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ABSTRACT

The booklet covers the identification, diagnosis, and clinical treatment of newborns with Phenylketonuria (PKU), an inborn error of metabolism, which, if untreated, can lead to mental retardation. An initial section considers biochemical and genetic factors of PKU including a diagram of aromatic amino acid hydroxylation systems. Screening procedures for detecting phenylalanine in the blood are reviewed, and followup actions are considered. The relation of infant age and time of initial blood screening is examined. Initiation of treatment is discussed and dietary management aspects described. Medical services, including diet termination and genetic counseling, are considered. Two appendixes, on dietary guidelines and diagnostic confirmation, are included. (CL)

MANAGEMENT OF NEWBORN INFANTS WITH PKU

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Health Services Administration
Bureau of Community Health Services

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Introduction

These guidelines for management of newborn infants with phenylketonuria have been prepared by a committee of the Collaborative Study of Children Treated for Phenylketonuria (PKU), comprising Kenneth N. F. Shaw, Ph. D., Richard Koch, M.D., George N. Donnell, M.D., and James C. Dobson, Ph.D., Children's Hospital of Los Angeles; Margaret E. O'Flynn, M.D., Children's Memorial Hospital, Chicago; and Phyllis Acosta, Dr. P. H., University of New Mexico, Albuquerque. The publication reviews current understanding of PKU, offers an approach to therapy, and provides guidelines for professionals in the health sciences, including medicine, nutrition, nursing, social work, psychology, and biochemistry, and for allied health personnel and others interested in this disorder. The information is for those dealing on a day-to-day basis with PKU patients as well as new students in the field. A comprehensive approach to care is stressed.

For the reader interested in the broader aspects of screening for PKU and other metabolic disorders, other publications are recommended. The most recent is entitled "Newborn Screening for Genetic-Metabolic Diseases: Progress, Principles and Recommendations," by Neil A. Holtzman, M.D., of Johns Hopkins University.[*1*] It is published by the Bureau of Community Health Services, Health Services Administration, U.S. Department of Health, Education, and Welfare, Washington, D.C. A more comprehensive reference for an overall approach is a book entitled "Genetic Screening: Progress, Principles and Research," published by the National Academy of Sciences, 1975 [2].

The present publication is based upon the experiences developed within the Collaborative Study of Children Treated for Phenylketonuria [*3*]. This project, in its 10th year, is a joint effort of 15 clinical programs coordinated through Children's Hospital of Los Angeles and supported by the Bureau of Community Health Services through funds appropriated under title V of the Social Security Act.

What is PKU?

Phenylketonuria (PKU) is an inherited metabolic disorder in which the affected individual lacks the ability to convert the amino acid phenylalanine to the amino acid tyrosine due to a defect in, or absence of, phenylalanine hydroxylase [*4*]. Untreated classical PKU patients have very high levels of phenylalanine and its metabolites in blood, urine, and other body fluids. This leads to serious mental retardation, neurological changes, and other clinical problems such as eczema. These adverse effects can be avoided only if PKU is detected soon after birth, and long-term therapy is initiated promptly.

Treatment of PKU is primarily dietary [*4*]. A special formula low in phenylalanine is utilized in order to maintain phenylalanine blood levels in the range of 2–10mg%. This will supply enough phenylalanine and other necessary nutrients to insure normal growth and development [*5*].

In contrast to classical PKU patients, some individuals have been found with a limited but appreciable ability to convert phenylalanine to tyrosine [*6*]. They show lesser elevation in levels of phenylalanine and its metabolites in blood and urine, and have been designated as hyperphenylalaninemic variants (HPV). Variants generally have no mental disability or other clinical problems, and they usually do not require dietary treatment. Because they lack abnormal clinical signs and symptoms, such variants usually escaped detection before the establishment of screening programs during the last decade.

Differentiating the PKU patient from the HPV as early as possible is desirable to insure that dietary treatment is applied only where needed so that unnecessary emotional strain to the patient and expense to the family are minimized. Biochemical challenge procedures which test the patient's phenylalanine tolerance and metabolic abilities at age 3 months and again at 1 year have been used to separate PKU and HPV patients with reasonable success, and are described later in this publication [*7*].

BIOCHEMICAL CONSIDERATIONS

The more important pathways of phenylalanine are outlined in diagram 1. Phenylalanine and tyrosine are derived from the digestion of proteins in various foods, and in turn are used along with other amino acids from the

diet to build necessary body proteins. Most of the phenylalanine not needed for protein synthesis normally is converted by the liver enzyme phenylalanine hydroxylase to tyrosine. Excess tyrosine subsequently is transformed through homogentisic acid to carbon dioxide and water. In the normal individual, a small amount of phenylalanine is transaminated along a secondary path to give phenylpyruvic acid which does not appear in the urine but is converted entirely to two other urinary metabolites, namely, phenylacetylglutamine (50–470 mg/day [*8*]) and *ortho*-hydroxyphenylacetic acid (1–2 mg/day).

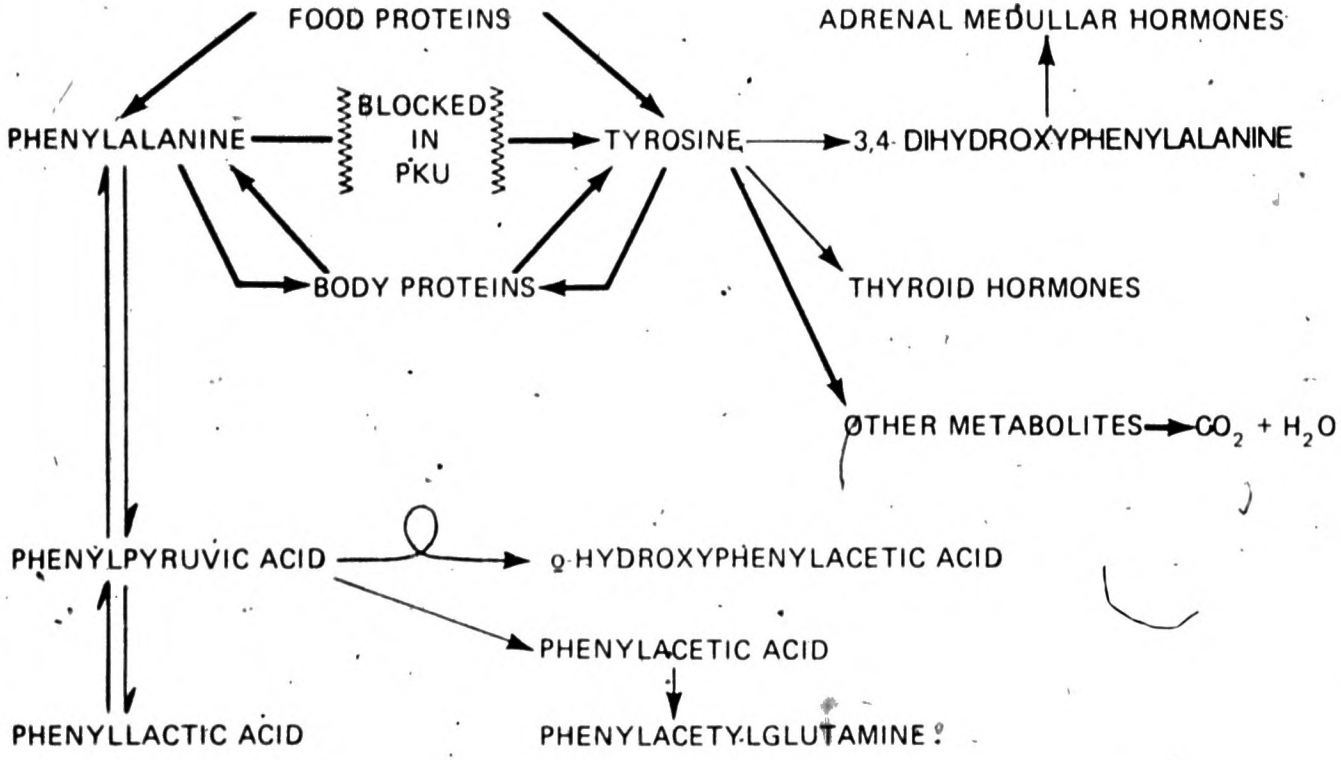
PKU patients are not able to convert phenylalanine to tyrosine to a significant extent. The consequent block in the primary metabolic path (heavy arrows), like a dam in a stream, results in a rapid increase in the concentration of phenylalanine in blood, urine, and various tissues of the body in the days following birth. Phenylalanine then is diverted into the secondary metabolic path (light arrows) to produce relatively large amounts of phenylpyruvic, phenyllactic and *o*-hydroxyphenylacetic acids, and phenylacetylglutamine.

These compounds, together with phenylalanine, are excreted in the urine of the untreated patient in large quantities a few weeks following birth. When the dietary intake of phenylalanine is carefully controlled, this unusual pattern soon reverts to near normal, in parallel with a decease in the serum level of phenylalanine.

Recent work [*9*] has shown that phenylalanine hydroxylase is not a single enzyme, but may be a mixture of three isozymes. In the normal individual, all three isozymes are present and functional in the liver. In the PKU patient, these isozymes are almost devoid of activity because of the genetic defect. In the HPV, one or more of the phenylalanine hydroxylase isozymes has appreciable activity. The total enzyme activity may be significantly less than in the normal individual, but is sufficient to metabolize a substantial proportion of the excess dietary phenylalanine. The levels of phenylalanine and its metabolites in blood and other tissues therefore are distinctly less than in the classical PKU patient on the same dietary intake, and thus, the HPV usually escapes major damage to the central nervous system. The current findings on isozymes permit speculation that phenylalanine hydroxylase may be controlled by more than one gene. The actual significance in terms of PKU, however, must await further research.

A new type of hyperphenylalaninemia has been described recently [*10*]. The biochemical hallmarks—abnormally high levels of phenylalanine and its metabolites in urine, blood, and other body fluids—may be the same as in classical PKU, although patients with lesser elevations of phenylalanine have been reported [*11*]. These abnormalities diminish when the dietary intake of phenylalanine is curtailed; however, the clinical picture of chronic myoclonic seizures and progressive mental retardation does not improve. Studies on liver enzymes have demonstrated presence of an active phenylalanine hydroxylase, but a dearth of the pteridine cofactor resulting from a genetic defect in the second enzyme of the system—*dihydropteridine reductase* (DPR). This same cofactor and its reductase are required for

Diagram 1. SOME PATHWAYS OF PHENYLALANINE METABOLISM



hydroxylation in the synthesis of 3, 4-dihydroxyphenylalanine (DOPA) from tyrosine and 5-hydroxytryptophan (5HTP) from tryptophan. (See diagram 2.) Patients with DPR defect therefore suffer from three simultaneous metabolic blocks: they are unable to convert phenylalanine to tyrosine (as in classical PKU), and also cannot make DOPA or 5HTP which are essential precursors of the neural hormones dopamine, norepinephrine, epinephrine and serotonin. A low-phenylalanine diet supplemented with DOPA and 5HTP is a logical therapeutic approach to patients with DPR defect: its long term effectiveness, however, is still uncertain because of scant clinical experience with this new disorder. Measurement of DPR [11] in fibroblast cultures has been suggested as part of the initial evaluation in any infant with persistent elevation of phenylalanine. A possible alternate biochemical approach, which still requires validation for diagnosis of DPR defect and monitoring efficacy of multiple dietary treatment, is measurement of homovanillic (HVA), vanilmandelic (VMA), and 5-hydroxyindoleacetic (5HIAA) acids in urine, in addition to ortho-hydroxyphenylacetic acid (*o*-HPAA), phenylpyruvic acid (PPyA), and phenylacetylglutamine (PAG).

GENETIC CONSIDERATIONS

PKU is considered to be an autosomal recessive disorder. The PKU individual is a homozygote, with two defective genes for phenylalanine hydroxylase. The parents, then, are obligatory heterozygotes for the condition, each with only one defective gene and no detectable clinical abnormality. In a mating of PKU heterozygote parents, the statistical chances at the time of *each* birth are 1 in 4 for a genetically normal child, 2 in 4 for clinically normal carriers like themselves, and 1 in 4 for an affected PKU homozygote. Should the isozyme data be substantiated, it may change this conventional explanation for the inheritance pattern of PKU.

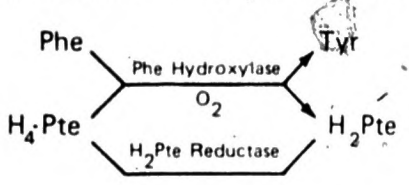
The incidence of classical PKU in the United States now is estimated to be about 1 in every 13,000 live births [*12*]. Data are fragmentary and less reliable concerning HPV but indicate an incidence of 1 in 23,000.

BENEFITS OF TREATMENT

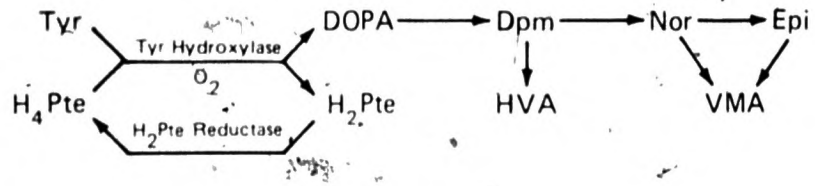
Untreated or late-treated children with PKU usually become mentally retarded. Therefore, it is imperative that the diagnosis be established within the neonatal period before the onset of mental retardation. The Guthrie screening trials [*13*] have paved the way for the establishment of newborn screening programs all over the world. Some 44 States now have screening programs.

Diagram 2. AROMATIC AMINO ACID HYDROXYLATION SYSTEMS

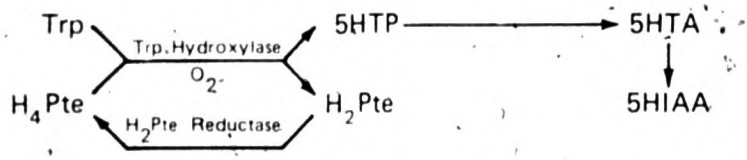
1. Conversion of Phenylalanine to Tyrosine



2. Conversion of Tyrosine to 3,4-Dihydroxyphenylalanine



3. Conversion of Tryptophan to 5-Hydroxytryptophan



List of Abbrevations

Phe: phenylalanine	Dpm: dopamine
Tyr: tyrosine	Nor: norepinephrine
DOPA: 3,4-dihydroxyphenylalanine	Epi: epinephrine
Trp: tryptophan	HVA: homovanillic acid
5HTP: 5-hydroxytryptophan	VMA: vanilmandelic acid
H_2Pte: dihydropteridine	5HTA: 5-hydroxytryptamine (serotonin)
H_4Pte: tetrahydropteridine	5HIAA: 5-hydroxyindoleacetic acid

When treatment is instituted prior to 3-4 weeks of age, normal development can be anticipated. Some years ago there was a controversy regarding the value of the phenylalanine-restricted diet. [*14*] However, today this has subsided as a result of increased experience gained from the establishment of newborn screening programs throughout the world. Admission of PKU individuals to State institutions serving the mentally retarded has almost been eliminated [*15, 16*].

Additional evidence supporting the value of treatment is being developed [*17, 18*]. While this long-term investigation is still in progress, preliminary reports suggest that PKU children develop intellectually nearly on a par with their siblings when the diagnosis and treatment are established before 30 days of age and treatment is begun promptly thereafter. These data support firmly the need for early detection and treatment.

It is of interest to examine some of the findings which thus far have emerged from the Collaborative Study.

1. *Levels of Serum Phenylalanine:* IQ test scores at years 4 through 6 showed no dependence upon, or correlation with, serum phenylalanine levels maintained between 1 and 10 mg%. [illegible] children who were maintained in the lower half of that range did not seem to differ intellectually from those controlled in the upper half of the range; indeed, both subgroups fell within normal IQ limits.
2. *Growth Parameters:* Annual growth measurements on these treated PKU children up to 4 years of age do not differ significantly from normative means with regard to height, weight, or head circumference.
3. *Age of Dietary Inception:* When PKU was diagnosed and treated within the first 30 days of life, no relationship was observed between age of dietary inception and IQ test scores at 4 through 6 years of age. However, when treatment was delayed until the second month of life or later, IQ test scores at 4 years suggested lower intelligence quotients.
4. *General Health of Treated PKU Children:* Initial pediatric evaluation performed at less than 121 days of age and 1 year followup indicated no higher occurrence of congenital disorders or disease than is expected in the population of non-PKU patients, with one exception. Pyloric stenosis was more frequent than should have occurred by chance.

HOW IS PKU DISCOVERED?

Initially PKU was identified by Fölling in individuals who were mentally retarded and who exhibited phenylpyruvic acid in the urine [*19*]. Subsequently Jervis discovered that this disease was due to an absence of phenylalanine hydroxylase activity in the liver, with associated elevated blood phenylalanine levels [*20*]. The possibility of preventing mental retardation by

treatment with a low phenylalanine diet stimulated the search for a screening test to detect PKU in the newborn period [*21*].

Dr. Robert Guthrie, who was associated with the treatment program for PKU at Buffalo Children's Hospital carried on under Dr. Robert Warner, developed an ingenious technique to measure blood phenylalanine by an inhibition assay utilizing *Bacillus subtilis* grown in agar in the presence of an inhibitor (β-2-thienylalanine). This method proved to be useful for monitoring phenylalanine levels in treated PKU children and was extended to the screening of newborns for early identification of PKU. His efforts resulted in large-scale field trials, which demonstrated the effectiveness of this method [*13*].

The Department of Health, Education, and Welfare [*22*] and the American Academy of Pediatrics Committee on the Fetus and Newborn [*23*] include the following basic requirements for an effective newborn PKU screening program.

1. Specimen collection and storage should be simple and easy.
2. The procedure and method used for screening must be established as reliable and reproducible.
3. Screening tests should be performed in laboratories experienced in recognition of abnormal findings. A system of quality control on a statewide or regional basis is an essential adjunct.
4. The initial specimen should be obtained at least 24 hours after adequate formula ingestion, and as close as possible to the time of nursery discharge, but no later than 14 days after birth. If the baby is discharged before 24 hours of milk feeding, the test should still be obtained, but arrangements should be made with the parents to have the baby retested during the second week of life.
5. A followup test should be carried out when the infant is 1–4 weeks old, particularly when the first test was obtained before the fourth day of life.
6. All presumptive positive tests should be validated promptly by specific confirmatory procedures.
7. Adequate procedures for prompt referral to a major treatment center should be clearly delineated for followup medical services to insure that affected infants receive appropriate treatment.
8. Urine screening alone is not recommended [*24*].

Evaluating the Screening Test

SCREENING FOR PHENYLALANINE IN BLOOD

The Department of Health, Education, and Welfare [22] recommends the Guthrie bacterial inhibition assay [*13*] or the McCaman-Robins fluorometric test [*25*] for screening newborns because both are simple, proven, and relatively inexpensive. The Guthrie assay uses a phenylalanine-requiring mutant of *B. subtilis* and β-2-thienylalanine as a specific growth inhibitor. Schleicher and Schuell No. 903 or Whatman 31 ET paper is recommended in collecting blood spots for the Guthrie assay; other types of filter papers have variable effects on the growth zone of *B. subtilis*. The automated McCaman-Robins procedure of Hill et al [*26*] offers the same advantages of ease in specimen collection, mailing, and storage as the Guthrie assay; however, it is slightly more expensive. Some degree of interference and consequent error also may be encountered with this method because of nonspecific fluorescence contributed by other serum components, especially at lower phenylalanine levels.

Availability of a centralized screening laboratory with both experience and reliability is a highly desirable prerequisite in the development of a mass screening program. This facility should be able to analyze a sufficient number of specimens to permit detection of several PKU patients per year so as to ensure adequate skill in laboratory diagnosis. A centralized laboratory results in maximal efficiency, in contrast to a large number of small laboratories, any one of which alone may miss detecting a PKU patient because of inexperience. Quality control on a statewide or regional basis is essential to ensure reliability of results. Routine screening with urine tests is not recommended because of a high rate of error involving false negatives.

PROTOCOL FOR FOLLOWUP OF PRESUMPTIVE POSITIVE SCREENING TESTS

Newborns who show serum phenylalanine levels exceeding 4 mg% by the Guthrie method within the first week of life should be considered presumptive PKU patients.

The following important points then should be considered:

1. Maturity of infant, i.e., full-term or premature. In the latter, transient elevations are more common with associated tyrosinemia.
2. Age at time of testing.
3. Nutrition at time of testing, i.e., how long has infant been fed with breast milk or formula?
4. Sensitivity of the test method and reliability of the laboratory.
5. The possibility of hyperphenylalaninemia without PKU.
6. Assurance of a later confirmatory test to confirm the presence of PKU before treatment.

Full-Term Infants ***(>2,500 g birth weight)***

1. A blood specimen (heel-stick) should be obtained for determination of phenylalanine at the time of discharge from the hospital (3–5 days of age). The infant should have been fed breast milk or formula for at least 48 hours before the specimen is collected.
2. For infants born at home, a blood specimen on the 5th–10th day of life is recommended.
3. The result may be considered negative if the blood phenylalanine level is less than 4 mg%. (Cutoff levels vary in laboratories from 2–6 mg%.)
4. If the specimen is obtained before the fourth day, a second screening test is indicated after the first week and before the fourth week of life.
5. When the blood phenylalanine level exceeds the cutoff value, the child should be tested again as soon as possible.
6. When the repeat test is positive, referral to a medical center experienced in evaluation of PKU is strongly recommended.
7. Full-term infants with confirmed serum phenylalanine levels exceeding 20 mg% should be placed on a phenylalanine-restricted diet as soon as possible. They should be challenged with increased phenylalanine intake at 3–6 months of age to confirm their diagnosis by use of a milk diet for 2–3 days. (See appendix 2 on Confirmation of Diagnosis for directions for the challenge procedures.)

Premature Infants ***(<2,500 g birth weight)***

1. A blood specimen should be obtained for determination of phenylalanine at 2 weeks of age or at the time of discharge from the hospital, whichever is earlier. If the infant's weight is less than 2,500 g at 2 weeks of age, and the initial test is negative, the analysis should be repeated at the time of discharge.
2. Premature infants with blood phenylalanine levels ranging from 4–15 mg% should be retested repeatedly until their clinical status is certain. It has been shown that occurrence of false positives is more frequent in the premature infant due to its immature enzyme system. At the same time, blood tyrosine levels also should be measured to rule out the

possibility of transient tyrosinemia (see para. 4.) in which moderate elevations of phenylalanine levels also have been reported.

3. Prematures who show a rising level of blood phenylalanine with normal values for tyrosine on repeated determinations should be considered presumptive PKU. They should be referred and followed as noted above for full-term infants. Those with confirmed serum phenylalanine levels exceeding 20 mg% should be given a phenylalanine-restricted diet, but require frequent clinical and biochemical monitoring to avoid hypophenylalaninemia with its attendant hazards.
4. Prematures with elevated blood tyrosine levels (up to 50 mg%) should be evaluated nutritionally for protein intake because tyrosinemia may reflect an excessive protein intake, insufficient vitamin C, enzyme immaturity, or combinations of these factors. In these cases, use of breast milk or a similar standard commercial formula (Enfamil, Similac, Bremil, etc.) to control protein intake is recommended, together with an oral vitamin C supplement (100 mg/day). Infants with transient tyrosinemia usually revert to a normal metabolic pattern on this regimen within 1 week (rarely requiring 4–5 weeks).

Siblings of Known PKU Patients

1. Phenylalanine should be measured in cord blood in the case of newborn siblings of known PKU patients, and again in specimens collected at 3–5 days of age. If levels rise appreciably, proceed as described above. Cord blood levels often are slightly higher than maternal levels. Furthermore, the pregnant mother may show somewhat higher blood phenylalanine levels and other amino acids than during a nonpregnant state.
2. Blood should be obtained from older siblings and parents of newly recognized PKU patients for determination of phenylalanine in order to rule out possible PKU.

RELATION OF INFANT AGE AND TIME OF INITIAL BLOOD SCREENING

The minimum period following ingestion of milk after which serum phenylalanine levels are reliable has been stated variously as 24, 48, and 72 hours [*27, 30*]. The range reflects innumerable variables: the genotype of the newborn, the maturity of his phenylalanine hydroxylase enzyme system, and the amount of protein ingested (dilution of formula, amount of formula taken). The results of the Guthrie field trials [*13*] showed that three PKU infants had phenylalanine levels between 6 and 10 mg% one day after feeding. In seven infants, levels of 10–20 mg% were reported 2 days after their first milk feedings. One patient was tested 2 days after his first milk

feeding, but PKU was missed. Fortunately, he was diagnosed at 4 weeks of age on a repeat test. Three days after initial feedings, 11 infants with PKU had levels exceeding 20 mg%. Three other PKU infants, however, showed levels between 10 and 12 mg% at 3 days after their first milk feedings, but developed higher levels soon afterwards.

ROUTINE FOLLOWUP TEST FOR INITIAL DIAGNOSIS

False negative results may be encountered in the neonatal Guthrie screening test, with an incidence as high as 10% or higher [*30*]. In addition to possible technical factors, a variety of other reasons may be pertinent. Many newborn infants are not fed during the first 12 hours, and some not during the first 24 hours of life. Initial feedings are small, and starting formulas are dilute in most nurseries. A policy of early discharge is common to many maternity hospitals. These features may result in some newborn PKU infants being sent home before they have received sufficient dietary protein to provoke an appreciable increase in their serum phenylalanine level. A second screening test is strongly recommended by 4 weeks of age to ensure diagnosis of PKU patients who may have escaped detection in the neonatal test because of inadequate protein intake, or other causes.

A false negative result should also be suspected in any infant with a mousey urinary odor, eczema, mental retardation, cerebral palsy, convulsions, or secondary microcephaly. Since an increased occurrence of pyloric stenosis is associated with PKU, infants with this diagnosis should also be suspect.

Establishing the Diagnosis

The biochemical criteria for the diagnosis of PKU depend upon measurement of phenylalanine in blood and also its metabolites in urine, rather than upon assay of phenylalanine hydroxylase. Liver biopsy is required for measurement of the enzyme activity, and most clinicians are unwilling to subject infants to this procedure.

The criteria for admission to the Collaborative Study for Children Treated for Phenylketonuria are finding two serum phenylalanine levels greater than 20 mg% at least 24 hours apart while the infant is on a regular diet with adequate protein intake, normal tyrosine levels in the blood of less than 5 mg% and, in addition, the demonstration of certain metabolites of phenylalanine in the urine. (See appendix 2.) The last may not be helpful initially because in an occasional patient urinary metabolites of phenylalanine may not rise until 4–6 weeks of age.

Data from the Collaborative Study have revealed that these criteria will include a few subjects who are not classical PKU patients. To confirm the diagnosis, a challenge is recommended for all patients by increasing their dietary phenylalanine intake to 180 mg/kg of body weight/day. This is administered for 3 successive days along with monitoring of serum phenylalanine twice daily. (See appendix 2 for details.) The challenge should be carried out between the third and sixth month of life. In the classical PKU infant, serum phenylalanine levels rise progressively and typical metabolites of phenylalanine appear in the urine. In case of an equivocal result, the challenge is repeated at 1 year of age. These tests can be carried out on an ambulatory basis. Some clinical centers recommend hospitalization while the diagnosis is being established.

The following general principles are offered for a comprehensive followup of PKU patients:

1. Confirmed cases should be offered multidisciplinary services for comprehensive long-term clinical, laboratory, and allied health services.
2. Family financial needs also must be recognized. If necessary, the family should be referred to appropriate sources of financial help. In many States, the Crippled Children's Services may pay for professional care, laboratory services, and dietary supplies.
3. Genetic implications should be explained to the parents.

Initiation of Treatment

When a diagnosis of PKU is made, the phenylalanine-restricted diet should be instituted promptly. The decision regarding hospitalization should be individualized. When parents are capable and the family lives near the clinical treatment resource, the diet can be initiated on an outpatient basis. Some clinicians prefer to hospitalize all infants to monitor initiation of therapy. A medical history (including a genetic history) and physical examination, including height, weight, and head circumference, should be routinely obtained. Serum phenylalanine and tyrosine levels are monitored daily. An electroencephalogram is done as a baseline for possible future need. If the urine chromatography procedure for phenyalanine metabolites is available, this study also should be done.

The objective of dietary management in PKU is to control the serum phenylalanine at a level that will not interfere with development of intellectual potential. At the same time, the diet must supply adequate nutrients and energy for optimal growth. The purpose of this section is to outline effective methods for prescribing and calculating the phenylalanine-restricted diet.

If a child with PKU were to be given sufficient protein from natural foods to meet minimal daily needs, the amount of phenylalanine ingested would be excessive. Consequently, the intake of phenylalanine must be regulated by the choice of foods, including the use of a special protein source.

Phenylalanine occurs in all proteins of animal and plant origin, although in somewhat lesser proportion in vegetables and fruits than in seeds, meat, milk, and eggs. It is important to avoid foods which are rich in protein, and therefore in phenylalanine. It is for this reason that meat and meat products are not used. In the United States, a satisfactory phenylalanine-restricted diet can be based upon Lofenalac®, a casein hydrolysate specially processed by the Mead-Johnson Laboratories (Evansville, Indiana), to remove 95% of its phenylalanine content.

The serum phenylalanine level can be decreased most rapidly after diagnosis by feeding Lofenalac at normal dilution with no phenylalanine added. This initial approach should be used *only when the infant is hospitalized and daily blood specimens for determination of serum phenylalanine can be analyzed promptly by the laboratory.* Otherwise, a precipitous drop in the serum phenylalanine level below the normal range can escape observation, and the infant may develop phenylalanine deficiency [4]. It must be em-

phasized that once the level has dropped to satisfactory clinical levels, phenylalanine must be added to the diet to meet essential needs of body metabolism and growth.

The infant or child who cannot be hospitalized following diagnosis, or for whom only weekly serum phenylalanine levels can be obtained, should be placed on a maintenance Lofenalac formula supplemented by an adequate amount of phenylalanine from an appropriate source such as evaporated milk. (See table 1.)

Table 1

RECOMMENDED DAILY AMOUNTS OF PHENYLALANINE, PROTEIN, AND ENERGY FOR INFANTS WITH PKU

Age (mo)	Phenylalanine [a] (mg/kg/day)	Protein (g/kg/day)	Energy (kcal/kg/day)	Percent protein from Lofenalac	Amount of Lofenalac [b] (ms/kg)	Evaporated Milk [c] (oz)	(ml)
0-3	58 ± 18	4.4	120	85	2.5-3	1-3	30-90
4-6	40 ± 10	3.3	115	85	2-2.5	0.5-2	15-60
7-9	32 ± 9	2.5	110	90	1.5-2	0.5-1.5	15-45
10-12	30 ± 8	2.5	105	90	1.5-2	0.5-1	15-30

[a] R. Koch, et al. "Collaborative Study of Children Treated for Phenylketonuria." in *Treatment of Inborn Errors of Metabolism* ed. by J. W. T. Seakins, R. A. Saunders, and C. Toothill. (Churchill, Livingstone, Edinburgh and London, 1973) pp. 3-18.

[b] One measure of Lofenalac equals 10 g or 1 tablespoon.

[c] One ounce (approximately 30 ml) of evaporated milk contains 106 mg phenylalanine, 2.2 g protein, and 44 calories.

A satisfactory diet for the infant or child with PKU must include adequate amounts of phenylalanine, protein, and energy. The prescribed intake of phenylalanine is based on serum phenylalanine analysis which reflects the child's tolerance for this amino acid. Recommended amounts are given for infants in table 1; however, these are baselines for beginning treatment [5]. The prescription must be adjusted to fit the individual needs determined by blood phenylalanine measurements of each patient, and frequent changes in the diet prescription are necessary, particularly during the first 6 months of life, to meet the nutritional requirements of rapid growth.

The phenylalanine intake should be gradually modified according to the maximum commensurate with maintaining a serum phenylalanine level of 2–10 mg%. This approach is in sharp contrast to former diet management techniques under which a serum phenylalanine value of 2–4 mg% was considered optimal. When the serum phenylalanine level continues to rise following an increase in dietary intake, then phenylalanine intake should be decreased accordingly. Periodic increases in intake of phenylalanine, protein, and energy are usually necessary during early infancy to maintain desired serum phenylalanine levels, to prevent hunger, and to promote adequate growth.

In order to meet the phenylalanine needs for growth during the early months of life, the Lofenalac formula must be supplemented with evaporated milk or other selected foods containing phenylalanine. Evaporated milk is preferable to pasteurized whole milk because it has been heat-treated and is more digestible [*31*]. Other natural foods are introduced as additional sources of phenylalanine and energy when the child begins to take various solids.

The nutrient composition of Lofenalac is listed in detail in table 2. Lofenalac is well fortified with fats, carbohydrates, vitamins, and minerals. Some amino acids which were partially removed during commercial processing also have been restored. No supplemental vitamins or minerals, with the exception of fluoride, are necessary for the child receiving adequate amounts of Lofenalac because sufficient quantities have been added [*32*].

Table 2

COMPOSITION OF LOFENALAC

Nutrients	Content (100g powder)
Kcalories	450
Protein Equivalent (g)	15
Fat (g)	18
Carbohydrate (g)	57
Amino Acids (g)	
L-Arginine	0.34
L-Histidine	0.27
L-Isoleucine	0.78
L-Leucine	1.45
L-Lysine	1.58
L-Methionine	0.51
L-Phenylalanine	0.08
L-Threonine	0.81
L-Tryptophan	0.20
L-Tyrosine	0.82
L-Valine	1.19
Minerals (mg)	
Calcium	648
Chloride	561
Copper	0.4
Iodine	0.047
Iron	8.6
Magnesium	54
Manganese	1.4
Phosphorus	450
Potassium	719
Sodium	324
Zinc	3

Table 2

COMPOSITION OF LOFENALAC—Continued

Nutrients	Content (100g powder)
Vitamins	
A (IU)	1,439
D (IU)	288
E (IU)	7.2
Ascorbic Acid (mg)	37
Folic Acid (mcg)	36
Niacin (mg)	6
Riboflavin (mg)	0.72
Thiamine (mg)	0.4
B_6 (mg)	0.4
B_{12} (mcg)	1.8
Biotin (mg)	0.02
Pantothenic Acid (mg)	2
Inositol (mg)	72
Choline (mg)	61

Table 3

AVERAGE NUTRIENT CONTENT OF SERVING LISTS

List *	Phenylalanine (mg)	Protein (g)	Energy (kcal)
Vegetables [b]			
Strained and junior	15	0.5	20
Table	15	0.5	10
Fruits [b]			
Strained and junior	15	0.6	150
Table and juices	15	0.6	70
Bread and cereals [b]	30	0.6	30
Fats [b]	5	0.1	60

* Complete lists of foods with serving size providing specified amount of phenylalanine are presented in "Diet Management of PKU for Infants and Preschool Children" by Phyllis B. Acosta and Elizabeth Wenz.

[b] When analyses were not available, the phenylalanine content was calculated on the following basis:

Breads and cereals	Phenylalanine 5% of protein
Fat	Phenylalanine 5% of protein
Fruits	Phenylalanine 2.6% of protein
Vegetables	Phenylalanine 3.3% of protein

Serving lists have been prepared to simplify the planning of phenylalanine-restricted diets for families and for professional persons guiding them (table 3). The lists are comparable to the diabetic exchange lists in that foods of similar phenylalanine content are listed together and can be exchanged one for another within a list to provide variety in the diet.

A two-part serving list is included in "Diet Management of PKU for Infants and Preschool Children" by Phyllis B. Acosta and Elizabeth Wenz [*33*]. It shows food groups, type of food, amount in a serving, and phenylalanine, protein, and energy content for a specified serving size. (Guidelines for dietary implementation are in appendix 1.

DIETARY MANAGEMENT

The usual procedures for assessment of growth and development must be followed carefully. The first year of life is the period of most rapid growth and greatest vulnerability to nutritional deprivation.

Effective dietary management of the patient with PKU requires frequent monitoring of the serum phenylalanine level to ensure an adequate dietary intake of phenylalanine. Weekly analyses are suggested to determine the infant's tolerance for phenylalanine and to ensure serum phenylalanine levels within the acceptable range (2–10 mg%). The influence of diurnal fluctuation in the serum phenylalanine level can be minimized by collecting blood specimens at a standard time convenient for the patient's family. Laboratory analyses must be prompt and accurate to be useful in making the needed dietary adjustments.

Parents should be instructed to record carefully all foods ingested in the 3 days before blood is drawn for serum phenylalanine determination. The amount of phenylalanine, protein, and energy ingested, the infant's health status, and the serum phenylalanine level should be correlated before a dietary change is prescribed. Further detailed information on dietary management is available [*33, 34*].

Reconfirmation of Diagnosis

After initiation of the phenylalanine-restricted diet, it is recommended that the diagnosis of PKU be reconfirmed when the infant is 3–6 months of age. It is now known that approximately 15% of cases initially considered to be PKU are hyperphenylalaninemic variants (HPV) who will not need treatment. Discrimination is done by use of the challenge procedure, which deliberately increases the phenylalanine intake for 2–3 days. The procedure for the challenge is in appendix 2. If dietary treatment suggests that the infant can tolerate more phenylalanine than the average PKU patient, a variant of hyperphenylalaninemia should be suspected.

The response of a classical PKU infant to the challenge is dramatic. The serum phenylalanine level will rise rapidly to above 20 mg% and by the third day usually will be above 30 mg%. Simultaneously urinary metabolites of phenylalanine will increase. Irritability, nausea, and occasional vomiting may occur. In variant patients the serum phenylalanine will slowly increase but only to levels of 15–20 mg%.

Occasionally, an infant will exhibit intermediate values of 20–25 mg%. These infants should be considered as potential PKU patients and should remain on diet therapy.

Rechallenging all PKU infants is recommended again at 1 year of age to be certain of the diagnosis. Occasionally an infant will appear to have PKU on the 3–6 month challenge but not at 1 year.

Medical Services

Medical services for PKU patients should be directed by a physician experienced in management of this disorder and knowledgeable about the nutritional needs of infants and children. However, this does not preclude a private pediatrician assuming primary responsibility for the management of the patient's metabolic disorder with consultation by specialists in a clinical center. In a team approach to comprehensive care, the leadership role must be both directive and coordinative to insure that all facets of the problem of providing medical care for these patients are considered.

Frequent monitoring of blood levels and assessment of growth are essential to guard against too-restrictive or too liberal an allowance of phenylalanine. Phenylalanine is an essential amino acid required for building protein, and too little or too much in the diet can be harmful. Experience gained in the course of 10 years within the PKU Collaborative Study indicates that serum phenylalanine levels maintained between 2–10 mg% are consistent with normal development.

Poor weight gain and a consistently low serum phenylalanine (below 1–2 mg%) suggest excessive restriction of dietary phenylalanine. In severe cases, excessive catabolism of body protein results, and a transient, paradoxical rise in serum phenylalanine levels may occur. This is a serious state which cannot be corrected unless more dietary protein and phenylalanine are provided promptly.

Too much dietary phenylalanine mitigates against the therapeutic objectives of maintaining safe levels of phenylalanine during the crucial period of early development. Requirements for phenylalanine vary considerably from patient to patient as well as for the individual patient from time to time. In general, more phenylalanine is required during early infancy and during other periods of accelerated gowth.

DIET TERMINATION

Different clinical centers vary in their opinions regarding termination of the low phenylalanine diet. There is little agreement at present. Most clinicians discontinue diet around 6 years of age, but long-term studies validating this approach are lacking. The PKU Collaborative Study has begun to evaluate this question. Many clinicians continue to give girls with PKU

a small amount of Lofenalac to maintain a taste for the product in case future dietary-resumption may be necessary due to pregnancy. Pregnancy in the untreated PKU woman usually results in birth of a baby with microcephaly and other associated anomalies. While authorities agree that a diet restricted in phenylalanine is probably beneficial, long-term results validating this recommendation are as yet minimal.

COUNSELING

Confidentiality of diagnosis may be important in some families who prefer that their relatives, neighbors, and school authorities are not informed. This is their right, but in practice it is better that the parents understand the help that relatives and friends can provide in dietary surveillance.

Since PKU is an autosomal recessive disorder, the usual counseling regarding the recurrent risk of 1 in 4 for each pregnancy applies and needs reinforcement. The most difficult problems are those faced by PKU patients as they grow to adulthood. PKU females have the risk of bearing mentally retarded babies. A low phenylalanine-restricted diet during pregnancy may be helpful, but this approach is not only uncertain, it is difficult to carry out. Many clinicians are advising these individuals not to have their own children, but rather to adopt. Birth control measures are recommended and some may choose sterilization. If pregnancy occurs, and the woman is unable to resume the phenylalanine-restricted diet, abortion may be considered. The mental retardation seen in offspring of PKU women probably is related to the altered biochemical environment in utero to which the fetus was exposed.

Cost Benefits to Society

The usual course of an untreated PKU child is to develop moderate to severe mental retardation. Before the advent of newborn screening programs, about 1% of individuals admitted to institutions for the mentally retarded were PKU children.

Institutional care is expensive. In California, for example, the annual cost of the care of a single patient in an institution averages $16,000 today. Statistics indicate that 55% of the known PKU population in California was institutionalized for an average period of 13 years. Thus, each of the 168 PKU persons in the various institutions in California cost the State $34,-944,000 for the group for that period of time. Newborn screening costs are minimal in comparison to these expenditures. The costs of screening are on the average of $1–$2 per test. These cost figures do not take into account the productivity lost when a person is mentally retarded, or the family heartache and anguish caused by the presence of a mentally retarded person in the home.

Appendix 1
Guidelines for Dietary Implementation

The following guidelines are offered to facilitate prescription and calculation of the phenylalanine-restricted diet.

1. *Establish the infant's phenylalanine, protein, and energy needs according to his age.* Requirements vary from infant to infant. Write the diet prescription to include the amounts of all three. Table 1 gives average recommendations by age. The phenylalanine intake suggested is that which maintained serum phenylalanine levels between 2 and 10 mg% in infants participating in the Collaborative Study [*3*]. The energy intake is that recommended for normal infants by the Food and Agriculture Organization [*35*].
2. *Establish the amount of Lofenalac to be given daily.* The Lofenalac prescription is based upon the total protein intake recommended for the infant. Protein recommended is greater than that suggested by National Academy of Sciences Recommended Dietary Allowances [*36*] to insure adequate utilization of a casein hydrolysate. Of the total protein ingested, 85–90% *must* be provided via Lofenalac because natural foods contain too much phenylalanine (Table 1).
3. *Indicate to the parents the amount of Lofenalac to be given in both metric and household measuring units.*
4. *State the volume of milk (if any) to be added in both ml and oz (Table 1).*
5. *State the volume of water to be used in mixing the Lofenalac powder in both ml and oz: e.g., "Water to make —ml (—oz)."* The volume specified is determined by the fluid requirement for the infant's age, his preference for fluids, and his taste for Lofenalac, but usually should not exceed 1 liter or 32 fluid oz daily. Fluids must be provided in sufficient volume to prevent dehydration (130–200 ml/kg). Lofenalac is used in a concentration of 1 measure to 1 oz of water or 33 g/100 ml as a concentrated source of protein and carbohydrate, and children using this formula tend to have greater thirst than others taking regular formulas or milk.
6. Subtract the phenylalanine, protein, and energy supplied in the Lofenalac-milk mixture from the total prescription. The remaining allow-

ance of phenylalanine, protein, and energy is to be given in solid foods (Table 3).

Providing an adequate energy intake is essential. Nonprotein sources of energy, such as Dextri-maltose®, corn syrup, or sugar, and other suitable nonprotein foods can be added to maintain energy intake, and to satisfy the infant's hunger without affecting serum phenylalanine levels. Solid foods should be prescribed in numbers of servings and introduced at appropriate ages in the forms usually provided for normal infants. It is important to offer a variety of foods to establish taste patterns for later life, to meet increasing phenylalanine requirements, to develop jaw muscles needed for speech, and to provide exercise for the teeth and gums [*37–38*].

7. *Calculate the phenylalanine, protein, and energy in the serving of solid foods.* (*Table 3*). These figures added to the values derived from the Lofenalac-milk mixture should equal the total amounts prescribed.
8. Monitor each infant regularly to determine blood phenylalanine level and change prescription when necessary to maintain level between 2 and 10 mg%.

Appendix 2
Confirmation of Diagnosis

DIRECTIONS FOR THE 3- TO 6-MONTH CHALLENGE

Nutritional and Clinical Background of the Patient

1. Dietary intake should be evaluated at least 4 weeks prior to the challenge. Any necessary adjustments of the dietary prescription should be made at that time. The diet should *not* be altered just prior to the challenge. Occasionally a child may have particular nutritional problems not amendable to easy modification; in this instance, no major adjustment in diet should be attempted.
2. The patient must be in good health to ensure an effective biochemical challenge. The challenge should not be started if the patient has been ill or subjected to a medical procedure provoking metabolic disturbance (e.g., vaccination, surgery, IVP) within the preceding 3 days. Likewise, the procedure should be discontinued and specimens discarded if illness develops during the course of the challenge. A new challenge should be carried out when the patient is known to be in good health again.
3. The following items should be excluded from the diet for 2 days before and during the 3 days of the challenge: chocolate, ice cream, other vanilla-flavored foods, and bananas. Likewise, the patient should not receive salicylates (aspirin, Liquiprin, etc.), or rubbing liniments containing oil of wintergreen (methyl salicylate) for 2 days before or during the 3 days of the challenge. These materials provoke excretion of exogenous urinary metabolites which interfere seriously with quantitative analysis for *o*-hydroxyphenylacetic and phenylpyruvic acids.

Administration of the Challenge

1. It is recommended that the challenge procedure be started at 0800 hours on day 1 and continued for 48–72 hours. The challenge may be discontinued prior to 72 hours *if the child's serum phenylalanine level rises rapidly above 25 mg%.*

2. The basal Lofenalac diet is discontinued completely during the period of the challenge. However, a small amount of Lofenalac powder (approximately 10 gm/4 oz bottle) may be added to the whole or evaporated milk formula to facilitate easy acceptance of the challenge formula (3 or 4 bottles thus modified usually suffice).
3. In place of Lofenalac, 24 oz of whole milk/24 hours is given, *or* 12 oz of evaporated milk diluted with 12 oz of water/24 hours. If the infant was premature or has growth measurements less than the 97th percentile, the actual intake of L-phenylalanine (mg/kg/day) should be calculated and adjusted to attain but not exceed 180 mg/kg/day.
4. The milk is provided in 4 feedings spaced throughout the day to maintain any loss by emesis and to distribute the biochemical stress uniformly.
5. Water is encouraged ad lib during the challenge.
6. Dextri-maltose or any equivalent nonprotein source of carbohydrates may be used ad lib, as indicated clinically, to ensure adequate caloric intake during the challenge.
7. The original basal diet is resumed at the end of the challenge period.
8. The time of feeding, the volume of milk *actually* consumed, and emesis should be recorded accurately on appropriate diet and challenge records. The exact time of collection of blood and urine specimens also should be recorded. If the patient is challenged at home, the parents should be instructed specifically what to record.
9. For best results, hospitalization is recommended for this procedure.

Collection of Blood Specimens

Blood specimens should be collected on the day before starting the milk challenge. A suggested schedule for specimen collection follows this section of the appendix. (Table 4.)

Collection of Urine Specimens

1. The first specimen (volume at least 15 ml) is collected on the day before the milk diet is started.
2. The second specimen is collected at some time during the period 60–72 hours after the start of the milk diet (earlier, if two blood phenylalanine levels 24 hours apart have exceeded 20 mg%).
3. Urine specimens should be frozen immediately after collection and kept frozen (preferably in a deep freeze) until analyzed by the local laboratory. If analyzed elsewhere, urine specimens should be adjusted to pH 2.0 and mailed to the urine reference laboratory as soon as possible.

Types of Responses

1. A patient with classical phenylketonuria *under effective clinical management* may be expected to show the following changes:
   a. Serum phenylalanine rises from a basal level of 2–12 mg% to well above 20 mg%, even as high as 40–45 mg% at 72 hours in some patients.
   b. Urinary phenylalanine excretion increases from 2–5 times normal up to 8–20 times normal.
   c. Urinary *o*-hydroxyphenylacetic acid levels often rise from 1–10 times normal up to 100–300 times normal.
   d. Urinary phenylpyruvic acid goes from zero (normal) to as high as 3000 mg/g of creatinine.
   e. Urinary phenylacetylglutamine increases from normal up to as high as 12 times normal.

   Absolute normal values are not listed because they vary to different degrees with age. Individual variation is to be expected because four parallel metabolic paths are involved, and only one of them is defective in classical PKU.
2. In the event of obvious discrepancies in values for serum phenylalanine or urinary metabolites, or poor correlation between the serum and urine patterns, the following possibilities should be examined carefully:
   a. Actual phenylalanine intake may be substandard or erratic due to loss by vomiting, variable appetite, or otherwise may not conform to prescribed intake.
   b. Patient may have been ill before or during challenge. Even minor illness (or vaccination) can provoke an atypical metabolic response.
   c. Drugs (e.g., aspirin) or other medications or other medical procedures (e.g., IVP) immediately before or during the challenge may have an adverse effect upon patient metabolism, or may interfere seriously with analytical procedures.
   d. Technical error may occur between ingestion of diet and collection, storage, or shipment of specimens. Technical error when suspected in the laboratory is tested promptly by repeat analysis on a new specimen aliquot. When one of these factors is evident, the biochemical challenge should be repeated as soon as feasible.
3. The hyperphenylalaninemic variant usually will show a distinctly lesser biochemical response than the classical PKU patient, but some variation may be anticipated in accord with the degree of liver phenylalanine hydroxylase activity:
   a. Serum phenylalanine levels usually will rise to less than 20 mg%. A downturn beyond the peak value may be noted in some variant patients, possibly because of enzyme induction and metabolic re-equilibration.
   b. The rise in urinary phenylalanine excretion may be quite similar to or even exceed that seen in the classical PKU patient.

c. *o*-Hydroxyphenylacetic acid excretion usually rises to a distinctly lesser extent (15–70 times normal) than in the classical PKU patient.

d. Phenylpyruvic acid often is not present in the urine of some variants before the challenge and its excretion generally rises no higher than 500 mg/g of creatinine in the course of the challenge.

e. Phenylacetylglutamine excretion may remain in the normal range, or increase to no more than 3 or 4 times normal.

The greater phenylalanine tolerance in variants can be expected to result in a fairly wide span in levels of urinary phenylalanine metabolites. A small proportion of variant patients may be expected to show "borderline" challenge results. Their response may change with increasing age, and thus a later challenge may provide more distinctive results.

Table 4

SUGGESTED SCHEDULE FOR SPECIMEN COLLECTION
3–6-MONTH CHALLENGE

Day	Time,[a] hours	Challenge, hours	Urine number	Blood number	Remarks
0	2000	pre	1	1	Baseline specimens on day before challenge
1	0800	0	—	([b])	Start challenge diet[c]
1	2000	12	—	([b])	
2	0800	24	—	2	
2	2000	36	—	([b])	
3	0800	48	—	3	
3	2000	60	—	([b])	
			2[d]		
4	0800	72	—	4	End challenge. Resume original diet.

NOTES:

[a] Specimen collection time is indicative, not absolute, but *actual time* should be recorded accurately.

[b] Extra blood specimens are optional at the discretion of the clinic director.

[c] The challenge diet should consist of 24 oz of whole or 12 oz of evaporated milk (diluted 1 to 1 with water) per day, distributed in small portions throughout the day and supplemented with water ad lib, and Dextri-maltose or other non protein sources of carbohydrate ad lib. Patient is essentially in a continuous postprandial state.

[d] Urine number 2 may be collected at any convenient time between 60 and 72 hours.

DIRECTIONS FOR THE 1-YEAR CHALLENGE

Patients may be challenged at 12–15 months of age by measuring the biochemical responses to a total L-phenylalanine intake of 180 mg/kg body weight/day.

Nutritional and Clinical Background of the Patient

1. Dietary intake should be evaluated at least 4 weeks prior to the challenge. Any necessary adjustments of the dietary prescription should be made at that time. The diet should *not* be altered just prior to the challenge. If a child has particular nutritional problems not amenable to easy modification, no major adjustment in diet should be attempted.
2. The patient must be in good health to ensure an effective biochemical challenge. The challenge should *not* be started if the patient has been ill or subjected to a medical procedure provoking metabolic disturbance (vaccinations, surgery, IVP) within the preceding 3 days. Likewise, the procedure should be discontinued and specimens discarded if illness develops during the course of the challenge. A new challenge should be carried out when the patient is known to be in good health again.
3. The following items should be excluded from the diet for 2 days before and during the 3 days of the challenge: chocolate, ice cream, any other vanilla-flavored foods, and bananas. Likewise, the patient should not receive salicylates (aspirin, Liquiprin, etc.), rubbing liniments containing oil of wintergreen (methyl salicylate) for 2 days before or during the 3 days of the challenge. These materials provoke excretion of exogenous urinary metabolites which interfere seriously with quantitative analysis for *o*-hydroxyphenylacetic and phenylpyruvic acids.

Calculation of the Daily Challenge Diet and Compilation of Diet Records

1. Multiply the weight of the child expressed in kg by 180. This gives the total daily intake of L-phenylalanine in mg to be prescribed for the challenge (call this quantity X).
2. Calculate as accurately as possible the daily intake in mg of phenylalanine provided by both Lofenalac *plus* the natural foods in the basal diet which is being given to the child at the time of the challenge (call this quantity Y).
3. Subtract Y from X, then $X-Y=Z$. Z is the amount of additional L-phenylalanine in mg, which is to be added *each day* in the form of pure L-phenylalanine or natural protein foods to the current basal diet.
4. The parents should be instructed to record dietary intake in detail for each of the 3 days immediately preceding the challenge.
5. The parents or nutritionist should record the intake of *each* challenge day on separate diet records. A 3-day prechallenge and a 3-day challenge diet record should be obtained.

6. *Actual* intakes of phenylalanine, protein, and kcalories should be calculated and recorded by the nutritionist.

Preparation and Administration of the Challenge

1. For patients challenged on an outpatient basis, the L-phenylalanine needed each day should be calculated and the appropriate quantity and type of phenylalanine-containing food added to the basal diet. These foods should be spaced out throughout the day.
2. The total amount of formula needed for a 24-hour period should be prepared at one time. The total volume of formula utilized each day should be accurately recorded. The formula should be stored in the refrigerator and stirred or shaken well before each feeding.
3. The formula should be offered to the patient per his usual feeding schedule. It is recommended that the challenge procedure be started at 0800 hours on day 1 and continued for 48–72 hours.
4. The time of feeding, the volume of formula and quantities of solid foods offered, *actual* intake, and emesis should be recorded accurately on the diet records and challenge forms. The exact time of collection of blood and urine specimens should also be recorded. If the patient is challenged at home, the parents should be instructed specifically concerning what to record.

Collection of Blood Specimens

Blood specimens should be collected in the evening, 12 hours before starting the challenge, and at 12, 36, and 60 hours from the start of the challenge. Specimens may be collected more frequently if the clinician desires.

Collection of Urine Specimens

1. A random postprandial specimen (volume at least 15 ml) should be collected approximately 2 hours after dinner on the day before starting the challenge.
2. A second random postprandial specimen should be collected 2 hours after dinner on the third day (60 hours after starting the challenge). See suggested Schedule for Specimen Collection. (Table 5.)
3. Urine specimens should be frozen immediately after collection and kept frozen (preferably in a deep freezer) until analyzed by the local laboratory. If analyzed elsewhere, urine specimens should be adjusted to pH 2.0 and mailed to the urine reference laboratory as soon as possible.

Types of Responses

1. A patient with classical phenylketonuria *under effective clinical management* may be expected to show the following changes:
   a. Serum phenylalanine rises from a basal level of 2–12 mg% to well above 20 mg%, even as high as 40–45 mg% at 72 hours in some patients.
   b. Urinary phenylalanine excretion increases from 2–5 times normal up to 8–20 times normal.
   c. Urinary *o*-hydroxyphenylacetic acid levels often rise from 1–10 times normal up to 100–300 times normal.
   d. Urinary phenylpyruvic acid goes from zero (normal) to as high as 3000 mg/g of creatinine.
   e. Urinary phenylacetylglutamine increases from normal up to as high as 12 times normal.

   Absolute normal values are not listed because they vary to different degrees with age. Individual variation is to be expected because four parallel metabolic paths are involved, and only one of them is defective in classical PKU.
2. In the event of obvious discrepancies in values for serum phenylalanine or urinary metabolites, or poor correlation between serum and urine patterns, the following possibilities should be examined carefully:
   a. Actual phenylalanine intake may be substandard or erratic due to loss by vomiting or variable appetite, or otherwise may not conform to prescribed intake.
   b. Patient may have been ill before or during challenge. Even minor illness (or vaccination) can provoke an atypical metabolic response.
   c. Drugs (e.g., aspirin) or other medications or medical procedures (e.g., IVP) immediately before or during the challenge may have an adverse effect upon patient metabolism, or may interfere seriously with analytical procedures.
   d. Technical error may occur between ingestion of diet and collection, storage, or shipment of specimens. Technical error when suspected in the laboratory is tested promptly by repeat analysis on a new specimen aliquot. When one of these factors is evident, the biochemical challenge should be repeated as soon as feasible.
3. The hyperphenylalaninemic variant will usually show a distinctly lesser biochemical response than the classical PKU patient, but some variation may be anticipated in accord with the degree of liver phenylalanine hydroxylase activity:
   a. Serum phenylalanine usually will rise to less than 20 mg%. A downturn beyond the peak value may be noted in some variant patients, possibly because of enzyme induction and metabolic re-equilibration.

b. The rise in urinary phenylalanine excretion may be quite similar to or even exceed that seen in the classical PKU patient.
c. *o*-Hydroxyphenylacetic acid excretion usually rises to a distinctly lesser extent (15–70 times normal) than in the classical PKU patient.
d. Phenylpyruvic acid often is not present in the urine of variants before the challenge and its excretion generally rises to no higher than 500 mg/g of creatinine in the course of the challenge.
e. Phenylacetylglutamine excretion may remain in the normal range, or increase to no more than 3 or 4 times normal.

The greater phenylalanine tolerance and consequently lesser degree of dietary control in variants can be expected to result in a fairly wide span in levels of urinary phenylalanine metabolites. A small proportion of variant patients may be expected to show "borderline" challenge results. Their response may change with increasing age, and thus a later challenge may provide more distinctive results.

Table 5

SUGGESTED SCHEDULE FOR SPECIMEN COLLECTION ONE YEAR CHALLENGE

Day	Time,[a] hours	Challenge hours	Urine number	Blood number	Remarks
0	2000	pre	1	1	Baseline specimens on day before challenge. Collect specimens approximately 2 hours after dinner.
1	0800	0	—	([b])	Start challenge diet [c]
1	2000	12	—	2	Collect specimen approximately 2 hours after dinner.
2	0800	24	—	([b])	
2	2000	36	—	3	Collect specimen approximately 2 hours after dinner.
3	0800	48	—	([b])	
3	2000	60	2	4	Collect specimen approximately 2 hours after dinner.
4	0800	72	—	([b])	End challenge. Resume original diet.

NOTES:

[a] Postprandial specimens are preferable from both the biochemical and nutritional standpoints, with collection time approximately 2 hours after dinner. Thus, 2000 hours is indicative, not absolute, but *actual time* should be recorded accurately

[b] Extra blood specimens are optional at the discretion of the clinic director, or may be substituted *only if* the times indicated for blood specimens 1, 2, 3 and 4 are completely incompatible with local clinical routine.

[c] The challenge diet consists of the patient's usual regimen of Lofenalac and natural foods, supplemented with sufficient pure *L*-phenylalanine to provide a total of 180 mg/kg/day in breakfast, lunch, and dinner (including any midmorning and midafternoon snacks). Thus intake for a given day should be completed by dinner.

CHALLENGE FOR CHILDREN OVER 2 YEARS OF AGE

Some older (>2 years), treated PKU patients who have a low phenylalanine tolerance may show an unduly rapid rise in levels of phenylalanine and its metabolites in urine and serum when given 180 mg L-phenylalanine/kg/day. They may also show some toxic manifestations such as vomiting, irritability, and ataxia. For these reasons, use of a total intake of 120 mg of phenylalanine/kg/day is recommended for the biochemical challenge of the older PKU child.

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