*Archives of Disease in Childhood*, 1978, **53**, 674–683

# *Short reports*

## Tetrahydrobiopterin therapy of atypical phenylketonuria due to defective dihydrobiopterin biosynthesis

Phenylalanine-4-hydroxylase, tyrosine-3-hydroxylase, and tryptophan-5-hydroxylase need 5, 6, 7, 8-tetrahydro-L-biopterin ($BH_4$) as a coenzyme. $BH_4$ is formed *in vivo* from guanosine-triphosphate by several enzymatic steps leading to dihydrobiopterin (DHB). The latter is transformed into (6 S)—or (6 R)—$BH_4$ by the action of dihydropteridine reductase (DHPR). The lack of any one of the enzymes involved in the $BH_4$ biosynthesis results in a deficiency of $BH_4$ leading biochemically to non-functional hydroxylating systems and clinically to variant forms of phenylketonuria (PKU) (Kaufman *et al.*, 1975; Bartholomé *et al.*, 1977). This paper concerns an infant with atypical PKU, who was treated successfully with chemically pure tetrahydrobiopterin.

### Case report

A baby girl weighed 2210 g at birth. The Guthrie test for PKU was negative on the 6th day of life, despite correct blood sampling and sufficient protein intake. At age 6 months severe muscle hypotonia and mental retardation were observed. At this time the serum phenylalanine (McCaman and Robins, 1962) was 20 mg/100 ml (1·21 mmol/l). A repeat test of the preserved Guthrie test paper confirmed $<2$ mg/100 ml (0·121 mmol/l), indicating that a very slow rise in blood phenylalanine had occurred after birth. A low phenylalanine diet was started. After 3 months no clinical improvement was observed and, therefore, further diagnostic procedures were initiated. The phenylalanine-4-hydroxylase activity in a liver biopsy (Bartholomé *et al.*, 1975) was 22 μmol/g protein per hour (normal mean $\pm$ SD: 35·2 $\pm$ 11·1). $K_m$ for phenylalanine was 0·035 mmol/l (normal). DHPR was 67 nmol NADH/mg protein per min (normal, 70·2–91·1, Bartholomé *et al.*, 1977, courtesy of S. Milstien and S. Kaufman). The phenylalanine-4-hydroxylase *in vivo* test (Curtius *et al.*, 1972, 1977; Zagalak *et al.*, 1977), showed only 2% of the activity found in a control group.

### Tetrahydrobiopterin administration, IV and oral

After having received the consent of the parents, the diet was interrupted and a therapeutic trial with $BH_4$ was begun. For four days on a normal diet (about 120 mg phenylalanine/kg per day) the serum phenylalanine increased from 2·1 to 20·4 mg/100 ml (0·127 to 1·234 mmol/l). Chemically pure (6 R, S)—$BH_4$· 2 HCl, synthesised by Schircks *et al.* (1977) was administered intravenously (25 mg $BH_4$· 2 HCl, corresponding to 2·5 mg/kg, in 2 ml isotonic buffer of pH 3·0, containing 25 mg ascorbic acid, 20 mg lactic acid, pH adjusted with 1 N NaOH). Three hours later, the serum phenylalanine decreased to 2·1 mg/100 ml. Six hours and 24 hours after the injection, the phenylalanine values were still 0·9 and 2·1 mg/100 ml (0·05 and 0·127 mmol/l) respectively. 24 hours after the first injection, a second injection of $BH_4$ was given. The serum phenylalanine remained below 2·1 mg/100 ml during the next 36 hours.

In a second therapeutic trial, 25 mg $BH_4$· HCl were administered twice within 6 days (Figure) through a gastric tube. Again $BH_4$ was supplemented with ascorbic acid (1:4, w/w) and dissolved in 20 ml water deaerated with $N_2$. A striking decrease of the serum phenylalanine concentration was also observed. The therapeutic trial was then discontinued and the phenylalanine-restricted diet was restarted, together with L-dopa, carbidopa, and 5-hydroxytryptophan (Bartholomé *et al.*, 1977).

### Discussion

The following findings strongly suggest a defective biosynthesis of dihydropterin in our patient: normal activities of the apoenzymes of phenylalanine-4-hydroxylase and DHPR in liver biopsy; abnormal *in vivo* assay of phenylalanine-4-hydroxylase with a clinical picture similar to PKU; and the patient's prompt biochemical response to the administration of $BH_4$. Moreover, the synthetic $BH_4$, given intravenously, proved to have a potent effect in lowering the phenylalanine concentration in blood to normal values in this patient. The fact that it was equally effective when

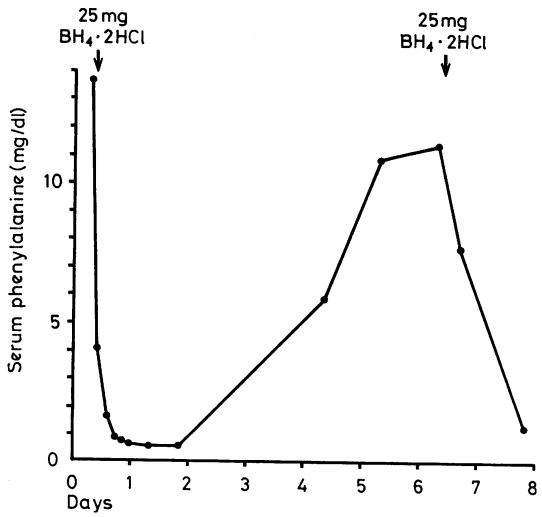


Figure *Decrease of serum phenylalanine concentration after oral administration of $BH_4 \cdot 2$ HCl-ascorbic acid* (1:4, w/w). *The child was on a normal diet. The first administration was before, and the second after a meal of milk.*

given orally proves that $BH_4$ is absorbed by the intestine.

In a patient with suspected DHB reductase deficiency, Danks *et al.* (1975) found a rather low response to IV administration of a $BH_4$ preparation, and no response at all to oral administration of 1 mg $BH_4$.

$BH_4$ is an essential coenzyme, not only for the formation of tyrosine from phenylalanine in liver, but also for the formation of catecholamines and serotonin which occurs at the synapses within the brain. A patient with a defect in the biosynthesis of $BH_4$ would lack these biogenic amines in the brain if the $BH_4$ administered could not penetrate the blood–brain barrier. Using the same $BH_4$ preparation as Danks *et al.* (1975) in experiments on rats, Kettler *et al.* (1974) thought the evidence suggested that $BH_4$ did not penetrate the brain. However, the $BH_4$ preparation used by these authors presumably had low biological activity, and the question of the penetration of $BH_4$ into the brain must be considered. This being so, patients with defective $BH_4$ biosynthesis should be treated not only with $BH_4$ but also with L-dopa, carbidopa, and 5-hydroxytryptophan. We conclude that it should be possible to replace the low phenylalanine diet for such patients, with a normal diet and a $BH_4$ supplement.

**Summary**

A patient with atypical phenylketonuria (defective $BH_2$ synthesis), detected at age 6 months because of severe muscle hypotonia and serum phenylalanine of 20 mg/100 ml, had normal activities of phenylalanine-4-hydroxylase and DHPR in liver biopsy, but only 2% activity in the phenylalanine-4-hyroxylase *in vivo* test using deuterated phenylalanine. After IV administration of 2·5 mg/kg chemically pure tetrahydrobiopterin bis hydrochloride ($BH_4 \cdot 2$ HCl), serum phenylalanine decreased from 20·4 to 2·1 mg/100 ml within 3 hours. Administration of 25 mg $BH_4 \cdot$ HCl and 100 mg ascorbic acid through a gastric tube decreased serum phenylalanine from 13·7 to $<1 \cdot 6$ mg/100 ml within 3 hours and it remained $<2$ mg/100 ml for 2 days.

This work was supported by the Swiss National Science Foundation, Grant No. 3.642–0.75.

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# Diagnosis of phaeochromocytoma after ingestion of imipramine

We describe a case of phaeochromocytoma of the adrenal medulla in one of identical twin girls, diagnosed after the ingestion of imipramine.

## Case report

An 11-year-old girl was admitted to hospital with a history of pallor and profuse sweating for 24 hours after a single dose of imipramine syrup (Tofranil 50 mg). There had been a similar episode 2 months earlier after medication with a tablet of imipramine (50 mg). This drug had been prescribed by the family doctor for the treatment of nocturnal enuresis of a year's duration. After the first dose the girl had sweated profusely for about 12 hours. Her mother stopped her medicine but decided to reintroduce it 2 months later as bed-wetting had persisted. Within 6 hours the girl developed the symptoms which led to her admission.

On examination she was 6 cm shorter and 5 kg lighter than her twin, despite being slightly taller and heavier a year earlier. She was pale, cold, and drenched in sweat, with a temperature of 36·2°C. Her pupils were widely dilated but briskly reactive to light, and she had a pulse rate of 160/min. Her blood pressure was 110/85 mmHg and there were no other abnormal findings on physical examination. A random blood sugar was within normal limits and ECG showed sinus tachycardia.

By the next day her diastolic pressure had risen to 100 mmHg and she continued in a state of sympathetic overactivity. A phaeochromocytoma was suspected.

Investigations included measurement of urinary hydroxy-methoxy-mandelic acid (HMMA) and metadrenaline levels. Both levels were raised at 30 μmol/24 h and 45·9 μmol/24 h respectively. Aortography confirmed the presence of a right-sided adrenal tumour. Her hypertension was controlled with oral phenoxybenzamine, and adrenalectomy was performed with full anaesthetic precautions (Crout and Brown, 1969). Histological examination of the tumour confirmed the diagnosis of phaeochromocytoma. After surgery her blood pressure returned to normal and there was no further enuresis. Urinary HMMA and metadrenaline levels fell to normal. The screening test for phaeochromocytoma was normal in her twin sister.

## Discussion

Phaeochromocytoma is a rare condition and has been found once in a thousand necropsies in adults (Herman and Mornex, 1964). It is estimated that 0·5% of adult hypertensive patients in the USA have this tumour. For every 10 patients successfully treated, it is thought that there is one death from hypertensive crisis in a patient whose phaeochromocytoma is demonstrated at necropsy (Harrison, 1976). This tumour is considerably rarer in children (Wotherspoon *et al.*, 1974), most reports being of single cases. Symptoms of the disease may be provoked by agents which stimulate release of amines, thereby producing abnormal pressor responses in the tumour. Such pressor agents may be used in provocative tests for the diagnosis of phaeochromocytoma, and include histamine, tetraethylammonium, and methacholine (Beeson and McDermott, 1971).

Imipramine is a tricyclic antidepressant which is commonly used to treat nocturnal enuresis in children. It possesses anticholinergic properties, and reported side effects include dryness of the mouth, dizziness, tachycardia, palpitations, and urinary retention. Excessive sweating has been reported although the mechanism is not known. Tricyclic antidepressants have also been shown to enhance the effects of catecholamines by blockade of active transport from extracellular fluid to cytoplasmic mobile pool (Axelrod *et al.*, 1961).

Our patient may have had an idiosyncratic reaction to imipramine. This may have been the result of direct adrenal stimulation by the drug, or in response to excess catecholamine production after blockade in the re-uptake mechanism by imipramine. We are unaware of any previous report of phaeochromocytoma diagnosed after the ingestion of imipramine.