Diagnosis of Phenylketonuria (Phenylalanine Hydroxylase Deficiency, Temporary and Permanent)*

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It is becoming evident that the diagnosis of classical phenylketonuria cannot always be made with certainty before it is judged necessary to prescribe a low-phenylalanine diet (Schneider and Garrard, 1966; Berry and Wright, 1967). If the diagnosis remains uncertain the results of dietary treatment cannot be properly assessed, and there remains the unwelcome possibility that harm may be done by depriving normal children of phenylalanine (Bessman, 1966). This paper reports a striking case of temporary phenylalanine hydroxylase deficiency as an illustration of the problems involved. Later we sought evidence of persisting phenylalanine hydroxylase deficiency in a group of children who have received low-phenylalanine diet. The results of treatment of these children are described elsewhere (McBean and Stephenson, 1967).

Methods

Serum phenylalanine (and tyrosine) was estimated by the snake venom L-amino-acid oxidase method of La Du and Michael (1960) or as modified by Henry (1964), except where indicated in the text. Blood phenylalanine was estimated by the Guthrie test (Guthrie and Susi, 1963). Urine phenylpyruvic acid: standard qualitative ferric chloride and Phenistix tests (Woolf, 1963) graded 1+ to 3+ (slight to strong). Urine orthohydroxyphenylacetic acid: one-dimensional paper chromatography.

Psychometric tests: Griffiths quotients (G.Q.) and Stanford Binet Form L-M intelligence quotients were measured by psychologists of the department of child psychiatry.

Phenylalanine Hydroxylase Deficiency of Early Infancy (Temporary "Phenylketonuria"): Case Report

The patient, date of birth 2 June 1966, was the second male child of unrelated parents. He was delivered by elective caesarean section at term because of previous obstetrical difficulties. Birth weight 3.04 kg. He was fed on National Dried Milk from 3 June and was slightly jaundiced during his first week. Hyperphenylalaninaemia was demonstrated by routine Guthrie test at the age of 5 days.

Investigations.—7 June—Guthrie test: blood phenylalanine 12 mg./100 ml. Urine: tyrosyluria. 20 June—Guthrie test: blood phenylalanine 50 mg./100 ml. 27 June—Urine: orthohydroxyphenylacetic acid present; no tyrosyluria. 30 June—Guthrie test: blood phenylalanine 40 mg./100 ml. 5 July—Urine: $FeCl_3$ 3+; Phenistix 2+. Serum phenylalanine 32.6 mg./100 ml. Serum tyrosine 3.0 mg./100 ml.

On 5 July, when he was clinically normal and weighed 3.4 kg., he was started on low phenylalanine diet (Minafen, cow's milk, Ketovite syrup, and blackcurrant syrup). His progress is summarized in Table I.

After the first month of treatment the serum phenylalanine did not rise above 2 mg./100 ml., but he maintained a steady weight gain. During the second month of treatment he suffered a series of convulsions (not hypoglycaemic) and developed hypochromic anaemia with normal serum iron. Despite progressive increase in phenylalanine intake up to 116 mg./kg./day his serum phenylalanine did not rise to 3 mg./100 ml. The diagnosis of phenylketonuria was therefore questioned, and normal milk diet was offered after four months of restricted phenylalanine. Non-fasting serum phenylalanine levels rose only slightly above normal, but orthohydroxyphenylacetic acid was detected in the urine at this time. During the following three months no phenylpyruvic acid was detected in the urine.

TABLE I.—*Progress of Temporary "Phenylketonuric" on Low-phenylalanine Diet*

Age in months	Month of Diet	Phenylalanine Intake/Day		Serum Phenylalanine (mg./100 ml.)	$FeCl_3$	OHPAA*	G.Q.†	Clinical Impression
		mg.	mg./kg.					
1		1,000	300	32·6	+	+		Normal
2	1	280	64	0·0–2·8				
3	2	350	60	0·5–1·0				Convulsions. Anaemia
4	3	470	70–78	0·0–2·0				
5	4	470–870	64–116	1·8–2·0				
6		1,500	200	3·1–4·0		+	62	
7		Unrestricted diet		0·8 (fasting)			72	Plump. Sociable. Probably retarded

* Orthohydroxyphenylacetic acid.
† Griffiths quotient.

On 20 January 1967 an oral phenylalanine tolerance test was carried out, with 100 mg. of L-phenylalanine per kg. body weight, administered by gavage (Table II). Serum estimations were made in duplicate.

TABLE II.—*Phenylalanine Tolerance Test on Patient Aged 33 Weeks. L-phenylalanine 100 mg./kg. by Gavage at Zero Time*

Time	Serum Phenylalanine mg./100 ml.)	Serum Tyrosine (mg./100 ml.)	Urine OHPAA
Fasting	0·8	1·1	None
1 hour	3·4	1·2	
2 hours	3·6	2·5	
3 „	2·3	1·8	Very faint trace
4 „	2·1	1·4	

Comment

The fasting serum phenylalanine was unequivocally normal and the rise in serum tyrosine after phenylalanine loading confirmed the presence of phenylalanine hydroxylase activity. The diagnosis of classical phenylketonuria was therefore excluded.

At the time of writing the child was 8 months old, well nourished, and socially responsive, but the clinical impression of a degree of psychomotor retardation was supported by psychometric tests (Table I).

The child's parents have normal serum phenylalanine levels, but their genotype has not yet been established.

Reassessment of Presumptive Phenylketonurics

The recognition of temporary "phenylketonuria" prompted a review of the diagnosis of other presumptive phenylketonurics.

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In this report we include only those children who have received low-phenylalanine diet and have been studied until after their first birthday (McBean and Stephenson, 1967). Because of this minimum follow-up period cases detected by mass neonatal screening by the Guthrie test are not included, nor are infants who have removed to other districts. Table III shows the clinical features of those infants detected in the first three months of life.

TABLE III.—*Infants Detected in First Three Months: Clinical Features*

Case No.	Sib. with PKU	Clinical Features Before Treatment					I.Q. < 80 After 1 Year (lowest)
		Vomiting	Eczema	Retardation	Convulsions	Others	
1	Yes						
2	,,						+(73)
3	,,					"Looks like sib with PKU" (mother)	
4*		+				Pyloric stenosis	+(73)
5†			+	+			+(79)
6*						Anorexia. Screaming. Thrush. Pyuria	
7†			+		+		
8†				+			

* Presented with symptoms.
† Detected by napkin-Phenistix screening test.
PKU = Phenylketouria.

Partington (1961) has stressed the frequency of early symptoms in phenylketonuria (vomiting, feeding problems, colic, ? pyloric stenosis, irritability, eczema and other rashes, suspected retardation, and, later, seizures). It is evident that none are specific, but it may be that such symptoms will be found to be more frequent in true phenylketonurics. Certainly in two of these patients the symptoms were valuable in bringing the child to hospital at an early age. Routine urine testing then revealed the diagnosis. Case 4 was detected in this way in January 1961, after pyloromyotomy (for marked hypertrophic pyloric stenosis). The other infants were detected either because an older sibling was known to have classical phenylketonuria or through routine napkin-Phenistix testing by the local health authorities (Stephenson and McBean, 1967).

Table IV gives some of the biochemical findings. The degree of positivity of the Phenistix reaction on fresh urine is recorded in preference to the ferric chloride reaction because it is more easily defined. In all cases the unacidified urine also gave a typical green colour with aqueous ferric chloride (Woolf, 1963).

TABLE IV.—*Infants Detected in First Three Months: Biochemical Features*

Case No.	Age at Test (days)	Biochemical Features Before Treatment		Highest Serum Phenylalanine After One Year (mg./100 ml.)
		Urine Phenistix	Serum Phenylalanine (mg./100 ml.)	
1	11	+ + +	30·0*	25·2
2	20	+ + +	65·0	50§
3	26	+ + +	57·4	20·0
4	47	$FeCl_3$ 3+	37·1†	34·0
5	59	+ + +	69·0	22·6
6	82	+ + +	30·0*‡	23·0
7	77	+ + +	70·2	66·2
8	82	+ + +	60·6	> 20§

* Chromatographic method (Berry, 1957).
† Five days after pyloromyotomy.
‡ Four days after diet started.
§ Guthrie inhibition assay.

It is evident that all these children, including those who were asymptomatic, have a persisting tendency to hyperphenylalaninaemia. In three (Cases 1, 6, and 7) dietary control has been excellent since early infancy, but at ages 3½, 4½, and 2 years, respectively, hyperphenylalaninaemia was induced by feeding a normal phenylalanine diet for a few days. (Complan was substituted for Cymogran for this test. The use of ordinary foodstuffs for such a trial is contraindicated in case the phenylketonuric child loses his taste for the low-phenylalanine diet.) In the remaining five cases[1] the high serum phenylalanine levels resulted from dietary indiscretions (excess phenylalanine intake).

The older patients (Cases 11–33), detected at age 6 months to 10 years, all gave evidence of permanent enzyme defect. In Cases 11–30 this was demonstrated by hyperphenylalaninaemia over the age of 1 year. Three earlier cases (Nos. 31–33), with severe mental retardation, did not have serum phenylalanine estimations, but two of them had typical phenylketonuric siblings, and the urine of the third contained phenylpyruvic acid, which disappeared during low-phenylalanine diet. In all cases the urine gave a typical positive reaction with ferric chloride, and the initial I.Q. was always below 80. Table V shows the distribution of serum phenylalanine levels, which tend to be lower than in the young infants.

TABLE V.—*Distribution of Serum Phenylalanine Levels in Phenylketonurics Diagnosed Age 6 Months to 10 Years (Cases 11–33)*

Serum Phenylalanine	No. of Children
Under 20 mg./100 ml.	1*
20— ,,	5
30— ,,	9
40— ,,	2
50— ,,	3
Not known	3

* 18 mg./100 ml. at age 10½ years.

No attempt was made to assess the likely genotype of these children by testing their parents, but the course in all cases suggests the diagnosis of homozygous phenylketonuria.

Discussion

When infant screening was introduced it was assumed that the new-found phenylketonurics would be differentiated from the remainder of the population as sharply as was the case in older children and adults. There is now increasing concern about the validity of this assumption (Bessman, 1966; Schneider and Garrard, 1966; Perry *et al.*, 1967; Berry and Wright, 1967). In the first place, it may be that the biochemical abnormalities of heterozygotes for the usual gene of phenylketonuria overlap with those found in (homozygous) phenylketonurics, making definitive diagnosis in the asymptomatic neonate impossible (Schneider and Garrard, 1966). Secondly, and perhaps more important, there might be delayed maturation of the enzyme phenylalanine hydroxylase even in infants of normal genotype. We have presented evidence to show that the infant whose case is described above had just this disorder. Until treatment was started in the fifth week of life he had the biochemical syndrome of severe phenylalanine hydroxylase deficiency (serum phenylalanine over 30 mg./100 ml., with urine phenylpyruvic acid and orthohydroxyphenylacetic acid), but by the age of 5 months his phenylalanine tolerance was normal. Indeed, the results of his fasting serum estimations (Perry *et al.*, 1967) and tolerance test (Hsia *et al.*, 1956) suggest that he is unlikely even to be heterozygous for the usual gene of phenylketonuria, irrespective of the probable genotype of his parents.

We may speculate briefly on the probable sequence of events in this case. Originally he had tyrosyluria, and could be classed as the type 2 phenylalaninaemia of Berry and Wright (1967), with presumptive deficiency of both phenylalanine hydroxylase and parahydroxyphenylpyruvic oxidase systems. The two enzyme systems then matured at different rates, parahydroxyphenylpyruvic oxidase rising to normal some weeks before phenylalanine hydroxylase. In our reassessment of presumptive phenylketonurics we have found no further cases of temporary enzyme deficiency, but the report of the Cincinnati conference on the treatment of phenylketonuria (Berry and Wright, 1967) suggests that milder examples may not be rare.

[1] Cases 9 and 10 are not described: follow-up details are not available.

A probable instance was reported by Moncrieff and Wilkinson (1961), though at the time of diagnosis the method for serum phenylalanine was not yet entirely satisfactory.

The effect of a low-phenylalanine diet on our case of temporary phenylalanine hydroxylase deficiency is of particular importance. Despite a normal gain in weight throughout treatment, and a phenylalanine intake never below 60 mg./kg./day, serum phenylalanine levels remained persistently low. There is only limited information on the phenylalanine requirements of normal infants, but Snyderman *et al.* (1955) suggest that less than 90 mg./kg./day might be unsafe. This figure was derived in part from observations on the weight gain of infants on differing phenylalanine intakes. However, it may be possible, as in the case described above, for normal weight gain to occur despite phenylalanine deficiency. If so, the optimum phenylalanine intake for a normal child may be much higher than 90 mg./kg./day. The particular concern is that harm might be done to the developing brain by phenylalanine deprivation (Bessman, 1966). There is suggestive evidence that general undernutrition during infancy will inhibit subsequent intellectual development (Stoch and Smythe, 1963), and isolated phenylalanine deficiency might do likewise. This is supported by the delayed psychomotor development in our case and in those reported by Rouse (1966) and Moncrieff and Wilkinson (1961). A fall in developmental quotient may also be seen in phenylalanine-deficient phenylketonuric infants (Brimblecombe *et al.*, 1961, and personal observations). In both the normal and phenylketonuric infants there is a tendency for the developmental quotient to rise after the phenylalanine deficiency is corrected, but the possibility remains that some damage may have been done.

In this somewhat disturbing situation, with no satisfactory criteria for making the diagnosis of phenylketonuria in early infancy, and with the danger of harming normal children by phenylalanine deprivation, some would advocate that the low-phenylalanine diet should not be given (Bessman, 1966). We would strongly oppose this, for the undoubted benefits of treating true phenylketonurics from early infancy would be lost (McBean and Stephenson, 1967). Well-controlled dietary treatment might indeed prevent brain damage even in temporary "phenylketonurics" if transient hyperphenylalaninaemia (and its biochemical consequences) is harmful. Rather one should concentrate on avoiding phenylalanine deficiency, using the strict criteria of Umbarger *et al.* (1965), whose well-controlled patients have a serum phenylalanine of 3 mg./100 ml. or more, no urine orthohydroxylphenylacetic acid, and urine phenylalanine 25–150 μg./ml.

Perhaps initial tyrosyluria may be a common accompaniment of temporary phenylalanine hydroxylase deficiency, as in our case, and may serve as a warning. If during treatment a serum phenylalanine concentration of 3 mg./100 ml. is difficult to sustain, or frequent changes in phenylalanine intake are found to be unnecessary (Umbarger *et al.*, 1965), or one of the parents can be shown not to be heterozygous (Hsia *et al.*, 1956; Perry *et al.*, 1967; Woolf *et al.*, 1967), then the diagnosis of phenylketonuria must be reconsidered. At the present time we would recommend a diagnostic trial of normal milk intake for young infants, and a substitution of Complan for Cymogran in older children, for a maximum of 10 days, with biochemical monitoring. In conclusion, we emphasize that the diagnostic difficulties and the consequences of error are so great that all suspected cases of phenylketonuria should be referred to special centres.

Summary

Temporary phenylalanine hydroxylase deficiency is described in a child thought to be genetically normal on the basis of his later response to phenylalanine loading. The condition is indistinguishable from phenylketonuria in early infancy, and might remain unrecognized during low-phenylalanine diet. Because it is possible that mental subnormality may result from clinically mild phenylalanine deficiency in such infants, the diagnosis of phenylketonuria should be re-examined when a serum phenylalanine concentration of 3 mg./100 ml. is difficult to sustain or where the genotype of one of the parents is in doubt.

Thirty-one children who had received low-phenylalanine diet were reassessed. Evidence of phenylalanine hydroxylase deficiency persisting beyond the first birthday was obtained in all.

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