N/B) was useful not only for the diagnosis of PTPS deficiency but also for the detection of heterozygotes for PTPS deficiency.

Case reports

Case 1. The first patient, a boy, was born on Oct. 12, 1973 at term after an uncomplicated pregnancy, as the second child of unrelated healthy parents. Birth weight was 2650 g. The infant developed normally until 3 months of age. He could not control his head movements at the age of 4 months, and starting then, convulsions occurred every 7 to 10 days. At the age of 6 months, anticonvulsant drugs (phenobarbital and diphenylhydantoin) were started because of hypsarrthythmia in the electroencephalograms (EEG), but the convulsions were not well controlled. At the age of 8 months, the patient was brought to this hospital. Head control was poor and the head circumference was very small (−2SD). The hair was brown rather than black and the skin was pale. The patient was expressionless and there were severe hypotonia, weak tendon reflexes, and feeding difficulties. The infant's development quotient was 24. Results of the ferric chloride reaction were positive and the serum level of phenylalanine was 16 mg/dl (0.97 mmol/l); the patient was diagnosed as having PKU. A low-phenylalanine diet (with a daily intake of 120 mg/kg) was started and after 3 days of the diet, the serum level of phenylalanine had decreased to 2 mg/dl (0.12 mmol/l), so that starting when the patient was 9 months of age, his phenylalanine intake was increased gradually until the daily intake was 120 mg/kg (the normal amount). The serum level of phenylalanine remained in the vicinity of 8 mg/dl (0.48 mmol/l). However, a high-protein diet (4 g/kg daily) started at this time increased serum phenylalanine to about 16-20 mg/dl (0.97-1.21 mmol/l), and the patient was diagnosed as having hyperphenylalaninemia.

The clinical signs improved temporarily when treatment was started, but later, signs worsened again, including the EEG findings, and the convulsions continued. When the patient was 18 months old, his cultured fibroblasts were sent to Dr. S. Kaufman (NIH) for measurement of DHPR activity, because of the report that "malignant PKU" involves DHPR deficiency (7). The test results showed normal activity, so the patient did not have DHPR deficiency. When the child was 3 and a half years old, a report was published that among patients with malignant PKU, some have normal DHPR activity, although the clinical signs in such patients improve when the same treatment as for DHPR deficiency is given. The patient was therefore given 160 mg/kg L-DOPA and 20 mg/kg 5-HTP. His convulsions became less frequent and the hypsarrthythmia disappeared. In the meantime, the serum level of phenylalanine was maintained at about 4 mg/dl (0.24 mmol/l) without a low-phenylalanine diet. The hypotonia was unchanged and speech did not begin, but the child's facial expression improved. The child weighed 9 kg when he reached 6 years of age, and he never weighed more than 10 kg because of feeding difficulties and recurrent respiratory infections. Mild brain atrophy in the frontal and temporal lobes was found by computerized tomography scanning.

Case 2. The patient, a boy, was born March 16, 1980, at term after an uneventful pregnancy, as the first child of healthy parents who were second cousins. Birth weight was 2850 g. The Guthree test for phenylalanine showed 16 and 20 mg/dl (0.97 and 1.21 mmol/l) on days 5 and 7, respectively. The infant was transferred to another hospital, where he was diagnosed as having PKU because his serum level of phenylalanine was 24.3 mg/dl (1.47 mmol/l) on day 12 after birth. A low-phenylalanine diet (with a daily intake of 0-80 mg/kg) was started immediately, after which the serum phenylalanine level was 2-4 mg/dl (0.12-0.24 mmol/l) when tested. The patient developed normally until 3 months of age. At the age of 6 months, tonic seizures resembling Moro's reflex developed and convulsions were suspected, but findings from the EEGs were normal. At the age of 1 year the patient was admitted to our hospital. At first, the serum level of phenylalanine was 4-6 mg/dl (0.24-0.36 mmol/l), the ferric chloride reaction was negative, and results of liver function tests were normal. When the serum phenylalanine level was 4.2 mg/dl (0.25 mmol/l, the serum tyrosine level was 0.3 mg/dl (0.018 mmol/l). The clinical signs were severe truncal hypotonia, mild eczema, and brown rather than black hair. The patient's nutritional status was normal. Physical examination showed developmental delay; the patient could not control his head movements, roll over,

or sit alone without support, although deep tendon reflexes were normal and there were no pathological reflexes. The development quotient was 46 and sporadic single spikes were found in the EEG, but results of brain computerized tomography scans were normal.

Case 3. The patient, a girl, was born August 24, 1980, 2 weeks before term after an uneventful pregnancy, as the first child of unrelated healthy parents. Birth weight was 2100 g and neonatal asphyxia was absent but the amount of vernis caseosa suggested mild immaturity. The results of Guthree tests were positive and serum phenylalanine was as high as 20 mg/dl (1.2 mmol/l). The infant was admitted later to another hospital because of the results of screening and was diagnosed as having PKU because her serum level of phenylalanine was 40.9 mg/dl (2.48 mmol/l) and that of tyrosine was 2.6 mg/dl (0.14 mmol/l). A low-phenylalanine diet (with a daily intake of 70 mg/kg) was started immediately, after which the serum level of phenylalanine was 4-6 mg/dl (0.24-0.36 mmol/l). The infant developed normally until 2 months of age. After discharge from the hospital, the patient had recurrent respiratory infections, and she could not control her head movements even at the age of 6 months. After that, the patient became irritable and difficult to feed, and always leaned back when crying, while stiffening her extremities, although there was truncal hypotonia.

Subjects and Methods

These three cases of persistent hyperphenylalaninemia and 13 cases of classical PKU (all such patients seen at the Children's Medical Center of Osaka City) are summarized in Table 1. The heterozygotes for PTPS deficiency listed in the table were the two sets of parents of patients 1 and 2.

It is important to know the normal ranges of neopterin and biopterin (and the normal range of their ratio) in neonates. The control neonates whose test results were used here were 50 healthy infants born at term, at a mean gestational week of 40± 0.5 (range, 38-42). The mean birth weight was 3278± 403 g (range, 2615 to 4545), and the mean Apgar score at 1 min was 10. At the time of the blood sampling, 6 boys and 7 girls were 3 days old, 15 boys and 6 girls were 4 days old, 8 boys and 7 girls were 5 days old, and one boy was 6 days old. Nine male infants were about 1 month old, and their birth weight and gestational weeks are shown in Table 2. All controls except the newborns and persons 50

Table 1. Three cases of persistent hyperphenylalaninemia (HPA) or classical PKU.

Disorder	Case	Sex	Age	Serum Phe[a] (mmol/l)	Phe intake/d (mg/kg)
Persistent HPA	4	M	1 y	0.24-0.36	60
	5	M	3 m	0.36-0.67	–[b]
		F	3 y	0.36-0.48	60
Classical PKU	6	F	3 m	0.61-0.67	50-60
		M	15 y	0.61-0.67	50
		M	3 y	0.36-0.48	30
		M	5 y	<[c]-1.21	40
		M	5 y	0.48-0.61	40
		F	6 y	0.48-0.61	40
		F	6 y	0.97-1.21	50
		M	8 y	0.97-1.21	30
		F	10 y	0.97-1.21	50
		M	12 y	0.61-0.97	20
		F	15 y	0.97-1.21	30
		M	20 y	0.97-1.21	20
		F	23 y	1.21-1.70	40

[a]Serum phenylalanine, with minimum and maximum values of repeated assays; [b]not measured; [c]record unclear, but minimum was between 6 and 8.

Table 2. Urinary neopterin (N), biopterin (B), and N/B levels in nine control subjects with birth weight (BW) small for gestational age or appropriate for gestational age.

n	Age (days)	GW*	BW (g)	N (μmol/mol creatinine)	B (μmol/mol creatinine)	N/B
2	37, 50	34	1600, 1620	709, 2122	196, 207	3.6, 10.3
7	25-48	31-41	1580-3180	760± 304	144± 70	6.6± 4.0

*Gestational week
For pteridines, results are given as means ± SD.

Table 3. Analysis of neopterin (N), biopterin (B), and N/B in control urine of some control subjects.

Age	Sex	n	N (μmol/mol creatinine)	B (μmol/mol creatinine)	N/B
3-6 d	M	30	1396± 769	139± 96	12.7± 8.7
	F	20	1475± 650	130± 64	13.2± 6.3
3-8 m	M	1	559	297± 151	2.6± 2.1
	F	3	560± 65	288± 124	2.5± 1.7
1-5 y	M	10	428± 303	309± 147	2.5± 2.1
	F	7	312± 106	287± 140	1.4± 0.8
6-11 y	M	16	193± 86	154± 85	1.5± 0.8
	F	11	248± 132	190± 92	1.5± 1.0
20-35 y	M	7	152± 54	132± 44	1.2± 0.3
	F	1	147	117	1.3

Results are given as means ± SD.

years old or more are summarized in Table 3. Two men and five women also used as controls were aged from 50 to 70 years.

Blood and urine sampling was done after the permission of the subject or the subject's parents was obtained.

Urinary levels of pteridines were assayed by the method of Kohashi *et al.* (14). Urine was collected early in the morning. To the sample, 1% (v/v) 4 M HCl was added and the mixture was stored at −40℃ until measurement. The serum level of biopterin was assayed by the *Crithidia* (*C. fasciculata* ATCC 12857) bioassay of Iwai *et al.* (15). Blood samples were collected by venous puncture, and serum was obtained by centrifugation and stored at −40℃ until being assayed.

Oral loading tests with BH_4 (Dr. Schircks Laboratories, Jona, Switzerland) were done early in the morning for all three patients with PTPS deficiency, and for two of the three patients with persistent hyperphenylalaninemia and one of the 13 patients with PKU, chosen randomly. Oral loading tests with L-phenylalanine (Wako Pure Chemical Industries, Osaka, Japan) were done early in the morning for two of the patients with PTPS deficiency, the same two patients with persistent hyperphenylalaninemia, the same patient with PKU, all eight control adults, and the parents of patients 1 and 2 with PTPS deficiency. BH_4 or L-phenylalanine was administered to the subjects at the dose of 2.0-2.5 or 100 mg/kg, respectively.

PTPS activity in erythrocytes was measured by a method described elsewhere (12). Dihydroneopterin triphosphate was the gift of Dr. M. Masada at Chiba University, and sepiapterin reductase was the gift of Dr. S. Katoh at Meikai University.

Results

In the three patients with PTPS deficiency, serum levels of phenylalanine decreased during the first 3 h after oral BH_4 loading and became normal after 4 to 8 h. The two patients with persistent hyperphenylalaninemia and one patient with PKU tested had little change in serum levels of phenylalanine during the test (Fig. 2).

In the oral loading test of L-phenylalanine, all control subjects had a peak serum level of phenylalanine at 1 h, and the levels decreased until they entered the normal range 4 h after the loading. Patients with PTPS deficiency, persistent hyperphenylalaninemia, or PKU had higher serum levels of phenylalanine than control adults had both before and after loading, including 4 h after the loading, the time of the last test (Fig. 3A). After oral loading of L-phenylalanine, the eight control adults and four parents of patients with PTPS deficiency had a peak serum level of biopterin about 1 to 2 h later, and the levels gradually decreased until they entered the normal range 4 h after the loading. The patients with persistent hyperphenylalaninemia or PKU generally had higher serum levels of biopterin than the control adults both before and after loading, including 4 h after the loading (Fig. 3B). The two patients with PTPS deficiency had lower serum levels of biopterin than the control adults before loading, and the patients' levels did not increase after the loading despite their hyperphenylalaninemia. The eight control adults and four parents of patients with PTPS deficiency had little change in their neopterin levels after loading, but their biopterin concentration doubled, so that the mean N/B ratio decreased by half after the loading (Table 4).

Urinary levels of pteridines and serum levels of biopterin in the healthy controls changed with age

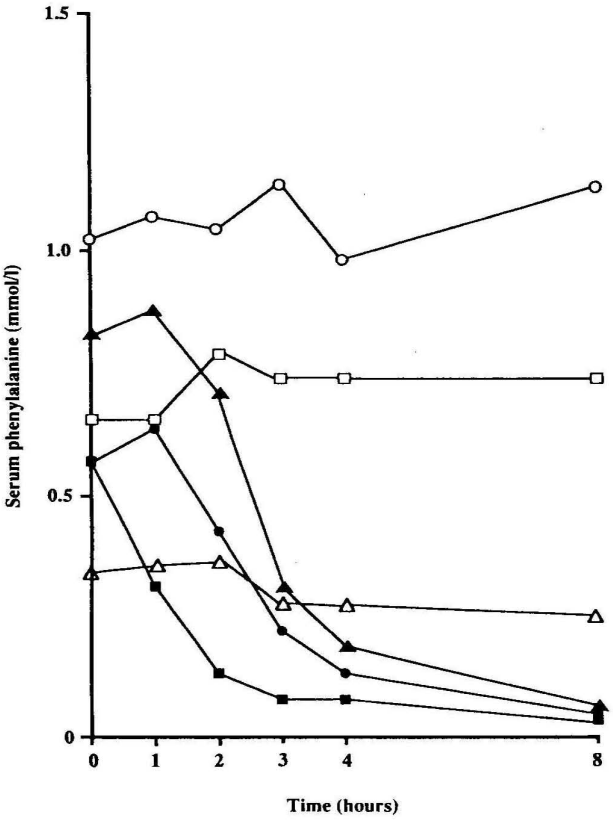

Figure 2. Response of serum levels of phenylalanine to oral tetrahydrobiopterin (BH_4) loading (2.4 mg/kg) of patients. ●, Case 1; ▲, case 2; ■, case 3 (BH_4 deficiency). ○, Case 4 and △, case 5 (persistent hyperphenylalaninemia). □, case 6 (classical PKU).

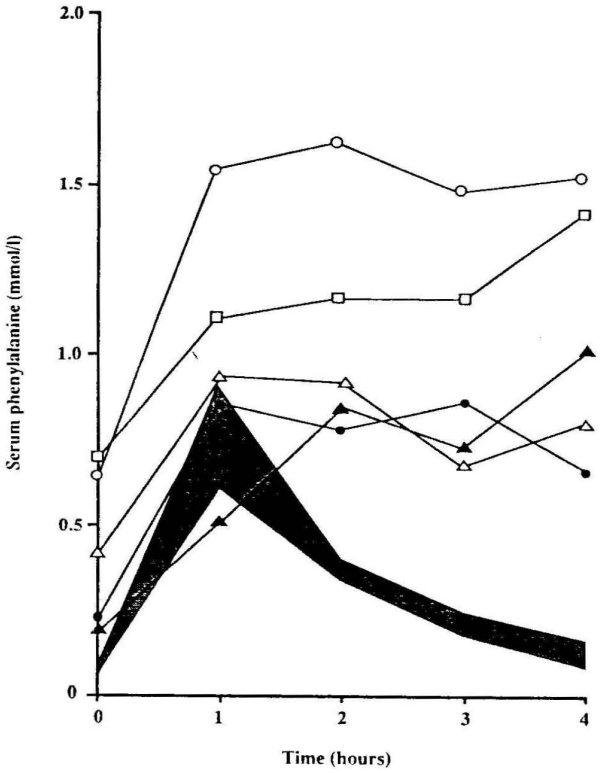

Figure 3 Response of serum levels of phenylalanine (A) and biopterin (B) to oral phenylalanine loading (100 mg/kg). ●, Case 1 and ▲, case 2 (BH_4 deficiency). ○, Case 4 and △, case 5 (persistent hyperphenylalaninemia). □, case 6 (classical PKU). The shaded area gives the control range for the substance being assayed; the results of the two sets of parents of cases 1 and 2 also were within this range.

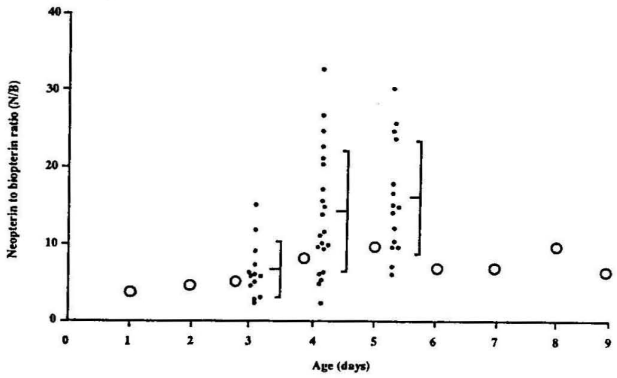

Figure 4. Daily changes in the urinary neopterin to biopterin ratio (N/B) during the early neonatal period for control subjects. Closed circles are for control neonates, and open circles are each from the same control neonate (40 gestational weeks, with birth weight of 2175 g). Means are shown with bars giving ± SD.

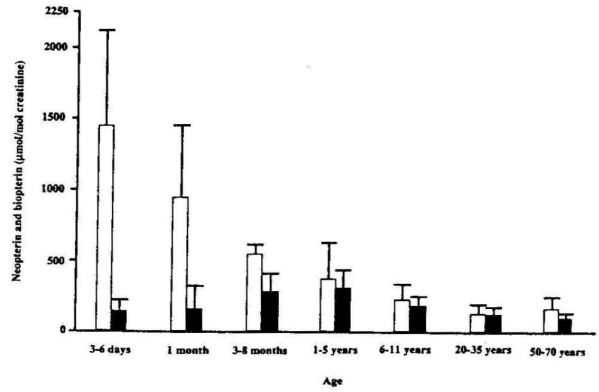

Figure 5. Age-related changes in neopterin (N) and biopterin (B) levels in control urine. Gray columns show urinary neopterin levels and solid columns show urinary biopterin levels. One standard deviation is shown with bars.

(Table 3). In the early neonatal period, the mean N/B ratio was greater in the infants aged 5 days than in those aged 3 days, and in the one subject studied longitudinally, a healthy newborn whose urine was collected daily for the first week after birth, the same tendency for the ratio to increase was found (Fig. 4). The urinary level of neopterin was higher in the early neonatal period than in adulthood, decreasing gradually with age, and the urinary level of biopterin level changed little (Fig. 5), so the N/B ratio decreased (Fig. 6). The serum level of biopterin was slightly higher during the neonatal period than in adulthood (Fig. 7).

The same age-related changes were found in persistent hyperphenylalaninemia, classical PKU, and PTPS deficiency, as well (Table 5). The urinary level of neopterin was higher in infancy than in the heal-

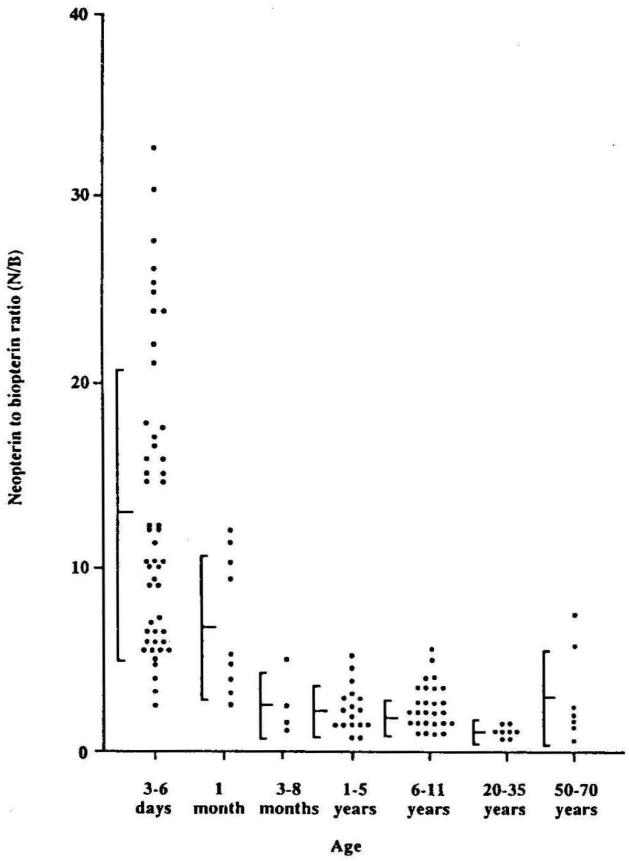

Figure 6. Age-related changes in the neopterin to biopterin ratio (N/B) of control urine. Means are shown with bars giving ± SD.

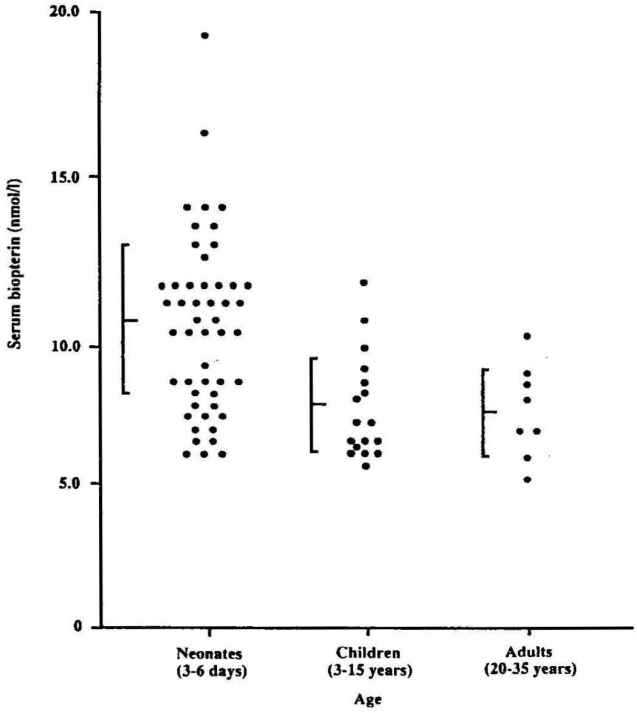

Figure 7. Serum biopterin levels in controls measured by a *Crithidia* bioassay. Means are shown with bars giving ± SD.

thy controls, but gradually decreased with age, as in the controls, and the urinary level of biopterin changed little so that the N/B ratio was high in infancy and decreased gradually thereafter. The serum level of biopterin was high in infancy.

In parents of patients with PTPS deficiency, urinary neopterin was high and urinary biopterin was almost the same as in the control adults, so the N/B ratio was twice that in the control adults.

PTPS activity in erythrocytes of case 2 was 0.1 μU/g Hb, less than 1% of the mean PTPS activity in the group of control adults younger than 50 years. The PTPS activity in the father and mother was 2.9 μU/gHb (17%) and 3.2 μU/g Hb (19%), respectively. This activity was 17% and 19% of the mean for younger control adults, and the parents were diagnosed as being heterozygotes for PTPS deficiency.

Discussion

In all three cases, signs were the same as those described by Smith *et al.* (3), despite treatment with

Table 4. Urinary neopterin (N), biopterin (B), and N/B and serum B levels in heterozygotes for 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency and controls before and after phenylalanine loading test.

Subjects	Age (years)	n	Phenylalanine loading test	Urine			Serum
				N	B	N/B	B
				(μmol/mol creatinine)			(nM)
PTPS heterozygotes	20-38	4	Before	275± 53	115± 12	2.4± 0.5	5.9± 0.8
			After	320± 34	210± 54	1.2± 0.2	21.9± 2.5
Controls	20-35	8	Before	152± 34	130± 35	1.2± 0.2	7.6± 1.3
			After	175± 40	300± 81	0.6± 0.1	30.0± 5.5

Results are given as means ± SD.

Table 5. Urinary neopterin (N), biopterin (B) and N/B, and serum B levels in patients with persistent hyperphenylalaninemia (HPA), classical PKU, or 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency and in heterozygotes for PTPS deficiency.

Disorder	Age	n		Urine			Serum
				N	B	N/B	B
		Patient(s)	Sample(s)	(μmol/mol creatinine)			(nmol/l)
Persistent HPA	3-8 m	2	3	1050, 2960	251, 1160	0.9, 11.2	25.3± 12.7
	3 y	1	2	452, 478	333, 516	0.9, 1.4	29.5± 12.7
Classical PKU	3 m	1	1	2610	540	7.7	3.8
	3-5 y	3	4	903± 163	595± 354	1.1± 0.3	48.9± 17.7
	6-10 y	4	7	689± 178	763± 205	0.9± 0.2	35.9± 11.8
	11-15 y	3	4	447± 149	522± 223	0.9± 0.2	33.3± 11.8
	20-23 y	2	3	122, 370	283, 601	0.4, 0.6	29.5, 50.6
PTPS deficiency	7 m	1		23.8×10^3	40	6.6×10^2	0.8
	1 y	1		15.6×10^3	41	3.8×10^2	1.7
	7 y	1		9.4×10^3	102	0.9×10^2	4.2± 0.8
PTPS heterozygotes	20-38 y	4	4	275± 64	115± 14	2.4± 0.7	16.9± 0.8

Results are given as means ± SD.

a low-phenylalanine diet, with delay in head control seen as the first clinical sign at 4 months of age, the appearance of truncal hypotonia, frequent convulsions starting at 6 months of age, severe hypotonia, developmental delay, untreatable convulsions, feeding difficulties, recurrent respiratory infections, and weakness of the deep tendon reflexes. These signs are very common in patients with hyperphenylalaninemia caused by a defective hydroxylation system for aromatic amino acids, and administration of BH_4 is necessary for both diagnosis and treatment (13).

The oral administration of BH_4 is the most useful test for the diagnosis of PTPS deficiency. In the BH_4 loading test, the results are similar to those of Curtius *et al.* (11), who recommend screening of all newborns found to have hyperphenylalaninemia for BH_4 deficiency by a single oral administration of BH_4 and measurement of serum phenylalanine before and 4 h after loading.

That the serum level of biopterin normally rises during phenylalanine loading tests was reported in 1976 by Leeming *et al.* (16), who found that a patient with malignant hyperphenylalaninemia had an abnormal biopterin response (no increase) to phenylalanine. In two patients (cases 1 and 2), phenylalanine loading did not increase the serum biopterin level despite the hyperphenylalaninemia. This lack of response to phenylalanine showed that these patients were not synthesizing biopterin. There was no difference in the increases in the biopterin levels between the healthy control adults and the parents of two of the patients with PTPS deficiency, but urinary neopterin levels were higher in the parents of the patients than in the control adults both before and after loading. Therefore urinary neopterin or the N/B ratio was useful to identify heterozygotes for PTPS deficiency. The N/B ratio was variable in the subjects aged 50 years or more, so that it is less useful for this purpose in older subjects. In old subjects, decreases in the biopterin level have been found when central nervous disease (Parkinson's disease, etc.) is present. There may be an increased N/B ratio and a decreased biopterin level caused by decreased PTPS activity.

In analysis of urinary pteridines, patients with hyperphenylalaninemia or classical PKU had the same age-related changes in neopterin and biopterin as the healthy controls, including a decreased N/B ratio with age. The usual increase in PTPS activity seen in healthy neonates seemed to be delayed in the patients, but in fact the high serum phenylalanine level in the patients probably stimulates BH_4 synthesis from GTP via dihydroneopterin triphosphate (NH_2P_3) so that neopterin levels are higher than biopterin levels.

The high level of neopterin and low level of biopterin in the three patients with PTPS deficiency showed that the deficiency is between the step of neopterin to biopterin, because BH_4 is synthesized from NH_2P_3 (17). Establishment of the normal ranges of neopterin and biopterin in neonates are necessary for the early diagnosis and treatment of hyperphenylalaninemia (18, 19).

Normal pterin values in urine and serum from neonates and their age-related changes throughout life have been published (20). Urinary neopterin levels decrease to two-thirds of the early neonatal level

after the first month, to one-third by the end of the first year, and thereafter gradually decrease to the adult values. Urinary biopterin levels were almost the same in early neonates as in adults, but were twice as high in young children aged 2 or 3 years as in the neonates, gradually decreasing to adult values. Therefore the N/B ratio decreased to half by the neonatal period and decreased to one-fifth of the early neonatal level by the end of the first year, reaching almost the adult levels during the second or third year. These results suggested that the PTPS activity in neonates was too low to produce much BH_4 from NH_2P_3 in spite of the large demand for BH_4 caused by the higher protein intake per unit body weight than in adults. However, the timing and rapidity of the increase in the N/B ratio (on days 4 and 5 of life) showed that the cause was not immaturity of PTPS but an increased demand for BH_4 in response to the phenylalanine loading arising from the increased intake of milk. During the early neonatal period, the PTPS activity is lower than GTP cyclohydrolase I activity. Therefore, even in patients with hyperphenylalaninemia with persistently high N/B ratios during infancy, it is possible to identify PTPS deficiency if there is a decrease in the N/B ratio after the serum level of phenylalanine is kept low for several days. The N/B ratios reported up to now for patients with PTPS deficiency have a lower limit of about 40 (21), and the values for healthy neonates have an upper limit of about 30, so it is possible to diagnose PTPS deficiency during the neonatal period by use of 40 as a cut-off value. However, elevated neopterin levels have been found in urine from patients with various viral infections (22), so that it is important to role out infection when diagnosis of PTPS deficiency is to be based on the N/B ratio.

For the detection of heterozygotes for the deficiency, estimation of protein (phenylalanine) intake must be accurate. Urine samples should be collected from subjects who had a low-protein meal for dinner the evening before and who fasted overnight. The first urine produced in the morning should be discarded.

The urinary N/B ratio was useful not only for the diagnosis of PTPS deficiency but also for the detection of heterozygotes for PTPS deficiency.

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