Phenylalaninaemia

Differential diagnosis

M. E. BLASKOVICS, GRACIELA E. SCHAFFLER, and SHIRLEY HACK

From Children's Hospital of Los Angeles, California

Blaskovics, M. E., Schaeffler, G. E., and Hack, S. (1974). Archives of Disease in Childhood, 49, 835. Phenylalaninaemia: differential diagnosis. A method is described for differentiating the phenylalaninaemias based upon blood phenylalanine (phe) responses to ingestion of natural protein diets with standard phe content. A classification scheme derived primarily from these studies is suggested which includes two forms of phenylketonuria (PKU) and four forms of phenylalaninaemia (variants) unrelated to abnormalities in tyrosine metabolism. Dietary therapy is mandatory for types I and II and possibly for type III, but does not appear to be necessary for types IV or V. Evidence provided by family studies supports the concept that the phenylalaninaemias are genetically distinct. The increased incidence of PKU consequent to newborn blood screening programmes may well be related to misdiagnosis as well as to increased recognition of PKU.

Physicians concerned with the diagnosis, management, and care of patients with phenylalaninaemia,* are acutely aware of their inability to differentiate all patients who require restrictive dietary therapy from those who do not. This is due primarily to the bias introduced when phenylketonuria (PKU) was first discovered and the sequence of the subsequent screening programmes developed to recognize this disorder during the neonatal period.

Time and experience have shown that some of the patients identified as PKU in early blood screening statistical reports were in all probability examples of a variety of atypical PKU patients (Blaskovics and Nelson, 1971; Levy, 1973). The term in vogue at the present time for all forms of atypical phenylalaninaemic patients is 'variants'. It is extremely important to recognize these variant patients because their prognosis without treatment is different from classical PKU. Most variant patients have been treated unnecessarily in the past, and many still are because of the prevailing attitudes before the use of blood screening programmes that all raised blood phenylalanine (phe) levels inexorably led to mental retardation. Since there was no previous experience for defining PKU on the basis of abnormal blood phe levels in normal newborn populations, different blood phe levels were arbitrarily adopted by clinicians and investigators throughout the world and equated with PKU. Up to the present time, reports still describe patients as having 'classical PKU' or as being 'hyperphenylalaninaemic' (in the present context equivalent to variant) without describing how the diagnosis was made, and even less, how it was confirmed. Most investigators still use a fixed blood phe level as the diagnostic criterion for PKU; for example, 15 or 20 mg/100 ml. Yet, it is eminently clear from individual case reports (Justice, O'Flynn, and Hsia, 1967; Blaskovics, 1971, 1973; Blaskovics and Shaw, 1971) and from the findings of the Collaborative Study of the Treatment for PKU (Koch et al., 1973) that an arbitrary and fixed serum phe level cannot predict permanence nor the degree of phe intolerance in all patients and, therefore, cannot predict PKU.

This paper describes, therefore, a method developed to aid in differentiating various forms of phenylalaninaemia. A classification also is suggested which is based on longitudinal observations and tolerance tests of approximately 25 patients with PKU and 35 patients with other phenylalaninaemic conditions. The subjects ranged from infancy (3 weeks) to adulthood (35 years). All patients were seen in the Child Development Division at Child-

Received 8 May 1974.

*Term recommended for any serum phenylalanine level above normal, as suggested by Bickel (1968).
Methods

To differentiate the phenylalaninaemic subjects, a challenge diet of natural foods with known phe content was given to each patient to determine the relative intolerance to phe. Each diet was calculated to provide approximately 180 mg phe/kg per 24 hours, which is an average phe intake for normal infants during the first 6 months of life. Lesser phe intakes did not reliably distinguish the different types of patients. Very high phe intakes also were associated with disturbed or ‘overload’ responses in some patients. Calories were prescribed according to the National Research Council recommendations, but protein intake was greater than is suggested for both children and adults. This high phe intake was generally given for 3 to 5 days. Serum phe levels were determined 2 to 3 times daily with duplicate specimens analysed in two separate laboratories by the McCaman-Robins method (1962). Specimens were obtained before meals and regularly at 7.00–8.00, 11.00–12.00, and 16.00–17.00 hours. When only two specimens were obtained the 11.00–12.00 specimens were omitted. Initially, 6 specimens per day were obtained, but experience suggested that only 2 determinations per day, 8 to 12 hours apart, were needed for purposes of discrimination. Urine was analysed by 2-dimensional paper chromatography and also spectrophotometrically (Shaw et al., 1971) for phe and metabolites in initial patient studies, but when it was confirmed that urinary metabolite levels generally reflected and were proportional to serum phe levels, as has been described by Armstrong and Low (1957), further urinary studies were discontinued. Furthermore, since some variant patients excrete PPyA (phenylpyruvic acid), which has been assumed to be the hallmark of PKU, this parameter, though helpful, was not judged to be a meaningful discriminant for the absolute diagnosis of PKU. Urine from each patient was analysed to exclude the possibility of a phe-transaminase defect.

This method was developed primarily to evaluate and differentiate phenylalaninaemia in infants (Blaskovics and Shaw, 1971), but it can be used for older children and adults. For infants the challenge diet is easily provided as whole milk or diluted evaporated milk. Natural protein was chosen over supplementation of the basal diet with L-phe, or the use of L-phe alone because it avoided the problem of limiting amino acids, transport inhibitions, and also the side effects associated with the ingestion of large quantities of pure L-phe, such as nausea, emesis, ataxia, etc. In addition, since the trend in PKU therapy is to discontinue dietary treatment when the patients are less than 10 years of age, this challenge also predicts the probable maximum serum phe level that a patient could have when receiving a normal diet. Most normal diets, however, never contain the amount of phe and protein ingested during the challenge period, except during infancy. A diet of balanced proteins, e.g., milk, meat, cheese, eggs, etc., calculated to provide a phe intake of 180 mg/kg per day to older children and adults generally requires 2 to 5 times their normal protein consumption. As a precautionary measure, all patients were screened with tests to assess renal and hepatic function before the loading studies. No serious or persistent untoward responses to the challenges have been observed. When a patient showed signs of illness, the studies were immediately terminated until the patient was clinically well. Some patients were studied when they were ill to determine the effect of illness on responses to loading. This has been reported elsewhere (Blaskovics, 1971). Challenges were begun with first morning feeds to minimize phe variations which might be related to circadian rhythms.

Almost all patients were studied at least twice, and most three or more times at different ages. Infants were usually studied at the time of initial referral, approximately 3 months later, and again at one year of age. Some patients had additional studies at yearly or 2-yearly intervals. Since all subjects were not studied during infancy, earlier levels of serum phe and of urinary metabolites for some patients were assumed on the basis of studies of other patients who were evaluated both during infancy and again at later ages. The clinical-biochemical attributes of each type of phenylalaninaemia are suggested principally from these patient studies, but some types necessarily are based on case reports, which are referenced, unless personally observed.

Phenylalaninaemias

Type I. Of all the phenylalaninaemias (Table), this group has the highest serum phe levels at any time in life, e.g., greater than 50 mg/100 ml. The urinary metabolites are quantitatively the most markedly abnormal. A typical challenge response during infancy can be seen in Fig. 1. During childhood and adulthood, with high protein and phe intakes similar to those received during infancy, the response appears to be similar. In adulthood on usual or normal food intakes phe levels generally are above 30 mg/100 ml. It is assumed that, if untreated, these infants will have severely abnormal EEGs, will typically have seizures, and will have severe retardation. If they are detected late (e.g. after 1 year of age), response to treatment is nil or poor. This type of phenylalaninaemia we consider to be ‘classical’ PKU. The enzyme defect is considered to involve hepatic phe-hydroxylase, with probably no measurable activity.

Type II. Serum phe levels in this group range between 30 and 50 mg/100 ml during infancy on normal diets. Urinary metabolites are characteristic of PKU, but the amounts present are less than are found in type I patients because of the lower serum phe levels. During childhood and adulthood serum phe levels on normal or usual diets stabilize.
## Phenylalaninaemia

### TABLE

<table>
<thead>
<tr>
<th>Type</th>
<th>Condition</th>
<th>Clinical course</th>
<th>Defect*</th>
<th>Blood</th>
<th>Urine†</th>
</tr>
</thead>
<tbody>
<tr>
<td>I PKU</td>
<td>Classical</td>
<td>Untreated infants become profoundly retarded and have abnormal EEGs frequently with seizures; odours and eczema common; response to therapy poor when diagnosed late, e.g. after 1 yr</td>
<td>Phenylalanine hydroxylase activity unmeasurable</td>
<td>Phe levels between 50–100 mg/100 ml during infancy on regular diets; normal serum tyrosine (&lt;5 mg/100 ml); phe levels between 30–50 mg/100 ml during adulthood on regular diet, &gt; 50 mg/100 ml on challenge diet</td>
<td>Very markedly abnormal levels or excretion rates of phe and metabolites</td>
</tr>
<tr>
<td>II PKU</td>
<td>Classical</td>
<td>Untreated become moderately to mildly retarded; EEGs frequently abnormal but seizures uncommon; odour and eczema like type I; improve with dietary therapy even when started late, e.g. after 2–3 yr</td>
<td>Phenylalanine hydroxylase activity probably minimal</td>
<td>Phe levels between 30–50 mg/100 ml during infancy on regular diets; normal tyrosine; phe levels between 20–30 mg/100 ml during adulthood on regular diet, &lt; 50 mg/100 ml in response to challenge diet</td>
<td>Markedly abnormal levels or excretion rates of phe and metabolites</td>
</tr>
<tr>
<td>III Variant</td>
<td>Persistent</td>
<td>Untreated usually have normal development; EEGs usually normal; patients have odours intermittently</td>
<td>Phenylalanine hydroxylase activity probably increased and appears to be inducible</td>
<td>Phe levels typically in 15–25 mg/100 ml range, but initially may be 30–40 mg/100 ml on regular diets during early infancy; phe levels in 12–20 mg/100 ml range in adulthood on regular diets; normal tyrosine; phe levels oscillate between 15–25 mg/100 ml in response to challenge diet during infancy and adulthood</td>
<td>Metabolites consistent with PKU but generally at lower levels; initially may suggest type II PKU; PPyA not present intermittently during adulthood on ordinary diet</td>
</tr>
<tr>
<td>IV Variant</td>
<td>Persistent</td>
<td>Untreated have normal development; EEGs normal; rarely have odour and no recognized except for screening programmes</td>
<td>Phenylalanine hydroxylase activity appears to be greater than type III and also inducible</td>
<td>Phe variably raised, but generally &lt;20 mg/100 ml during infancy on regular diets; levels may be raised just above normal (4–6 mg/100 ml) during adulthood on normal diets; phe levels in 12–20 mg/100 ml range in response to challenge and frequently with oscillating phe levels</td>
<td>During infancy phe excretion is increased but other metabolites at low levels and PPyA rarely detected; phe excretion and metabolites may be just above normal in adulthood</td>
</tr>
<tr>
<td>V Variant</td>
<td>Persistent</td>
<td>Untreated have normal development; EEGs normal; odour may be noted with illness during infancy; never recognized except for blood screening programmes</td>
<td>Phenylalanine hydroxylase activity probably just less than normal</td>
<td>Peak phe values during infancy on regular diets approach 10–12 mg/100 ml; by 2 yr phe values are generally less than 6–8 mg/100 ml; in adulthood phe values normal by usual screening methods; recognized during adulthood by deliberately challenging with high protein and phe intake</td>
<td>Phe and 'end' metabolites such as oHPAA and PAG may be just above normal during infancy and early childhood, phe and metabolites normal in adulthood except during ingestion of very high protein intakes, as during a challenge</td>
</tr>
</tbody>
</table>

*Defects are indicated by values during infancy. PKU, phenylketonuria; Phe, phenylethanol; oHPAA, o-hydroxyphenylacetic acid; PPyA, p-phenylpyruvic acid; PAG, phenylacetylglutamine.

†Urinalysis includes tests for oHPAA and PAG, which are usually normal in PKU but may be raised in other conditions such as oHPAA and PAG, which are usually normal in PKU but may be raised in other conditions.
**TABLE—continued**

<table>
<thead>
<tr>
<th>Type</th>
<th>Condition</th>
<th>Clinical course</th>
<th>Defect*</th>
<th>Blood</th>
<th>Urinet†</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>Transaminase Defect</td>
<td>Normal, no odour</td>
<td>Phenylalanine transaminase</td>
<td>Phe variably raised to 30 mg/100 ml; tyrosine normal</td>
<td>PHE excretion increased but other phe metabolites usually seen in PKU are conspicuously absent, e.g. PPYA, oHPAA</td>
</tr>
<tr>
<td>VII</td>
<td>Tyrosinaemia of neonate</td>
<td>Frequently premature infant, or term infant receiving high protein intake; rarely lasts longer than 1 mth</td>
<td>Vitamin C dependent and responsive para hydroxy phenylpyruvic acid oxidase; inhibited by substrate accumulation proximal to enzyme</td>
<td>Phe may temporarily rise to as high as 25 mg/100 ml; but generally 4-12 mg/100 ml; tyrosine from 5-50 mg/100 ml</td>
<td>Increased levels or excretion rates of tyrosine and metabolites; generally low or nondetectable levels of phe and metabolites</td>
</tr>
<tr>
<td>VIII</td>
<td>Tyrosinosis</td>
<td>Hepatomegaly, rickets, growth failure; there may or may not be jaundice; may have odour of methionine</td>
<td>Unknown</td>
<td>Phe may be in 4-6 mg/100 ml range; tyrosine generally between 3-10 mg/100 ml</td>
<td>Generalized nonspecific aminoaciduria with renal deToni-Fanconi syndrome; phe excretion inconsequential</td>
</tr>
</tbody>
</table>

*Reports (Barranger, et al., 1972; Kaufmann and Fisher, 1970) suggest that phenylalanine hydroxylase activity may be the sum of 2 or 3 isoenzymes.  
†The metabolites most frequently cited in association with PKU are phenylpyruvic acid, PPYA; ortho-hydroxyphenylacetic acid, oHPAA; and phenylactic acid, FAA (cause of the odour). However, many laboratories can routinely determine phenylacetilglutamine, phenyl-lactic acid, mandelic acid, n-acetyl phenylamine, m-hydroxyphenylacetic acid, and other metabolites.

![Fig. 1.—Type I phenylalaninaemia (classical PKU) in a patient aged 10 months. A linear increase in serum phe is noted in response to a constant high phe intake provided as natural protein. This response and the degree of intolerance appear to be constant with increasing age. Despite a low starting level (3 mg/100 ml) the serum phe rose rapidly to greater than 50 mg/100 ml in 72 hours.](image1)

![Fig. 2.—Type II phenylalaninaemia (classical PKU). Serum phe plateaux at less than 50 mg/100 ml. This patient (aged 19 years) has had no dietary restrictions, but has an IQ of 65 (Wechsler Adult Intelligence Scale). She was diagnosed at 19 years of age because of mild mental retardation and a history of having had a peculiar body odour as an infant. Despite the high serum phe level at the initiation of the challenge, her peak phe value at the end of 72 hours was only 37 mg/100 ml. Average phe intake from her regular diet was 80 mg/kg per day.](image2)
Phenylalaninaemia

839

separated from type I. The enzyme defect has been considered to involve phe-hydroxylase and, based upon tolerance to phe, with probably minimal activity.

Type III. Patients with this form of phenylalaninaemia generally are and have been included under the term PKU. However, they are the subjects who in the past have been called 'atypical PKU' patients. They are atypical because biochemically they are phenylketonuric 'by the usual criteria', but they have normal intelligence despite never having received any form of treatment. They are difficult to identify. On unrestricted intakes, serum phe levels during the early weeks of life may transiently rise as high as 40 mg/100 ml, but in response to a constant challenge intake their phe levels range between 15 and 25 mg/100 ml (Fig. 3–5). The metabolites associated with PKU are present in urine, but the amounts excreted are often surprisingly low as compared with types I and II. This is due to the unpredictable and fluctuating serum phe levels. Phe levels in adulthood, while ingesting regular diets, vary between 12 and 20 mg/100 ml. Phe levels, to an extent, are dependent on phe and protein intake. When phe tolerance is assessed, phe and protein intakes from 120 to 180 mg/kg per day have relatively little effect on serum phe levels. The progressive linear rise in serum phe, typical of type I and II PKU patients on the challenge diet, is not seen (Fig. 3–5). Characteristically, on a high phe intake, the serum phe level will rise initially and then either plateau or oscillate and usually decrease, which suggests a degree of adaptation or enzyme induction as a consequence of the high phe intake. They may have the odour of PKU intermittently, depending upon their serum phe level. If they have 'good treatment' when treated for PKU, their serum phe levels will not become subnormal. However, when phe intake is significantly decreased, serum phe levels also decrease as in types I and II. Minimal phe requirements appear to be comparable for all phenylalaninaemic groups. A clue to the diagnosis may be the remark-

Fig. 4.—Type III phenylalaninaemia (variant). The response to a high phe intake by this patient (aged 15 months), who erroneously was classified as type II because of an overload at 3 months of age, is typical of type III phenylalaninaemia. The oscillatory response is suggestive of variable and inducible enzyme activity.

Fig. 5.—Type III phenylalaninaemia (variant). This patient (aged 17 years) was identified at 8 years of age when a sib was detected by a urinary screening test. He never received any form of dietary therapy or had any dietary restrictions, yet is normal (WAIS = 128). He also has had intermittently positive urinary ferric chloride tests. He might be considered a classical 'atypical PKU' subject.
ably stable serum phe levels despite marked dietary indiscretions. Intermittent illnesses can cause their serum phe and urinary metabolite levels to rise noticeably as in true PKU. This response to illness may be seen in all of the phenylalaninaemias and, therefore, cannot be reliably used for differentiation. Untreated, these patients generally have no EEG abnormalities and their intelligence is normal.

Type III patients are generally identified after an infant sib has been detected via a screening programme and the family is then screened for other affected members. 5 patients in 2 families studied in this clinic with type III phenylalaninaemia, and who have previously been described (Blaskovics, 1974), were recognized in this manner. The similarity in responses to the standardized challenge by these 5 patients, and other patients studied in this clinic, strongly suggests that type III patients represent a distinct genetic population. The enzyme defect appears to be different from that of types I and II, regardless of whether or not it affects the same enzyme as in types I or II phenylalaninaemia (true PKU). Type III phenylalaninaemia is considered to be a variant disorder because of its generally benign nature. One patient who appears to have a type III response is retarded. It is conceivable that his retardation is related to the raised phe levels, but an older untreated sib with less tolerance to phe, but who has a precisely similar response to the challenge diet (Fig. 5), has superior intelligence (IQ 128) and is functionally normal in all respects.

Another form of phenylalaninaemia which has been included under the umbrella of 'hyperphenylalaninaemia' and is usually presented as one population is classified in the present studies as two subpopulations, designated types IV and V. Patients in both groups show normal intelligence without dietary restriction and EEGs, and other clinical parameters are almost always normal.

**Type IV.** In this group, serum phe levels on unrestricted dietary intakes during the early weeks of life range between 15 and 20 mg/100 ml. Urine may show metabolites associated with PKU, but intermittently, uncommonly, and at very low levels in proportion to the low serum phe levels. Serum phe levels during adulthood are normal or just above normal, e.g. 4 to 6 mg/100 ml. These patients are relatively easy to recognize in infancy because of their low serum phe levels while receiving normal or regular diets. A sustained phe intake of approximately 180 mg/kg per day often will cause serum phe levels to increase initially, then to decrease suddenly and stabilize between 12 and 20 mg/100 ml, a response similar to type III patients but at lower levels (Fig. 6). This response also suggests induction of enzyme activity. The PKU odour due to phenylacetic acid is infrequently present.

**Type V.** Type V phenylalaninaemia is the one most easily recognized during infancy. Serum phe levels on regular diets are almost consistently less than 10 mg/100 ml in the first year of life, except during illnesses, when they may rise as high as 20 mg/100 ml and metabolites typical of PKU may be found in urine. Overloading with phe, e.g. intakes greater than 225 to 250 mg/kg per day, usually does not cause a phenylketonuric chemical response in these infants (Fig. 7a and b). These patients develop normally without dietary restrictions and serum phe levels decrease early into a normal range. The eventually normal phe level is probably a consequence of the decreased protein and phe intakes per unit of body weight, which naturally occurs with increasing age. However, an increased phe intake can show the minimal impairment in phe metabolism even at older ages (Fig. 8 and 9). These seemingly normal subjects may be the adults who with L-phe challenges have abnormal phe responses and are sometimes called, erroneously, heterozygotes.
Phenylalaninaemia

The response typical of a normal control patient to a high phe and protein intake is shown in Fig. 10.

**Type VI.** This type of phenylalaninaemia has rarely been detected. It is recognized by the conspicuous absence of, or very low levels of, the typical urinary metabolites of PKU, e.g. PPyA and oHPAA (ortho-hydroxyphenylacetic acid), while the serum phenylalanine is markedly raised (30 to 40 mg/100 ml). The urinary metabolites usually seen in PKU are low or absent because the transaminase enzyme which converts phe to PPyA is inactive. oHPAA is low or not present because it is primarily derived from PPyA. This phenylalaninaemia was first described by Auerbach, DiGeorge, and Carpenter (1967). Another case was suspected by Anderson et al. (1966), and more recently Menkes and Holtzman (1970) and McBean (1970) have described patients suspected of having partial transaminase defects. Serum tyrosines were not raised. It has been suggested that these patients develop normally without dietary restrictions.
Type VII. Phenylalaninaemia associated with 'transient tyrosinaemia of the neonate' has no proven morbidity, but it is important because it is the most frequent cause of raised serum phe. The diagnosis is made by determining a raised serum tyrosine level (5 to 50 mg/100 ml) in conjunction with a raised serum phe (6 to 20 mg/100 ml). Both abnormal amino acid levels usually normalize within a week when protein intake is reduced. This reversible condition is noted most frequently in premature infants and in term infants receiving very high protein intakes. Though vitamin C has been shown to be a co-factor for the para-hydroxyphenylpyruvic acid oxidase enzyme which is inhibited by the accumulation of substrates present in transient tyrosinaemia, there is good evidence that the practice of supplementing these patients with excess vitamin C is of little value in decreasing the tyrosinaemia (Raine et al., 1972).

Type VIII. The phenylalaninaemia which is seen with tyrosinosis is clinically of no consequence. Serum tyrosine is only slightly raised (3 to 10 mg/100 ml), and the serum phe level is usually just above the minimum needed for a positive Guthrie test (4 to 6 mg/100 ml). The diagnosis is made on clinical grounds, e.g. hepatomegaly, failure to thrive, rickets, and the finding of generalized aminoaciduria of the pattern usually associated with a renal deToni-Fanconi Syndrome.

Other nonspecific causes of phenylalaninaemia. There are other causes of raised serum phe not included in this scheme. The infant born to a mother with PKU is one example (Forbes et al., 1966). Another example is galactosaemia, which can cause a transient and mildly raised serum phe level due probably to the liver damage of galactosaemia (R. Guthrie, personal communication, 1972).

Discussion

The first newborn PKU screening reports in Massachusetts suggested an incidence of 1:7000, whereas a more recent report from there indicated a probable PKU incidence of 1:16,000 (Levy, 1973). These discrepancies are due to the fact that the earlier newborn screening reports included patients with phenylalaninaemia due to causes other than true PKU.

The lack of unanimity about the definition of PKU makes it virtually impossible to arrive at true incidence figures. For example, in some centres (Bickel, 1968; McBean, 1970) phe levels of 8 to 10 mg/100 ml are considered consistent with mild PKU and treatment is initiated. Other centres (Berry, Sutherland, and Umbarger, 1966) consider a phe level of 15 or 20 mg/100 ml (J. Berman, personal communication, 1973) as diagnostic for PKU and as the criterion for treatment versus nontreatment. A definition of PKU based only on random or isolated blood phe levels is arbitrary and will be influenced by the experience and prejudices of the individual investigator.

The incidence of phenylalaninaemia types III, IV, and V cannot yet be given; screening reports suggest that approximately 1/3 to 1/2 of all patients with phenylalaninaemia are in these clinically benign groups (Blaskovics and Nelson, 1971; Levy, 1973). Most clinics have included patients with type III phenylalaninaemia under the heading PKU, or atypical PKU, because chemically they are 'phenylketonuric'. This scheme classifies type III phenylalaninaemia as a distinct entity because unlike PKU, in our experience, these patients appear to have a benign clinical course. Except in rare instances, patients with type III phenylalaninaemia are treated as if they had type I or II disease because they are difficult to recognize. This skews the statistics towards an increased relative incidence of true PKU.

The present classification evolved after studying serum phe responses to nearly constant high phe intakes provided as natural proteins from food that would be ingested in everyday life, and after longitudinal studies of patients with clinical and chemical PKU and other phenylalaninaemias who had escaped retardation despite lack of dietary restriction. It is appreciated that the scheme suffers the defect of making diagnoses on indirect evidence, i.e. predicting a genotype on the basis of a
phenotypic response to an extended tolerance test and clinical evidence. This is not unreasonable, however, because other inborn errors have previously been shown to have gradations in enzyme activity with variable clinical manifestations (Dancics, 1967). Eventually, no doubt, diagnoses in the phenylalanaemia will be made on the basis of total enzyme activity and/or enzyme components or isoenzymes.

The authors wish to acknowledge the help of all the members of the Child Development Division PKU Staff, the Clinical Research Center Staff, the Staff of the Chromatography Laboratories under the direction of Dr. K. N. F. Shaw, and Drs. Samuel Bessman and Ennis Layne for aiding in the serum phe determinations; and particularly Miss Betty Knoch for collecting serum phe specimens. This study was supported in part by NIH Grant No. R.R.-86, and in part by a Maternal and Child Health Service Grant, No. 911.

REFERENCES

Correspondence to Dr. M. Blaskovics, Children’s Hospital of Los Angeles, 4650 Sunset Boulevard, Los Angeles, California 90027, U.S.A.