Phenylalanine metabolites, attention span and hyperactivity\(^1,2\)

K Michals, RD, PhD and R Matalon, MD, PhD

ABSTRACT The metabolites of phenylalanine, phenylacetate, phenyllactate, phenylpyruvate and phenylethylamine, were measured in the urine of PKU patients. In general correlation was found between serum phenylalanine excretion of these metabolites. However, there were individual variations in the quantities and type of metabolites excreted that could not be explained by blood phenylalanine levels. In a PKU pregnancy large quantities of phenylalanine metabolites were found in urine despite a modest elevation of serum phenylalanine. Increase in the excretion of phenylalanine metabolites was found in patients who were considered to have good blood phenylalanine control. These preliminary studies indicate that the current practice of allowing a wide range of blood phenylalanine in the treatment of PKU may have to be reexamined. Since these metabolites are neurotoxic, they may afford a new parameter for the study of PKU not only regarding the prevention of mental retardation but also with regards to behavior and learning disabilities. *Am J Clin Nutr* 1985;42:361–365.

KEY WORDS Phenylalanine, phenylethylamine (PEA), aromatic acids of phenylalanine, phenylketonuria, behavior and learning problems

Introduction

Phenylketonuria (PKU) is an autosomal recessive disorder caused by a deficiency of the enzyme phenylalanine hydroxylase. As a result of this enzymic defect, phenylalanine is not hydroxylated to tyrosine. Subsequently, phenylalanine levels in blood and other body fluids increase. The aromatic acid derivatives of phenylalanine, phenylacetate, phenyllactate and phenylpyruvate, are increased in the untreated PKU patients. These derivatives have been shown to be neurotoxic (1). In addition to these metabolites, phenylethylamine (PEA), which is an endogeneous amine with pharmacological action similar to amphetamine, has been shown to be increased in untreated patients with PKU (2–4).

More recently deficiency of the cofactor tetrahydrobipterin (BH4) has been shown to cause hyperphenylalaninemia in some cases (5–7). Since BH4 is the cofactor not only for phenylalanine hydroxylase, but also for tyrosine and tryptophan hydroxylase, the level of the neurotransmitter derivatives of the latter amino acids, dopamine and serotonin, may be compromised (8–10). Thus, PKU and its variants can serve as a model for the correlation, and hopefully the understanding, of the effect of phenylalanine, its metabolites and the modulation of neurotransmitters in normal and disease states.

The detection of PKU through newborn screening programs and early treatment has resulted in the elimination of mental retardation associated with PKU. More recently attempts to screen for biotyperin defects have been implemented in this country as a part of the comprehensive PKU screening program (11–13). Early detection of these patients will help institute proper therapy with neurotransmitter precursors. Nevertheless, despite the improved outlook for early treated

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Received February 19, 1985.

Accepted for publication March 12, 1985.
PKU children, learning disabilities, behavior disorders and decline in academic performance have been reported in these children during school years (14–17).

This report correlates blood levels of phenylalanine with the urinary excretion of PEA and the aromatic acid derivatives of phenylalanine in PKU patients. Since the range of blood phenylalanine levels considered therapeutically safe varies considerably, and since these metabolites are neurotoxic, their measurement may provide a specific parameter for a better understanding of behavior and learning disabilities in early treated PKU patients. The study of phenylalanine metabolites may be of importance in pregnant women who have PKU. There is an increasing number of women treated in childhood for PKU, with normal intelligence who are now entering the childbearing age. High blood phenylalanine levels during pregnancy have been associated with fetal malformations and mental retardation (18). The nature of this association is unclear since there have been conflicting reports of the blood phenylalanine levels that cause such defects (18–22). The association of phenylalanine metabolites with the maternal PKU syndrome has not been studied. This report will include a study of these metabolites in one PKU pregnancy.

Methods

Patients

The aromatic acid derivatives of phenylalanine were studied in 22 urine specimens from 19 individuals with PKU with normal intelligence, 9 boys and 10 girls. These individuals ranged in age from 2 wk to 16 yr. Urinary phenylethylamine was measured in six PKU patients. There were three males and three females between the ages of 3–14 yr. All patients were on restricted phenylalanine intake and consumed either Lofenamic or Phenyl-Free (Mead Johnson Laboratories, Evansville, IN) or PKU-2 (Milupa Corporation, Darrien, CT). A pregnant female, 20 yr old, with PKU was started on a low-phenylalanine diet at 16 wk gestation. She was treated with PKU-3 (kindly donated by Milupa Corporation), and her urinary aromatic acid derivatives were assayed throughout the pregnancy.

Blood phenylalanine was determined by a fluorometric method (23). Urinary aromatic acids of phenylalanine were extracted by ethylacetate and determined by gas chromatography as the silyl derivatives (24). The identity of the various metabolites was verified by their characteristic ion mass, using gas chromatography/mass spectroscopy system (3992-B, Hewlett-Packard, Palo Alto, CA).

Phenylethylamine in urine was determined by the method of Brossat et al using high-performance liquid chromatography and pre-column derivatization with dansyl chloride. A radial compression module was used. (RCM-100, Waters, Milford, MA) (25).

Results

The excretion of phenylacetic, phenyllactic, and phenylpyruvic acid was studied in 22 urine samples from 19 phenylketonuric patients. These patients were divided into three groups based on their mean blood phenylalanine concentrations. The first group consisted of seven patients with a mean serum phenylalanine of 3.8 mg/dl (SD = 1.6). Eight urine samples were assayed from these patients. Small amounts of phenylacetate were found in two of the eight specimens. Phenyllactate was excreted in four of eight specimens and phenylpyruvate in three of the eight samples. Three patients did not excrete any of these metabolites. The mean excretion of these metabolites in this group of patients is summarized in Table 1.

The second group consisted of six phenylketonuric patients. These patients were on phenylalanine restricted diet and had a mean serum phenylalanine concentration of 9.3 mg/dl (SD = 2.1). One patient did not excrete phenylacetic acid but all patients excreted phenylpyruvic acid. The mean excretion of these metabolites was greater than in Group 1 as shown in Table 1.

There were seven samples assayed from six phenylketonuric patients in the third group. The mean serum phenylalanine concentration in this group was 17.2 mg/dl (SD = 2.7). All six patients excreted large quantities of the phenylalanine metabolites. There was a large range of individual differences in the excretion as indicated by the standard deviation shown in Table 1. There are also variations in the quantities of the various aromatic acid derivatives of phenylalanine in relation to blood phenylalanine levels.

The excretion of urinary phenylacetate and phenyllactate was studied in a pregnant PKU female. Dietary restriction of phenylalanine was started at 16 wk gestation. At the first clinic visit when blood phenylalanine was 12.0 mg/dl, the excretion of phenylacetic and phenyllactic acids was 251 and 103 mg/g.
Organic acids of phenylalanine in a PKU pregnancy

### TABLE 1
Organic acids of phenylalanine in phenylketonuric children under dietary control

<table>
<thead>
<tr>
<th>Number samples (patients)</th>
<th>Mean blood phenylalanine (SD)</th>
<th>Urine metabolites</th>
<th>Phenylacetate (SD)</th>
<th>Phenyllactic (SD)</th>
<th>Phenylpyruvic (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/dl</td>
<td>mean mg/g creatinine</td>
<td>mg/dl</td>
<td>mean mg/g creatinine</td>
<td>mg/dl</td>
</tr>
<tr>
<td>8* (7)*</td>
<td>3.8</td>
<td>2.6</td>
<td>23.7</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>6† (6)†</td>
<td>9.3</td>
<td>53.7</td>
<td>140.6</td>
<td>238.0</td>
<td></td>
</tr>
<tr>
<td>7‡ (6)‡</td>
<td>17.2</td>
<td>237.9</td>
<td>2294.2</td>
<td>829.7</td>
<td></td>
</tr>
</tbody>
</table>

* = Group 1.  
† = Group 2.  
‡ = Group 3.

Throughout the remainder of the pregnancy minimum excretion of these metabolites occurred with further phenylalanine restriction. Urine samples were monitored weekly to assure compliance, and the data in Table 2 are representative samples. Immediately following delivery, normal diet was resumed by the patient. Blood phenylalanine rose to 24.5 mg/dl and excretion of these metabolites rose as indicated in Table 2.

Phenylethylamine excretion was measured in urine samples from six PKU patients and three normal individuals. The phenylalanine concentrations ranged from 2.9–18.3 mg/dl in the PKU patients. In three controls PEA excretion was 0.6–0.13 ng/mg creatinine. In all six PKU patients phenylethylamine excretion was markedly elevated as shown in Table 3.

### TABLE 2
Organic acids of phenylalanine in a PKU pregnancy

<table>
<thead>
<tr>
<th>Blood phenylalanine (mg/dl)</th>
<th>Urinary metabolites</th>
<th>Phenylacetate (mg/g creatinine)</th>
<th>Phenyllactic (mg/g creatinine)</th>
<th>Phenylpyruvic (mg/g creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.0</td>
<td>251.0</td>
<td>103.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>0.6</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>0.5</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24.5</td>
<td>113.2</td>
<td>301.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

There is concern with reports of behavior problems and loss of academic skills in early treated PKU children (14–17). In past studies, mean blood phenylalanine levels have been correlated to global intelligence quotient in order to determine safe plasma phenylalanine levels for treatment of PKU. A wide range of safe blood phenylalanine levels have been described.

This study examined the excretion of phenylalanine metabolites in children with PKU with varying concentrations of blood phenylalanine. It is clear that phenylacetic, phenyllactic and phenylpyruvic acid excretion increased with serum phenylalanine concentration. When mean serum phenylalanine was 3.8 mg/dl some patients did not excrete any

### TABLE 3
PEA excretion in six PKU patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Blood phenylalanine (mg/dl)</th>
<th>PEA (ng/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.9</td>
<td>2.55</td>
</tr>
<tr>
<td>2</td>
<td>5.1</td>
<td>6.78</td>
</tr>
<tr>
<td>3</td>
<td>9.6</td>
<td>11.49</td>
</tr>
<tr>
<td>4</td>
<td>11.5</td>
<td>30.60</td>
</tr>
<tr>
<td>5</td>
<td>16.7</td>
<td>53.81</td>
</tr>
<tr>
<td>6</td>
<td>18.3</td>
<td>52.83</td>
</tr>
<tr>
<td>Control (n = 3)</td>
<td>0.8–1.0</td>
<td>0.06–0.13</td>
</tr>
</tbody>
</table>
of these metabolites and other patients excreted small amounts. In spite of low blood phenylalanine levels in this group, some children excreted these metabolites. Individual variation in the excretion of these metabolites may be the cause or the excretion may fluctuate with degree of compliance with diet. It is likely that the aromatic acid derivatives of phenylalanine lag behind the blood levels of phenylalanine which may be low during clinic visits, but the urine metabolites still remain elevated. Individual differences with rates of growth, weight gain or loss may also contribute to such variations. In the third group there was considerable variation in the excretion of these metabolites. It would be important to study the serial excretion of these metabolites to find if there is delayed clearing of these metabolites in relation to blood phenylalanine concentration.

Measuring the excretion of these metabolites during pregnancy may be of importance since they are neurotoxic (1). Reports of safe blood phenylalanine levels to be maintained during pregnancy vary greatly. According to some investigators blood phenylalanine of 12 mg/dl at 16 wk gestation would not require dietary therapy (21). However, in our pregnant PKU, when the baby was born it was small for gestational age, microcephalic and had a characteristic facial appearance reported by Lipson et al (20). In this case the excretion of phenylalanine metabolites may have been of important prognostic value, more than the actual serum phenylalanine level.

In addition to these aromatic acid derivatives of phenylalanine, phenylethylamine, a potent endogeneous amine, can be monitored in these patients. Preliminary studies show elevated excretion of this metabolite in patients with PKU. Further work must be completed to determine the levels of blood phenylalanine that result in close to normal PEA excretion.

The metabolites of phenylalanine may be of importance in determining a safe method for the treatment for PKU. It is possible that individual variation occurs and an acceptable blood phenylalanine for one patient may be unsafe to another patient. Further work on the excretion of these metabolites must be completed before behavior studies are attempted. However, it is likely that a relationship between the excretion of these metabolites, hyperactivity, and behavior problems exists.

References