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PHENYLKETONURIA
AN INBORN ERROR OF METABOLISM

by

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INTRODUCTION

Phenylketonuria (PKU) is an inborn error of metabolism that results in mental retardation. PKU was first described by the Norwegian physician and biochemist Asbjorn Fölling in 1934 when he discovered that the urine of two mentally retarded siblings turned green when treated with ferric chloride (1). Phenylketonuria was earlier called oligophrenia phenylpyruvica or phenylpyruvic amentia (2). This disease is inherited as a autosomal recessive character, achieving expression only in the homozygous form and occurring in the United States in approximately 1:11,000 live births (3,4). The classical clinical picture is of a fair-haired, fair-skinned child with severe mental and physical retardation (4-6). Patients with PKU lack the enzyme, phenylalanine hydroxylase, in the liver and therefore are unable to convert the essential amino acid, phenylalanine, to tyrosine (2,6). As a result, phenylalanine accumulates in the plasma and is thought to have a permanent damaging effect upon the developing brain (5-7). The only known effective method of treatment of phenylketonuria is dietary management (8). A patient with PKU is placed on a diet which restricts dietary intake of phenylalanine. The amount of phenylalanine provided by the diet must be sufficient to meet the metabolic requirements for growth; yet its intake must not permit an excess accumulation in body fluids of phenylalanine and its derivatives (9). The purpose of this paper is to review and discuss the causes, effects, and treatment of phenylketonuria.

METABOLISM OF PHENYLALANINE

Hydroxylation of phenylalanine to tyrosine. The natural form of phenylalanine, L-phenylalanine, is an essential amino acid for growth in the human being. In the growing child, 50% of the normal dietary intake of L-phenylalanine can be used for protein synthesis while the remainder must be oxidized primarily to tyrosine and to a much lesser extent to other metabolites. The major chemical conversion of L-phenylalanine in human tissue involves para-hydroxylation to form L-tyrosine (10-13). This hydroxylation of L-phenylalanine to L-tyrosine in the human being occurs in the liver and involves a complex enzyme system consisting of at least two essential enzymes, phenylalanine hydroxylase and dihydropteridine reductase, as well as an essential nonprotein coenzyme, tetrahydrobiopterin (5,12,14). Phenylalanine hydroxylase catalyzes the reaction:

$$\text{L-phenylalanine} + O_2 + \text{tetrahydrobiopterin} \longrightarrow$$
$$\text{L-tyrosine} + H_2O + \text{quinonoid dihydrobiopterin} \quad (15)$$

The enzyme, dihydropteridine reductase, catalyzes the reduction of quinonoid dihydrobiopterin back to tetrahydrobiopterin, allowing the cofactor to function catalytically (15). In the phenylalanine hydroxylase system where phenylalanine is converted to tyrosine, the first enzyme is a labile enzyme which requires NADH and the second enzyme is a stable enzyme which requires oxygen, NADPH and tetrahydropteridines

(5) (Fig. 1). The second enzyme, dihydropteridine reductase, is not involved in the hydroxylation reaction, but merely catalyzes a reaction which keeps the coenzyme in an active form. When a deficiency in the phenylalanine hydroxylase system occurs at the site of enzyme 1 (phenylalanine hydroxylase), the classical form of phenylketonuria occurs.

Minor metabolites of phenylalanine metabolism. Phenylalanine can be metabolized by minor pathways which form products other than tyrosine (10,11) (Fig. 2). Transamination to form phenylpyruvic acid is the best known initial step; the cosubstrate for the specific amino transferase is pyruvate. Phenylalanine concentrations in the plasma must be above 10 to 15 mg/100 ml (13) before phenylpyruvic acid is formed and excreted in the urine.

Phenylpyruvic acid can be converted to phenylacetic and phenyllactic acids (10,11). In man most of the phenylacetic acid is conjugated with glutamine before it is excreted into the urine. Phenylacetylglutamine is then excreted by renal tubular secretion. Phenylpyruvic acid is converted to its lactate derivative primarily by an aromatic alpha-keto acid reductase and not by lactate dehydrogenase. The keto acid can also be ortho-hydroxylated by p-hydroxyphenylpyruvic acid oxidase to produce o-hydroxyphenylacetic acid.

Another normal pathway for phenylalanine degradation is initiated by decarboxylation of the amino acid. The product of the reaction is phenylethylamine, a pharmacologically potent amine. An amine oxidase converts phenylethylamine

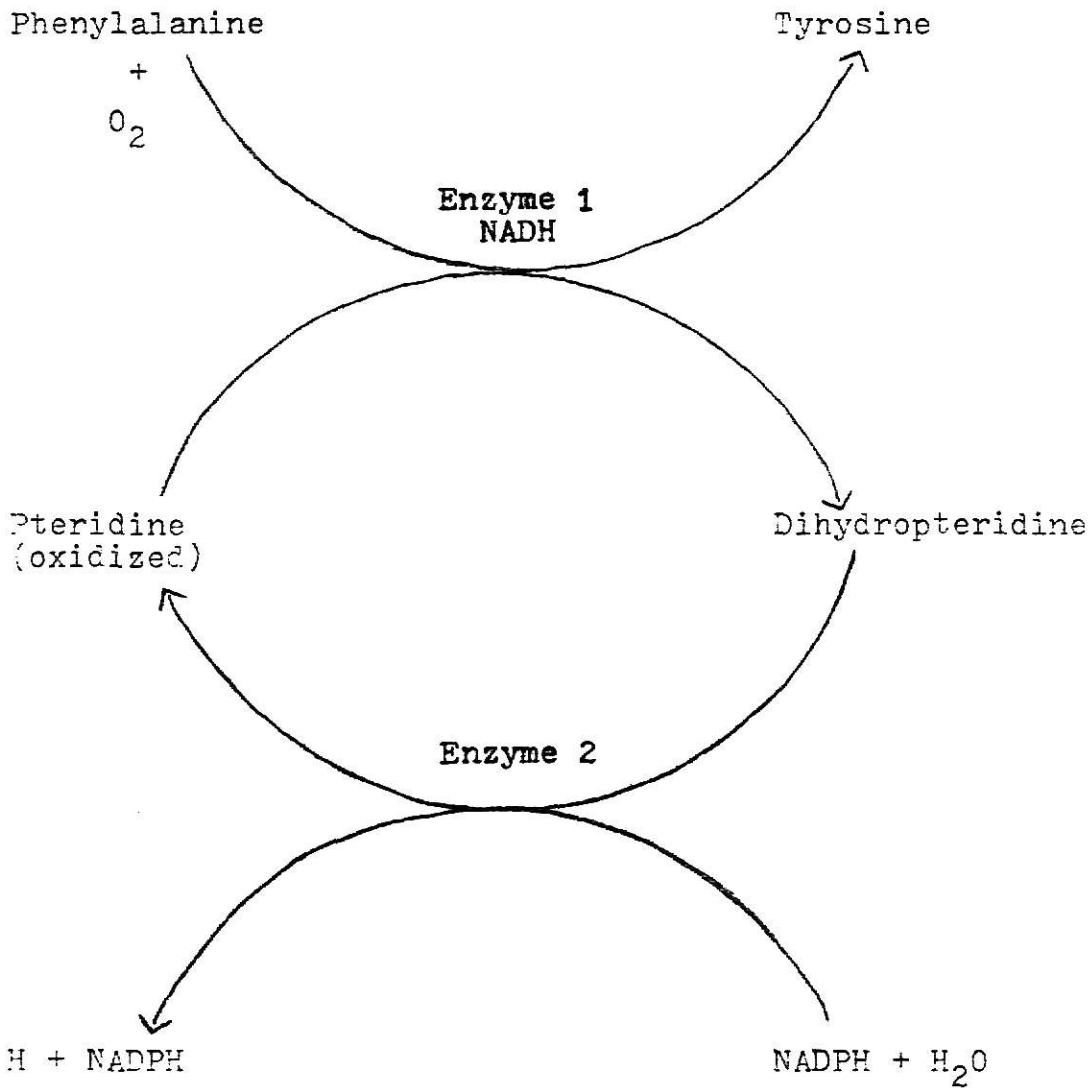

Fig. 1 Schematic representation of the metabolism of phenylalanine to tyrosine showing the relationship of important enzymes and cofactors to the hydroxylase system (5).

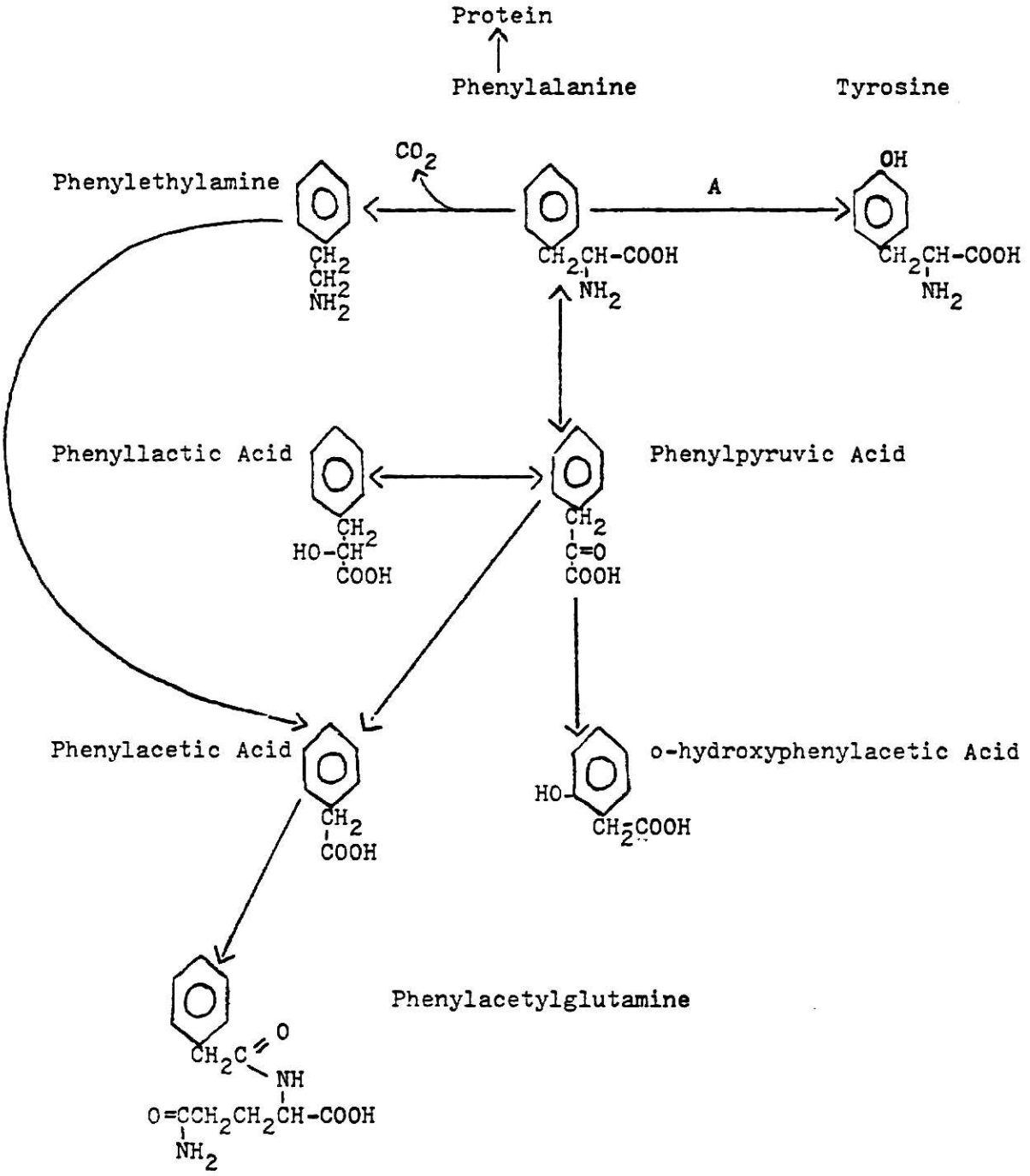

Fig. 2 Metabolism of phenylalanine by normal minor pathways. Incorporation of phenylalanine accounts for less than 50% of the disposal of phenylalanine entering the free pool, even at times of maximum growth. Site of block in phenylketonuria is shown at A (10,11).

to phenylacetic acid (10,11). These normal metabolites are often found in abnormal amounts in the urine of phenylketonuric patients (13).

BIOCHEMICAL ABNORMALITIES IN THE PHENYLKETONURIC PATIENT

Altered pattern and transport of amino acids. Excess accumulation of phenylalanine in the plasma may cause other biochemical abnormalities to occur in patients with PKU. It has been suggested that increased levels of phenylalanine in patients with PKU can competitively inhibit cells from capturing and utilizing other amino acids (13). The nutritional problem of cells in such a deficient medium is intensified by the high phenylalanine present. Because of the increased amounts of phenylalanine, transport of tyrosine and other amino acids into the brain may be inhibited. One such amino acid, 5-hydroxytryptophan, the precursor of serotonin, is an important substance for normal brain development. Not only can increased amounts of phenylalanine distort intracellular amino acid patterns in some tissues, it also has an inhibitory effect on amino acid absorption from the intestine (13). The absorption of the amino acid tryptophan is inhibited in PKU because accumulation of phenylalanine in the plasma will equilibrate with the intestinal lumen so that the concentration of inhibitor (phenylalanine) will be raised in the lumen and will impair substrate (tryptophan) absorption (10,11). For reasons not yet fully explained, indole products derived from tryptophan and tyrosine are also excreted in the urine of patients with PKU (5,10,11). It has been hypothesized that damage to the brain occurs in PKU when the brain is presented with an abnormal and deficient pattern of amino

acids from which it cannot correctly construct its essential and permanent components (13).

Tyrosine metabolism. Phenylketonuria also alters tyrosine metabolism (5,10,11,13). Tyrosine normally is metabolized to the pigment melanin. A decreased pigmentation in PKU is accounted for by the competitive inhibition of phenylalanine on the tyrosinase system (13). The impairment of melanin synthesis is reversible when phenylalanine concentration of plasma is lowered to the normal range (10,11). Due to the inhibition of tyrosine metabolism, there is also a decreased conversion of tyrosine to adrenalin. This disturbance in adrenalin production results in an unusually low blood adrenalin level in the PKU patient (5).

CLINICAL FEATURES OF PHENYLKETONURIA

It has been determined that the infant with PKU is not clinically abnormal at birth (13,16). A study comparing the birthweights of affected PKU babies with their unaffected siblings was performed by Rothman and Pueschel in 1976 (16). They found the mean birthweight for 52 affected children to be 3,300 gm compared to 3,390 gm for their unaffected siblings. This small difference in birthweight suggested that intrauterine physical growth of the PKU fetus proceeds normally. In the untreated PKU child a mental defect may not be detected until 4 to 12 months after birth (17). The earlier symptoms or signs of PKU are observed usually in over one-half of PKU infants prior to the appearance of a mental defect. During the first few weeks of life, the patient may show unusual irritability, have epileptic seizures, and exhibit vomiting which is severe enough to warrant medical attention (5,17). Detection of a musty or mousy odor is attributed to phenylacetic acid in urine and sweat, but, it may be absent if the majority of the acid is excreted as the glutamine conjugate (10,11). The transformation of an apparently normal baby into a severely defective one during the first year or so of life is the most striking clinical characteristic of the disease (13). Manifestations of these clinical and biochemical changes are observed in Fig. 3.

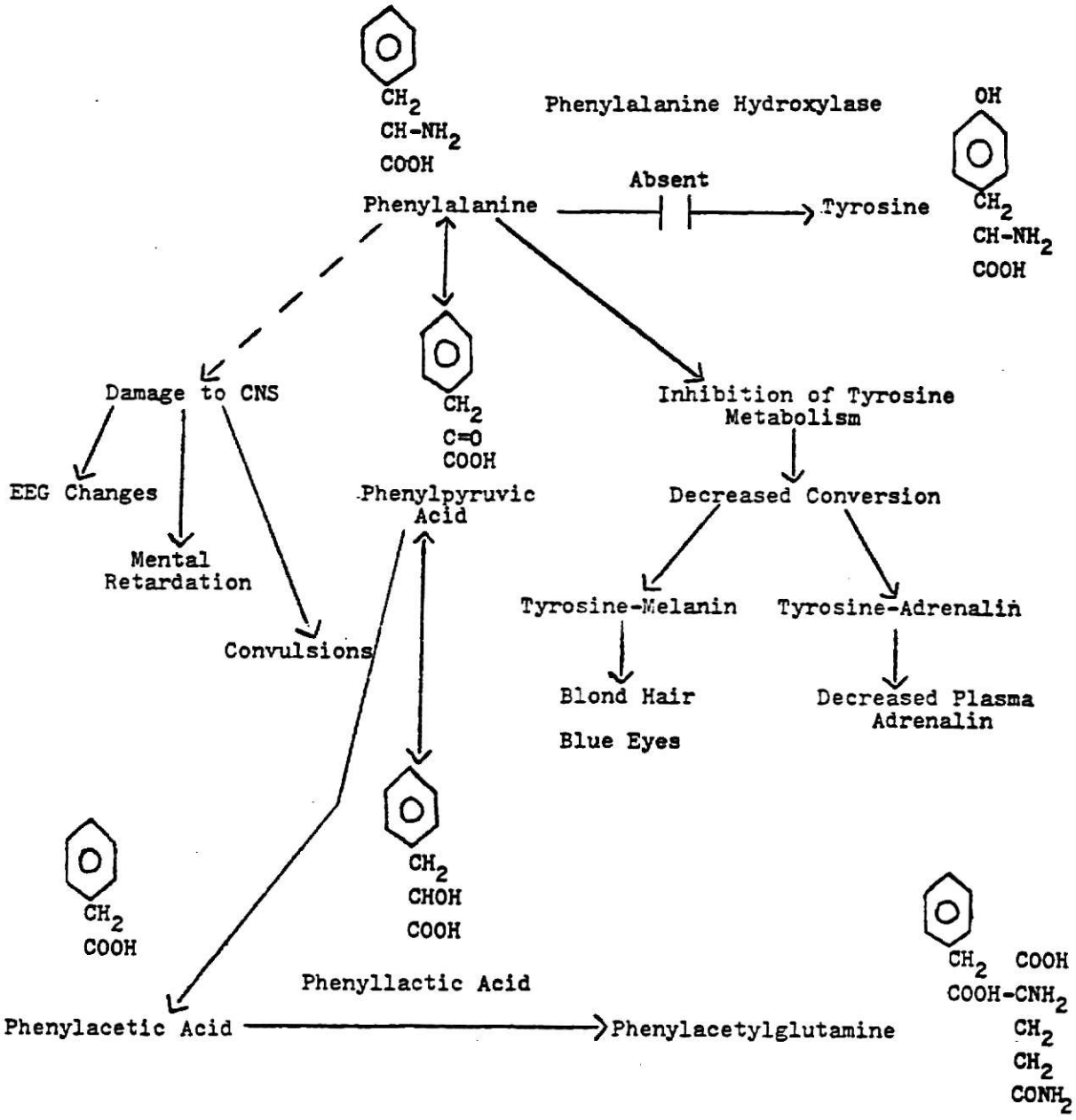

Fig. 3 Clinical and biochemical manifestations which may occur due to altered phenylalanine metabolism in the untreated child with PKU (5).

Skin lesions. Dermatitis, such as atopic eczema, seborrheic dermatitis or autosensitization eruptions may occur in over one-half of the patients with PKU (18). Eczema usually begins in infancy and may persist into adolescence or adulthood (13). About a quarter of PKU children show generalized infantile eczema and another quarter have dry skin with repeated minor inflammatory lesions or nonspecific rashes (5). In 1961, Partington (17) observed that 6 out of 36 PKU children experienced infantile eczema after 1 to 4 months of age. The skin changes periodically observed in PKU are due to toxic effects of phenylalanine and its decomposition products in the skin and are responsive to dietary restriction of phenylalanine.

Pigmentation. Although there are many exceptions, phenylketonurics tend to have blue eyes, blond hair, and fair skin (5,18,19). This decreased pigmentation is due to the inhibitory effect of the increased phenylalanine on the tyrosinase system. A decrease in tyrosine results in a decrease in melanin which is responsible for the pigmentation characteristics. Striking instances of blond PKU patients are observed in normally pigmented families of Sicilian and Spanish origin. Other striking cases are mulattos who are less pigmented than their parents and have sandy-blond hair and blue eyes. Phenylketonuric Japanese patients tend to have brown hair (13). The correct generalization is that each patient with PKU has a relatively lighter complexion

or pigmentation than expected for members of their family, ethnic or racial norm. Due to the decreased pigmentation of PKU patients, there is also a greater risk of sunburn because of the abnormal sensitivity to sunlight (10-12).

Electroencephalogram. About three quarters of patients with phenylketonuria show abnormal electroencephalogram (EEG) patterns (15). The most frequently seen abnormalities are spike and wave complexes, dysarrhymia, and petit mal variant type, which are found even in the absence of convulsive seizures. The most common finding is a mixture of high voltage fast and slow waves occurring more irregularly than in petit mal (5,13).

Behavior. The untreated phenylketonuric child rarely is described as friendly, placid or happy (13). Adjectives such as miserable, fretful, unhappy, irritable, disturbed, fearful and restless have been used to describe the PKU child. Parents of PKU children also may report that their children are always crying, subject to screaming attacks, and are nervous and easily startled (17). Symptoms such as these may be described even in the early months of infancy. Most untreated PKU children have characteristic behavior ranging from that of the shy, anxious and restless high-grade patient to that of the destructive and noisy psychotic. Uncontrollable temper tantrums are common. The hyperactivity, irritability, and uncontrollable tempers are the

usual reason given for admitting patients to institutions. Profile ratings by teachers of school behavior disclosed that PKU children who are mildly retarded after treatment are significantly more clumsy and awkward, more talkative and more hypersensitive than matched controls (13).

Body movements. A great variety of abnormal body movements have been described in the child with untreated PKU (5,13). These are voluntary, purposeless and repetitive motions of the whole or a part of the body. Hand posturing is a very striking characteristic, especially among those of low intelligence. These purposeless movements include rhythmic pill-rolling movements of the hands, irregular tic-like motion, aimless to-and-fro movements of the fingers, and frequent habitual fiddling of the fingers held close to the eyes. These movements are accompanied by a rhythmic rocking back and forth which may continue for hours. PKUs frequently show tremor of the hands and increased reflexes. It is believed that enhancement of their reflex activity is related to damage of developing pathways within the central nervous system. Increased muscle tone is described in 70% of PKU patients. The patients always seem ready to jump. This unrelaxed attitude may be responsible for the awkward or even rigid, short-stepped gait.

POSSIBLE CAUSES OF MENTAL RETARDATION IN PKU

Impaired transport of essentail amino acids into the brain. Perhaps the most devastating characteristic of the classical form of phenylketonuria is the severe mental retardation which develops when the disease goes untreated (Fig. 4) (13). The mechanism whereby phenylalanine accumulation may impair brain growth and development of cognitive function has yet to be explained by one single event (10). In fact, many theories have been proposed to explain how the deficiency in phenylalanine hydroxylase activity in the liver of patients with untreated PKU might lead to impaired brain growth or function. The more popular theories spring from observations that high concentrations of phenylalanine impair the transport into brain cells of tyrosine, 5-hydroxytryptophan, and various other essential amino acids (20).

In 1970, Aoki and Sigel (21) observed that polyribosome structure in cell-free preparations from developing rat brain was disrupted by concentration of phenylalanine in vitro equivalent to those found in the plasma of phenylketonurics. The disruptive effect was to some extent dependent on tryptophan depletion in vitro. A supportive study by Lindroos and Oja in 1971 (22) indicated that in vivo induction of hyperphenylalanemia also impaired cerebral protein synthesis in the rat. McKean et al. in 1968 (23) also showed the effects of injecting a compound which resembles phenylalanine chemically and metabolically and

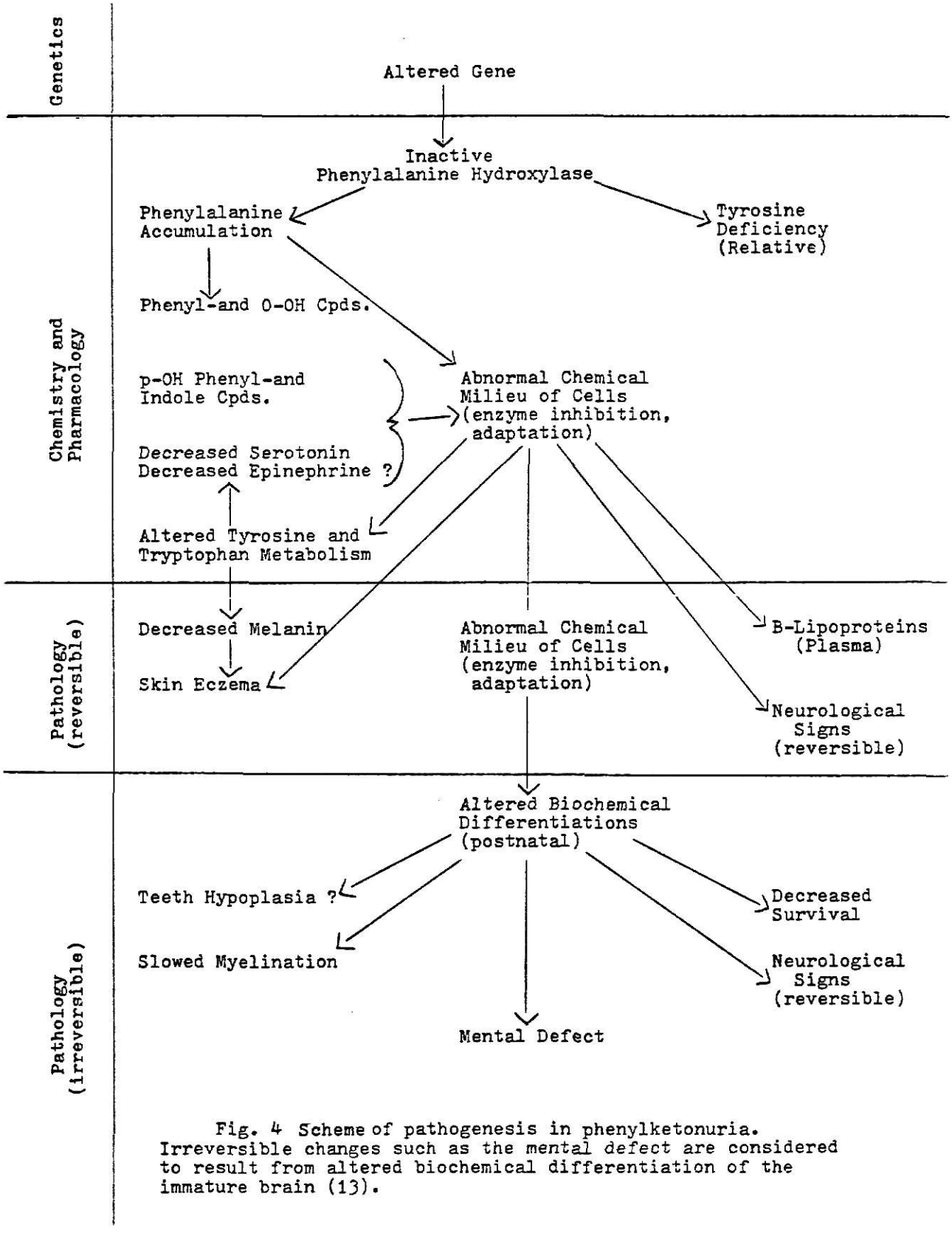

Fig. 4 Scheme of pathogenesis in phenylketonuria. Irreversible changes such as the mental defect are considered to result from altered biochemical differentiation of the immature brain (13).

found that its accumulation in the plasma causes depletion of such amino acids as threonine, valine, methionine, isoleucine, leucine, histidine, tyrosine, and tryptophan in the adult rat brain. The consequence of such a failure in transport of amino acids into the brain might include a deficiency of such probable central synaptic transmitters as norepinephrine and dopamine, a deficiency of serotonin, or failure to synthesize certain cerebral proteins. The transport of 5-hydroxytryptophan into the brain is of considerable importance because it is a precursor of serotonin, an important substance for normal brain development. Impaired synthesis of serotonin resulting in low serotonin concentrations in the brain has also been suggested as an explanation for the mental defect in PKU (24).

Glutamine deficiency. It was inferred by Perry and colleagues (20) that chronic glutamine deficiency in body fluids and in the brain may be a determinant of the mental retardation in PKU. Their proposal was based on the discovery of two PKU brothers, one with a severe mental defect and the other with superior intelligence. They found no significant differences in their degree of hyperphenylalaninemia, nor in their urinary excretion of phenylalanine metabolites. The one striking difference was the significant reduction in concentration of glutamine in the plasma of the defective brother. Taken in perspective with known depression of plasma glutamine in most untreated

patients, and the effect of high plasma concentrations on brain amino acids in animals, the presence of normal plasma glutamine in PKU seemed to be an important finding. The relationship between the two is probably not as simple as it appeared. In 1971, Wong and colleagues (25) reported that they could find no significant difference between plasma glutamine content of 8 untreated PKU patients with mental retardation and 4 similar patients without serious mental retardation. Colombo in 1971 (26) also found no correlation between IQ and plasma glutamine in 2 PKU siblings with borderline normal IQ levels. The major challenge to the glutamine hypothesis came from McKean and Peterson (27) who measured glutamine in plasma cerebrospinal fluid and cerebral gray and white matter of mentally retarded PKU patients and retarded patients without PKU. Plasma glutamine was decreased whereas glutamine was elevated in the cerebrospinal fluid of PKU individuals as compared with control patients. Brain glutamine also was increased in the former. A disturbance of influx or efflux of amino acids into the brain was implied, but glutamine depletion as the major cause of mental retardation could not be supported by the evidence (27).

Abnormal synthesis of structual proteolipids. Neuropathologic evidence of alterations in myelin in the brain of patients with PKU has recently raised the possibility of abnormalities in the synthesis of structural proteolipids as

an explanation of the mental defect. Chase and O'Brien (28) studied the effect of excess phenylalanine on the formation of the myelin lipid cerebral sulfate (sulfatide) in the newborn rat. Prolonged exposure to hyperphenylalaninemia resulted in decreased sulfatide in rat brain. Such findings are relevant to the abnormal lipid content observed in human phenylketonuria (29). Although the origin of the defect in cerebral lipid metabolism remains unknown, some investigators hope to explain that important abnormality. Shah et al. (30,31) found that deaminated metabolites of phenylalanine were potent inhibitors of glucose incorporation into the total lipid fraction of developing brain. As a result of reduced formation of such lipids as cholesterol and glycolipids, the hyperphenylalaninemic brain contains significantly less myelin than that of control rat brains, although gross composition (ratio of protein: cholesterol: galactolipids: phospholipids) of the myelin is unchanged. A suggestion was made that in hyperphenylalaninemic brains the reduced availability of cholesterol may cause a reduction in the formation of cholesterol-phospholipid-protein complexes in the brain, which in turn limits the formation of myelin (29).

Weber (32) stated that phenylalanine and its metabolites, particularly phenylpyruvic acid, inhibit glucose metabolism and oxidative phosphorylation. Very high concentrations of phenylalanine competitively inhibit brain pyruvate kinase and phenylpyruvate inhibits brain hexokinase. Any impairment of pyruvate availability and oxidation to acetyl-CoA may

compromise the synthesis of fatty acids and cholesterol and the yield of ATP from glycolysis and might account for the depression of myelin synthesis and decomposition which accompanies the mental retardation of PKU.

There remains a large deficit in the knowledge about the cause of mental retardation in phenylketonuria. None of the studies in vitro or in vivo in animals or in man have yet solved the riddle. It is likely that no single answer will be found, but rather that cerebral malfunction is the outcome of a number of chemical abnormalities occurring within the brain as a consequence of a deranged internal milieu present during a critical phase of development (10).

VARIANTS OF PHENYLKETONURIA

Hyperphenylalaninemia associated with the mental defect. Several variants of phenylketonuria have been described which do not fit the description of the "classical" form of PKU (33). These variants of PKU have been assigned various terms including: hyperphenylalaninemia without phenylketonuria, neonatal phenylalaninemia, transient hyperphenylalaninemia, and normal phenylketonuria. Since there is no common agreement on the classification of the variants of phenylketonuria, it is difficult to define their characteristics. Generally, patients with the several variants of PKU can tolerate greater amounts of phenylalanine than patients with the "classical" form of phenylketonuria. This greater tolerance for phenylalanine results in a slower rise of plasma phenylalanine levels after birth. The plasma phenylalanine levels generally remain lower throughout life and following a phenylalanine load. For convenience, most patients with persistant plasma phenylalanine levels of over 25 mg/100 ml are generally classified as phenylketonuric while those with levels persistently below 15 mg/100 ml are described as having one of the variants of PKU. Those patients with constant levels between 15 and 25 mg/100 ml are difficult to classify and many clinicians tend to make their decision based on a combination of family history, clinical course, phenylalanine tolerance curves, and ease of management with the diet, none of which are very accurate. Many investigations have been

made which attempt to explain the various forms of hyperphenylalaninemia not caused by PKU.

It is known that in classical PKU, phenylalanine hydroxylase is missing from the complex hydroxylating system of phenylalanine. Evidence indicates that variant forms of PKU might exist in which one of the other essential components of the hydroxylating system is missing (14,34). Recently several reports have described new variant forms of PKU in which neurological deterioration occurs despite dietary control of blood phenylalanine levels (35,36). The possibility remains that the disease could result from a lack of any of the other essential components of the system: tetrahydrobiopterin, dihydropteridine reductase or dihydrofolate reductase (35).

Deficient dihydropteridine reductase activity. In 1975 Kaufman et al. (14) investigated a child diagnosed with classical PKU. Despite excellent control of blood phenylalanine concentration from the third week of life, seizures and retardation became evident at 7 months of age. In order to evaluate the various enzymes involved in phenylalanine metabolism, an open liver biopsy was performed. A marked deficiency of dihydropteridine reductase in the liver, as well as in the brain and fibroblasts cultivated from the skin was indicated. In order to devise a more practical screening test for possible mutants in dihydropteridine reductase, a search was made for the enzyme in several other

tissues which were more readily available than in liver. No activity was detected in normal lymphocytes, leukocytes, or erythrocytes. Dihydropteridine reductase activity was detectable in normal human fibroblasts at levels of 20 to 30% of the specific activity of the liver (14,15). In 1976, a second child reported as hyperphenylalaninemic showed the same enzyme defect (15). No dihydropteridine activity was found in the fibroblastic cells of the child. Since dihydropteridine reductase is essential not only for the enzymatic conversion of phenylalanine to tyrosine, but also for the biosynthesis of the neurotransmitters, dopamine, norepinephrine, and serotonin, the neurologic symptom which developed in the two patients mentioned above could be due to impairment of the ability to synthesize those neurotransmitters (15,34). Because of those additional defects, dietary restriction of phenylalanine intake does not appear to be effective in the treatment of this new form of hyperphenylalaninemia (14,15,34-36).

Deficient tyrosine and tryptophan activity. Bartholome et al. (35) reported another form of phenylketonuria in which all of the known components of the phenylalanine hydroxylase system, phenylalanine hydroxylase, dihydropteridine reductase, and biopterin, were active in vitro, and yet the child showed marked hyperphenylalaninemia. Although the child's blood level of phenylalanine was well controlled from early infancy by restriction of dietary intake of phenylalanine,

neurological deterioration was evident at 7 months of age. Clinical examination of the child's urine revealed the lack of a serotonin metabolite, 5-hydroxyindol acetic acid. Evidence indicated that three different enzymes, phenylalanine, tyrosine and tryptophan hydroxylases, were not functioning normally in the patient. Although the primary molecular defect in the child was not known there were indications the defect lead to disturbances in phenylalanine metabolism and the biosynthesis of L-dopa and L-5-hydroxytryptophan. The administration of these two neurotransmitters brought about a notable improvement in the patient's neurological symptoms.

Hyperphenylalaninemia without the mental defect. Not all variants of untreated phenylketonuria result in mental and neurological defect (37). A number of children initially diagnosed as phenylketonuric on the basis of high blood phenylalanine levels in the newborn period have required virtually normal intakes of phenylalanine to maintain serum phenylalanine levels near normal and maintain normal growth (38).

Decreased phenylalanine activity. Some newborn infants have rather persistent elevations of their serum phenylalanine levels at intermediate ranges without other biochemical features of PKU for the first years of life (38). Early reports of patients with atypical PKU or hyperphenylalaninemia

have suggested, on the basis of responses to tolerance testing, that those patients may be heterozygotes for PKU (37-39). The increased blood levels of phenylalanine in those patients is attributed to partial deficiency of phenylalanine hydroxylase activity and difficulty in transaminating phenylalanine (45). Direct enzyme assays on liver biopsy specimens from patients with hyperphenylalaninemia have shown a reduction in phenylhydroxylase activity from one-tenth to one-third the normal value (41). Although it is not known conclusively whether moderate degrees of elevation of the blood levels of phenylalanine results in intellectual impairment, some centers are placing all patients with hyperphenylalaninemia on a low phenylalanine diet (39). Since neurological and intellectual deficits may result from too severe restriction of phenylalanine intake (42), the use of unnecessary dietary management may actually be harmful (38). The differentiation of hyperphenylalaninemia from PKU is thus of practical importance.

Hyperphenylalaninemia associated with hypertyrosinemia. Hyperphenylalaninemia during early infancy may be associated with hypertyrosinemia (33). Human infants, especially those of low birthweight have a functional deficiency of both tyrosine transaminase and p-hydroxyphenylpyruvic oxidase. This results in a marked accumulation of tyrosine and a moderate increase in phenylalanine. Excess tyrosine levels in low birthweight infants are not always associated with

increased phenylalanine levels, although most infants with high phenylalanine levels are shown to have elevated tyrosine levels. As a general rule, phenylalanine levels in hyperphenylalaninemia associated with hypertyrosinemia seldom exceed 10 mg/100 ml. Hypertyrosinemia and the slight hyperphenylalaninemia associated with that condition do not appear to be harmful to the infant. A single 100 mg dose of ascorbic acid prevents hypertyrosinemia and its associated hyperphenylalaninemia. This regimen is recommended to avoid a false-positive test due to hypertyrosinemia noted in screening programs.

SCREENING FOR PKU

Criteria for diagnosis of PKU. The infant with PKU, or any other form of postnatal hyperphenylalaninemia, is born with a normal level of phenylalanine in the blood plasma (43). The normal level may range between 1 to 4 mg/100 ml serum (4) but is rarely more than 2 mg/100 ml (1). In PKU, plasma phenylalanine rises after birth because the exogenous and endogenous loads of free phenylalanine cannot be oxidized normally and because less than half of the free phenylalanine can be incorporated in peptide linkage even during periods of rapid somatic growth. Therefore, the trait can be detected in the newborn period either by screening for hyperphenylalaninemia or by detection of phenylalanine metabolites in abnormal amounts in urine (10, 11). In PKU screening, a blood phenylalanine level of 4 mg/100 ml or higher is generally accepted as a requirement for follow-up testing (44). Because screening of newborns has led to the recognition that not all hyperphenylalaninemia is caused by PKU (1), diagnostic criteria in screening for PKU has become more stringent (45). The diagnostic criteria has risen from a blood phenylalanine concentration of greater than 10 mg/100 ml in 1964 to over 15 mg/100 ml in 1966 (46) to 20 mg/100 ml in 1971 (45). At the present time the diagnosis of the classical form of PKU is accepted if the following criteria are met (1,10,11,47): a) The plasma phenylalanine level rises from a normal of 1 to 4 mg/100 ml

at birth to 20 mg/100 ml or higher by the end of the first week of life and this marked hyperphenylalaninemia persists. b) There is normal serum tyrosine levels of 5 mg/100 ml or lower and plasma tyrosine does not rise after a challenge of phenylalanine. c) The urine contains ortho-hydroxyphenylacetic acid during the newborn period and later contains phenylketones, including phenylpyruvic acid, phenylacetic acid, and phenylacetylglutamine.

Screening for congential metabolic disorders in the newborn is carried out in order to permit diagnosis and institution of treatment within the first 4 weeks of life (3). Most countries now have laws requiring screening of all newborns for elevation of blood phenylalanine values (48). To prevent mental retardation due to phenylketonuria, a low phenylalanine diet is initiated early in infancy before symptoms are manifested. After the infant is started on a low-phenylalanine diet, frequent monitoring of serum or blood phenylalanine concentration is needed (47). Lower than expected levels suggest the possibility of a variant form of hyperphenylalaninemia. The diagnosis should be further confirmed by challenging the infant with a formula containing normal amounts of phenylalanine or by the demonstration from serial monitoring that the dietary phenylalanine tolerance cannot exceed 500 mg/day without provoking hyperphenylalaninemia in excess of 16 to 20 mg/100 ml.

Laboratory methods of testing for PKU. In PKU, blood levels of phenylalanine rise before the amino acid and its metabolites spill over in the urine (49). For this reason screening tests which depend on the detection of elevated blood levels are more specific and become positive earlier than those tests which depend on an amino acid and its metabolites in the urine. Several different methods have been used to detect PKU. One of the earlier test methods was the ferric chloride test (4,50). The preferred screening methods currently in use include the Guthrie bacterial inhibition assay, the McCaman and Robins fluorometric assay, and chromatographic precedures on either paper or thin layer plates (47).

Ferric chloride test. In the ferric chloride test 5 ml of fresh urine are added to a 5% ferric chloride solution and the color change is observed after 2 to 3 minutes (4,50). In a patient with PKU, phenylpyruvic acid produces an olive-green color. This test does not become positive until the child is several weeks old (over 3 weeks) by which time he has left the hospital and is less readily available for a routine screening test. In the older PKU patient, this test fails to give positive results when the blood levels of phenylalanine fall below 15 to 20 mg/100 ml (4,51). Because this test does not produce reliable results in the first few weeks of life, other methods which are more accurate have been devised.

Guthrie bacterial inhibition assay. The Guthrie inhibition assay follows the principle that inhibition of growth of Bacillus subtilis ATCC 6051 by B-2-theinylalanine in minimal culture medium is prevented by phenylalanine, phenylpyruvic acid and phenyllactic acid (52). A small amount of blood obtained by skin puncture (or a urine sample) is transferred immediately to filter paper. The spot is dried, autoclaved, punched out and placed over the agar surface containing inoculum and the inhibitor. The reading is made 16 hours later. The size of the halo surrounding the disk that represents the growth of Bacillus subtilis is relative to the increase in phenylalanine content (1,4,50). The amount of growth around the disc containing 4 mg/100 ml phenylalanine is used for comparison. This method of testing for high elevations of blood phenylalanine associated with PKU has been found to be a rapid and economical method for screening large numbers of infants in hospital nurseries (52). Screening of 682 infants (96% tested by 4 days of age) by the inhibition assay was reported by Guthrie and Susi (52). None of the blood phenylalanine values were found to be as high as 4 mg/100 ml and only 8% were above 2 mg/100 ml. These values indicate that a low rate of "false-positives" will be encountered during screening of the 10,000 or more infants that may be necessary to detect one case of PKU. It is recommended that any value of 6 mg/100 ml or above be considered positive and that confirmation by phenylalanine determination of a second blood specimen be required.

A modified form of the Guthrie inhibition assay, described by Haab and colleagues (53), is also useful for mass screening of PKU. This method replaces the agar as a gelling agent with hydroxyethyl cellulose, a spore-containing lyophilized medium which can be hydrated with water at room temperature. The hydroxyethyl cellulose is advantagous over the agar, because agar prohibits reconstitution at temperatures below 100 C. This modified method has been found to be as reliable as the Guthrie method. The prepackaged, lyophilized, spore-containing medium lends itself to mass testing procedures of phenylketonuria with a minimum of time and effort on the part of the clinician.

McCaman and Robins fluorometric assay. The fluorometric assay of phenylalanine in serum as first described by McCaman and Robins (54) is based on the enhancement of the fluorescence of a phenylalanine-ninhydrin reaction product by a peptide leucylalanine. The peptide, L-leucyl-L-alanine, is used because it gives 100% relative fluorescence of phenylalanine. This method allows phenylalanine to be measured quantitatively in the presence or absence of other amino acids at a final concentration of 10^{-7}M. Only 5 microliters of serum is required for analysis. This method has been applied to determine phenylalanine concentrations in adult, pediatric and PKU patients.

Various modifications of the McCaman and Robins spectrophotofluorometric method have been described. Wong and

colleagues (55) described a successful modified spectrophotofluorometric method, employing 25 microliters of serum, for detecting increased levels of phenylalanine and decreased levels of tyrosine commonly observed in PKU patients. Hill and colleagues in 1965 (56) also produced a successful adaption of the McCaman and Robins method by an automated procedure. The automated procedure is both simple and reproducible. This modified method of Hill et al. differs from that of the McCaman and Robins method in that dialysis rather than trichloracetic acid precipitation is used to remove protein and the reaction mixture is heated to 95°for 11 minutes rather than 60°for 2 hours. Capillary blood samples of as little as 20 microliters of whole blood can be used with a testing rate of 60 samples/hour. Hsia and colleagues (43) also described a modification of the spectrofluorometric method in which one technician can process 150 serum samples at a time to determine phenylalanine concentrations.

A rapid, specific and precise method for measuring elevated serum levels of phenylalanine also has been described by LaDu and Michael (57). This simple enzymatic spectrophotometric method permits simultaneous determination of phenylalanine, tyrosine and tryptophan in serum. It has been found to be useful as a confirmatory test in diagnosis of suspected PKU and in the evaluation of the effectiveness of a diet low in phenylalanine.

Paper chromatography. Paper chromatography is used also to detect phenylalanine and a series of amino acids in the blood (58,59). This method employs the use of dried blood on filter paper cards which provides for easy collection, mailing, and storage of samples (58). Paper chromatography is a rapid method, requiring only 20 minutes of working time (59). This method of testing for PKU requires only 0.12 ml serum, does not require a protein-free filtrate and does not involve use of expensive equipment. Culley et al. (59) observed that 15 PKUs tested by this method had an average serum phenylalanine level of 29 mg/100 ml, whereas serum from 5 fasting non PKUs averaged 2 mg/100 ml. Although Culley et al. (59) reported that chromatograms are semiquantitative, their accuracy is sufficient to detect PKU. Efron and colleagues (58) disputed this finding and reported that chromatograms can only be relied upon to detect phenylalanine concentrations in excess of 10 mg/100 ml. Because of the small increase in blood phenylalanine in the newborn period, PKU might well be overlooked when testing is done by chromatography. For this reason, and because chromatography is the least sensitive method for screening (47), chromatography should not be used as the major hospital test for phenylketonuria (58).

Factors related to the effectiveness of test results. An ideal program for the screening of PKU would detect all infants with PKU and a minimal number of false-positives (3).

In addition, it would also permit the initiation of treatment in time to prevent retardation.

Age at screening. A study by Holtzman and colleagues in 1974 (3) indicated that while virtually all newborns are screened in some states, less than 70% are screened in others. There are also indications that between 5 to 10% of infants ultimately proven to have PKU are not detected by newborn screening of blood samples (3,44,45,47). Holtzman and colleagues (3) reported on 23 infants from 8 states whose initial test results were negative, but who subsequently were proven to have PKU with maximum blood phenylalanine levels in excess of 20 mg/100 ml. The states or clinics reporting these false-negatives found (over the same time), 253 patients in whom the diagnosis of PKU was made as a result of screening. Therefore in these states approximately 92% of infants with PKU were discovered by screening. Fifteen of the 23 false-negatives, or 65.2% , were screened on or before the third day of age (11 on the third day and 4 on the second) although only 44% of all infants were screened by that age. Thus the probability of missing a case of PKU is greater if screening is performed early in the neonatal period (3,44,45,47). The detection of PKU among infants screened after the fourth day of life is 1.6 times higher than among infants screened earlier (3).

Because infants screened in the first few days of life have lower phenylalanine levels than those screened later

(60), the earlier a PKU infant is screened the greater the chance that the blood phenylalanine concentration will not be elevated. For PKU screening, a blood phenylalanine level of 4 mg/100 ml or higher on screening is generally accepted as requiring a follow-up test (44). A phenylalanine level of 20 mg/100 ml or higher is likely to be a manifestation of PKU, but infants with PKU may have lower levels, particularly if screening is done very early. A study of the effectiveness of screening for PKU (44) in the United Kingdom, Ireland, and the United States indicated that diagnosis is more likely to be missed in the latter two countries because of the earlier age at which infants are screened. In the United Kingdom, virtually all infants are screened between 5 and 14 days of life, whereas in Ireland and the United States, most are screened in the first 4 days of life. Effectiveness of the screening programs can be measured by determining the number of infants whose original screening test was negative but who were subsequently discovered to have PKU. Table 1 shows that no such infants screened by blood or serum assay were discovered in the United Kingdom, but that such infants were found in the United States and Ireland where screening was done on or before the third day of life.

Sex of child screened. In many infants with PKU the blood phenylalanine rises only minimally during the first three days of life and infants of both sexes may be missed by screening (60). During this period the rate of rise of

TABLE 1

Sensitivity of phenylketonuria detection (adapted from Starfield and Holtzman) (44).

Country	Year	Cases Missed By Screening	Cases Discovered	% Missed
Republic of Ireland	1966-72 (Blood)	1	73	1.4
United Kingdom	1964-72 (Blood)	0	251	0
	1968-72 (Urine)	2	7	22.2
United States	1962-71 (Blood)	23	253	8.3

blood phenylalanine is slower in females than in males and on the fourth day of life, significantly more males than females are discovered by screening. Phenylalanine may appear in the blood as a consequence of dietary intake as well as from tissue breakdown. It is possible that the catabolic phase normally observed in the immediate postnatal period is greater in males than in females. The influx of phenylalanine into the blood would then be greater in males and impairments in the removal of phenylalanine, even if equal in the two sexes, would cause higher phenylalanine levels in males than in females. The greater susceptibility to bacterial infection is also one factor which exaggerates the neonatal catabolic phase in newborn males. Although the initial rise of blood phenylalanine level in female infants with PKU is slower than in males, after day 4 the rate of rise is faster in females and their blood phenylalanine levels at 8 days of age are higher than those of males. In view of the age-dependent rise in blood phenylalanine concentrations during the first week of life, the probability of missing the diagnosis of PKU in both males and females is greater for those screened during the first few days of life.

Feedings prior to testing. The effect of feeding on the screening for PKU in infants is of great interest. A study by Dontanville and Cunningham (61) indicated there was no relationship between the phenylalanine content of the feedings prior to the PKU screening test and a positive test

result of greater than or equal to 4 mg/100 ml in 68 PKU, 14 atypical and 26 hyperphenylalaninemic infants. On the other hand, there is evidence that high protein feeding, especially of cow's milk formula, will increase the concentration of amino acids, including phenylalanine (12). Because breast milk or low protein artificial formulas are recommended for infants in their first 4 months of life, the use of those milks may cause difficulties in the diagnosis of phenylketonuria (62). Phenylalanine content of mature breast milk may provide intakes similar to those used in treating PKU. One investigation (62) was reported on two patients with PKU requiring treatment who were fed breast milk and other low protein milks. Both had blood phenylalanine levels below 20 mg/100 ml until given a phenylalanine challenge. Diagnosis of PKU is unlikely to be missed if screening is carried out on the sixth or seventh day of life because of higher phenylalanine in breast milk during the first week. Interpretation of screening tests requires careful investigation and knowledge of the infant's food when a blood phenylalanine level above 6 mg/100 ml in the absence of tyrosinaemia is observed.

Interpreting false-positives. Some infants may show elevated blood phenylalanine levels at birth, but do not actually have classical PKU (3,43,45). The vast majority of infants with elevated levels of phenylalanine on a screening test have normal blood phenylalanine concentrations by the time of follow-up (3). Among 6,662 infants whose

phenylalanine levels were elevated on the first test, 85% had a phenylalanine level of less than 6 mg/100 ml on follow-up; 9.9%, greater than 6 but less than 20 mg/100 ml; and 5.1%, greater than or equal to 20 mg/100 ml. The infants with phenylalanine concentrations between 6 and 20 mg/100 ml on the follow-up fall into two categories based on additional determinations. The first category consisted of those in whom moderate increases of phenylalanine persisted while on a normal diet, but without risk of retardation (one third of infants fall into this category). The second included those in whom the phenylalanine concentration fell within a few months. This group was further subdivided into infants with and without associated tyrosinemia. Tyrosine concentrations were reported in 85 of the infants whose phenylalanine level was between 4-20 mg/100 ml on the first follow-up examination. In 43.5%, elevated tyrosine levels (greater than 4 mg/100 ml) were reported. No tyrosine level elevations were found in infants whose follow-up phenylalanine value was greater than or equal to 20 mg/100 ml. Only 5.1% of infants with a phenylalanine increase on screening had blood phenylalanine levels greater than or equal to 20 mg/100 ml on follow-up, and were considered to have classical PKU. It is therefore possible to interpret positive screening tests in several ways (63). Results may indicate variant forms of PKU requiring completely different theraputic approaches than the typical form for which screening was established.

Delay in follow-up testing. Because the onset of irreversible mental retardation occurs relatively early in infants with PKU who are kept on regular diets, it is important to institute formulas low in phenylalanine before three weeks of age (44). Follow-up testing of infants with elevated screening levels takes significantly longer in the United States than in the United Kingdom due to differences in application of the screening process. The time required to retrieve an infant with abnormal test results is sometimes excessive (47). The mean interval between screening test and follow-up for 1,283 infants in the United States and North America whose initial phenylalanine concentration was elevated was 24.9 days (3). In 22.6%, the interval was more than 30 days. Irreversible brain damage might occur before diagnosis of PKU could be confirmed and low-phenylalanine diets instituted. Screening for PKU, as well as for other inborn errors of metabolism, will not prove beneficial unless rapid follow-up is assured.

Laboratory error. Because the blood phenylalanine concentrations in infants with PKU may be only minimally elevated in the first four days of life, laboratory error contributes to the failure to detect some affected infants (49). Differences in the performance and interpretation of the Guthrie bacterial inhibition assay test as well as the other testing methods used does exist (3,60). The problem of laboratory error is compounded by the fact that in some states

more than one laboratory performs the test (3). In California in 1967, at least 194 laboratories performed screening tests. Variability due to differences in laboratory methods could be reduced if fewer laboratories performed the test and strict quality control was imposed (3,44).

Recommendations for improving PKU screening programs. To improve the operation of the entire screening program for PKU there should be one authority (usually under the state health department) or a regional group with an advisory board or commision consisting of a representative of hospitals who sends specimens, a specialist in metabolic disease, a nurse and/or nutritionist involved in management of patients, a representative of practicing physicians, the director of the laboratory performing the test, and consumers (47). Because of the problems in the performance and interpretation of screening tests, specimens should be analyzed in a large, central facility. This may be one laboratory within a state, or when the number of births in a state is too small to permit efficient utilization, one laboratory serving several states (47). The United Kingdom and Ireland, who have more efficient screening programs for PKU than the United States, confirm that regionalization programs do promote rapid institution of diagnostic testing and therapy (44). The United States is slowly developing centralization of the public-health laboratory function for newborn screening

programs (48). Alaska, Montana, and Oregon have participated in such a program since 1975. Now, 5 New England states are currently participating in the New England Regional Screening Program at the State Laboratory Institute. Screening is done by obtaining filter-paper blood specimens from infants at the time of nursery discharge or at 3 to 5 days of age. These are forwarded to each state public health lab, respectively, and then delivered to the regional program by a commercial carrier. Negative test reports are mailed to each state lab. Abnormal results are telephoned immediately and confirmed in writing. The delay of 1 to 2 days inherent in the system of forwarding specimens through each state lab to the Massachusetts lab proved to be less important than the variability of time of collection and forwarding by the staff of newborn nurseries to the respective state lab. This method of screening helps to permit diagnosis and institution of treatment within the first 4 weeks of life. Centralization, consolidation of testing programs, the establishment of lines of communication between those collecting specimens, laboratories analyzing them, and those providing follow-up care could alleviate some of the problems associated with the screening of PKU (47).

Benefits of screening for PKU. The most worthwhile advantage of screening programs is the prevention of the mental defect associated with PKU. Since the widespread

screening program started in the United States in 1962, a large number of children with PKU who otherwise would have gone undiscovered until mental retardation and other signs of brain injury became evident, have been detected (64). Those infants were treated in early infancy with a restricted phenylalanine diet in the expectation that treatment would prevent brain damage. In order to evaluate the effectiveness of the screening program, a survey was conducted to determine the number of children institutionalized as a result of PKU. From 1962 to 1967, no children with PKU were admitted to institutions for the mentally retarded. This contrasts rather strikingly with admission records before screening programs. The early treatment made possible by screening of newborn infants seems a likely reason for the reduction in PKU admissions to institutions.

Cost of screening. Monetary cost of screening and treatment needs to be weighed against the results incurred when such preventative practices do not take place (65). The monetary cost to society of PKU screening is averted by the elimination of chronic care institutions for the mentally retarded. In 1974 it was estimated that $1 spent for screening and testing was balanced by saving $4 in institutional and other care costs otherwise given to retarded PKU patients. Marginal operational cost per test also decreases as the number of tests increase. Each state currently participating in the New England Regional Program contributes

to its support on a prorated basis according to expected number of live births per year (48). For the 1977 calendar year cost of amino acid screening ran $1.20/test. This relatively low cost of screening has the potential for avoiding expensive care of children with brain damage resulting from untreated phenylketonuria.

ASSESSMENT OF INTELLIGENCE IN TREATED PKU CHILDREN

It has been proven that appropriate dietary management in young children with PKU is effective in preventing mental retardation (66). By introduction of a phenylalanine-restricted diet in the neonatal period and maintenance of such a diet during the early years, damage to neural tissues and other manifestations can be prevented and normal physical growth and intellectual maturation can be expected (67). There is, however, conflicting evidence from comparisons of IQs of PKU children with those of their unaffected siblings about whether some degree of intellectual impairment occurs even with early treatment (66). Some authors (68-71) have reported that treated PKU children are much less intelligent than their normal siblings although their IQ still falls within the normal range. Other reports, such as that by O'Grady et al. (72) indicate that early treated PKU children do not differ significantly from nonaffected siblings. O'Grady et al. (72) reported early treated children with a median IQ of 114.5 as compared to a median IQ of 119.5 for nonaffected siblings. The general trend is toward a lower IQ the longer a PKU infant goes before being placed on a phenylalanine-restricted diet (70). In 1977, Dobson et al. (73) reported on the intellectual assessment of 11 four-year-old children with PKU. Their mean IQ on the Stanford Binet Intelligence Scale was 93. Females scored a significantly higher mean IQ than males (97 vs. 90). Those children for whom dietary treatment was initiated during the

the first month of life scored a mean IQ of 95 compared with 85 for those initially treated after 31 to 65 days.

In 1970, Kang and colleagues (74) reported the mean IQ of 27 PKU patients at the mean age of 3 years 10 months treated before 3 weeks of age was comparable to that of their unaffected siblings. The mean IQ of 12 patients at the mean age of 5 years 6 months treated between 3 to 6 weeks of age fell significantly below the mean IQ of their unaffected siblings. Nineteen patients treated after 8 months of age did not differ in their mean IQ at the end of treatment from 11 untreated patients, despite the observation that more than half of the late-treated patients achieved significant gains in IQ during the course of treatment. Dobson et al. (75), reported on the intellectual development of 36 PKU children placed on dietary treatment before 121 days of age (82% of those before reaching 30 days of age). An effort was made to maintain serum phenylalanine in the range from 1 to 10 mg/100 ml between birth and the age of testing. Each PKU child was matched against an unaffected sibling and given a Stanford Binet Intelligence test when they were approximately the same age. PKU index cases earned a mean IQ of 94 with a standard deviation of 17.6. The non-PKU siblings received an average IQ of 99 with a standard deviation of 15.0. In 24 of the 36 pairs, IQ of each PKU index case was below that of their sibling. Interpretation of these data indicated a small degree of intellectual damage apparently occurs from PKU even when the patient receives conventional diet therapy early in life. The deficit could result from a

a brief delay in achieving diagnosis, damaged sustained in urero, periods of dietary indiscretion during the early months of life, or even from intercurrent illnesses during early childhood which elevated blood phenylalanine despite dietary control.

While dietary treatment may mitigate intellectual retardation, some degree of impairment may remain (66). Koff and colleagues (66) reported that children with treated PKU who score within the average range of intelligence on the Wechsler Intelligence Score for Children (WISC) exhibited substantial impairment of perceptual motor-functioning as measured by performance on the Binder Gestalt test. The majority also had lower performance IQs than verbal IQs, a finding which has been thought to be associated with perceptual-motor dysfunction. These findings suggest the possibility of a specific deficit that could interfere seriously with academic process, but which is not signalled by obvious impairment of overall intellectual functioning.

DIETARY MANAGEMENT OF PKU

Since the recognition of phenylketonuria in 1934, there have been many attempts to cure and prevent mental deficiency associated with this disease. The first significant improvement came with the institution of low-phenylalanine diets during the 1950's. Soon after, it was demonstrated that early initiation of the special diet in infancy could prevent the mental deficiency of PKU (76). Dietary management of PKU is currently the only known effective method of treatment (8).

Requirements and nutritional adequacy of the PKU diet. Nutritional requirements for the child with PKU are the same as those required by normal infants at the same age (77). Although phenylalanine levels must be controlled by dietary inception in the child with PKU, it is important to provide adequate levels to maintain growth and development (78,79). Growth proceeds at optimum rates when the dietary source of protein provides amino acids in roughly the proportions in which they are required by the body (79). When one or several amino acids are present in either excess or deficient quantities an imbalance occurs and physical and mental growth suffer. It has been determined that the phenylalanine requirements in the newborn infant range from 65 to 90 mg/kg body weight (80,81). As the child grows the diet must be adjusted continuously to meet protein and phenylalanine needs

(81). At 2 years of age, requirements for phenylalanine may drop to between 25 to 35 mg/kg and by 10 years to 20 to 25 mg/kg (80,81).

The dietary treatment of phenylketonuria is based on the creation of a balance between the essential amino acids contained in a commercial phenylalanine-free (78) or a low-phenylalanine protein substitute, and supplemental phenylalanine provided from natural foods (81). The nutritional adequacy of a low-phenylalanine protein substitute was studied by Acosta et al. (82). Mean protein intake during the first year of life exceeded recommended dietary allowances (RDA) made by the Food and Nutrition Board of the National Research Council (83). Mean intake of protein (82) (total and on a body weight basis) was consistently less in treated PKU infants after the first quarter than that found in normal infants. In spite of the lower intake of protein, growth was adequate by all standards. This would suggest that a casein hydrolysate supplemented with phenylalanine from natural sources supplies adequate essential amino acids and nitrogen to support growth when the child was fed at mean levels of 4.2, 3.0, 2.5, and 2.5 g/kg at the mean ages of 2, 4.7, 7.6, and 10.4 months, respectively. Mean fat and carbohydrate intakes were found to be somewhat less than those found in normal infants. With effort on the part of the nutritionist and parents, energy intakes meeting the RDA can be achieved. Despite some apparent restriction in energy intake, growth of PKU infants continued to be adequate. Mean

intakes of the minerals, calcium and phosphorus, were considerably greater than the RDA in PKU infants of all ages (82). Iron, vitamin E and vitamin B_6 intake closely paralleled recommendations. Mean intakes of vitamin A, thiamin, riboflavin and ascorbic acid exceeded intakes suggested by the RDA.

Meeting nutritional requirements and initiation of the PKU diet. The nutritional requirements for the child with PKU can be met by providing a semisynthetic diet, derived either from a modified protein hydrolysate or from a mixture of L-amino acids, so the diet contains either an extremely low amount of the amino acid or is free of it (67). Other dietary sources of protein can furnish phenylalanine in an amount sufficient to sustain normal metabolism yet low enough to avoid toxicity. Requirements for other nutrients, (calories, fat, carbohydrates, minerals and vitamins) are met either by special dietary formulations added to the amino acid product or by further supplementation with natural foods of known composition.

Normally, infants in the first six months of life receive all or most of their nutritional requirements from breast milk or infant formula. During this period it is relatively easy to meet all the nutritional requirements of an infant with PKU by providing a semisynthetic dietary product prepared from suitably treated protein hydrolysates or amino acid mixtures and fortified with required additional

nutrients to meet the standards of an infant formula. Small amounts of milk may be added to meet the requirements of the restricted amino acid.

As the infant grows, other foods are introduced into the diet. The composition of these foods and the quantities ingested must be regulated to keep the amino acid composition of the diet under control and to assure provision of other nutritional requirements. Additional modified protein hydrolysates or specific amino acid mixtures low in, or devoid of, phenylalanine and fortified with modular components of the diet which supply vitamin and/or mineral mixtures and various sources of calories are necessary components of the diet. These products, when appropriately formulated, make it easy to meet the requirements for other amino acids and permit a wider variety of natural foods to be used in balancing the diet (78).

Food products used in treatment of PKU. At the present time there are several preparations on the market which may be used in the management of the patient requiring a low-phenylalanine diet (84). These products, as well as their composition, are listed in Table 2 (84). Albumaid-XP, Cymogran, Aminogran, and Minafen are European manufactured amino acid mixtures; Phenyltol and Lophemilk originated in Japan.

Lofenalac and Product 3229, produced by the Mead Johnson laboratory in Indiana, are the primary products used

TABLE 2

Special preparations for the low-phenylalanine diet (84).

Preparation		Phenylalanine	Protein	Fat	Carbohydrates	Calories	Vitamin Suppl.
		mg/100 g		g/100 g		Cal/100 g	
Albumaid-XP	(hydrolysate)[1]	0	30	0	50	330	D
Cymogram	(hydrolysate)[1]	10	30	9	42.7	400	Multiple
Aminogran	(amino acid mixture)[2]	0	80	0	0	330	Multiple
Minafen	(hydrolysate)[3]	20	11.5	31	46	540	A and D
Phenytol	(amino acid mixture)[4]	100	62	0	0	250	None
Lophe milk	(hydrolysate)[5]	67	16	18	59	443	Multiple
Lofenalac	(hydrolysate)[6]	80	15	18	57	450	Multiple
Product 3229	(amino acid mixture)[6]	0	20.3	20.3	66	406	Multiple

1) Scientific Hospital Supply, Liverpool, England. 2) Allen-Hansbury Ltd., London, England. 3) Trufood Ltd., Guilford, Surrey, England. 4) Take da-Jakuhin-Koggo KK., Osaka, Japan. 5) Snow Brand Milk Products, Tokyo, Japan. 6) Mead Johnson Laboratories, Evansville, Indiana.

in nutritional management of the child with PKU in the United States.

Lofenalac. Lofenalac, the first commercial product made available for the dietary management of PKU, contains a low (0.08 gm/100 gm) but significant amount of phenylalanine (78). Lofenalac is made from an enzymatic hydrolysate of casein. It also contains carbohydrate (corn syrup solids and tapioca starch), fat (corn oil), minerals and vitamins. In a powdered form, Lofenalac can be dissolved in water to make a milk substitute which will provide 80 to 90% of the protein requirement for the PKU infant (76). The exact composition of Lofenalac is shown in Table 3 (82).

Lofenalac is prescribed in amounts to meet a child's protein needs at a given age (81). The protein allowances used in calculation of amounts of Lofenalac have been based on the recommendations published by the Food and Nutrition Board, National Academy of Science-National Research Council. Table 4 column 3 (81) shows the recommended low-phenylalanine protein intake according to age expressed in level tablespoons of dry powder. Lofenalac contains 1.4 gm of protein and 7.5 mg of phenylalanine per tablespoon. Phenylalanine from natural food sources and from Lofenalac supplies the total phenylalanine requirement of 70-90 mg/kg at one month of age, decreasing to 35 mg/kg by two years of age and 25 mg/kg at ten years of age. The phenylalanine prescribed from natural foods is determined by subtracting that contained in

TABLE 3

Composition of Lofenalac (82).

Nutrients	Value (100 g powder)
Kcal	450
Protein equivalent (g)	15
Fat (g)	18
Carbohydrate (g)	57
Amino Acids (g)	
L-argine	0.34
L-histidine	0.37
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	0.02
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
Vitamins	
A (IU)	1439
D (IU)	288
E (IU)	7.2
Ascorbic acid (mg)	37
Folic acid (μg)	36
Niacin (mg)	5.7
Riboflavin (mg)	0.72
Thiamin (mg)	0.4
B_6 (mg)	0.4
B_{12} (μg)	1.8
Biotin (mg)	0.02
Pantothenic acid (mg)	2
Inositol (mg)	72
Choline (mg)	61

TABLE 4

Suggested Lofenalac and Phenylalanine Intake for PKU Children (81).

1 Age (Less Than)	2 Total mg of Phenylalanine[1]	3 Lofenalac Tbsp = mg Phenylalanine[2]	4 Natural Foods Phenylalanine[3] (mg)
1 mo	290	7 = 50	240
1 mo	320	8 = 60	260
2 mo	350	9 = 68	282
3 mo	385	11 = 80	305
4 mo	390	12 = 90	300
5 mo	390	13 = 98	292
6 mo	390	14 = 105	285
7 mo	385	14 = 105	280
8 mo	390	15 = 113	277
9 mo	365	16 = 120	245
10 mo	360	16 = 120	240
11 mo	360	17 = 128	232
12 mo	350	18 = 135	215
15 mo	375	19 = 140	235
18 mo	400	20 = 150	250
21 mo	420	21 = 158	262
24 mo	440	22 = 165	275
30 mo	475	23 = 170	305
36 mo	475	24 = 180	295
42 mo	510	25 = 188	322
48 mo	540	26 = 195	345
54 mo	570	27 = 207	365
60 mo	580	29 = 215	365
6 yr	640	33 = 248	392
7 yr	650	36 = 270	380
8 yr	680	40 = 310	370
9 yr	755	42 = 315	440
10 yr	825	43 = 325	500

[1]In preparing the table, all figures have been rounded off because of lack of percision in the available data.

[2]The suggested intake of Lofenalac is calculated on the basis of the recommended protein allowance for a given age.

[3]Column 2 minus column 3 equals column 4.

the commercial product from the total calculated daily phenylalanine requirement according to age and weight. Table 4 column 4 indicates the prescribed amount of phenylalanine from natural foods. Vitamin and mineral supplements are recommended as part of the Lofenalac diet. Lofenalac will meet the vitamin and mineral requirements for an infant if 16 or more tablespoons are taken daily. Since most infants do not consume that amount until 9 months of age, it is prudent to supplement the diet with vitamins and iron.

Diet therapy with Lofenalac is begun after the diagnosis of PKU has been established (81). The amount of Lofenalac initially offered should be based on the infant's protein requirement as determined by his weight. A natural source of phenylalanine is added to the formula to prevent a precipitous decline in serum phenylalanine level during initial treatment. Cow's milk is the most suitable source of phenylalanine for this purpose. Five to six fluid ounces of cow's milk, containing approximately 250 to 300 mg phenylalanine, added to the initial formula is successful in preventing a precipitous decline in serum phenylalanine. During the first month of treatment, the amount of cow's milk required to stabilize serum phenylalanine levels within treatment range of 5 to 10 mg/100 ml aids in the establishment of the initial amount of phenylalanine which will be allowed from natural foods. All infant foods are introduced at the same time for PKU infants as they would be for normal infants. Between 3 and 6 weeks of age the infant may be started on rice cereal in unlimited

quantities because of its low phenylalanine content. There should be a gradual progress to strained fruits, vegetables, meats and egg yolk by 4½ months. Meat and egg yolk are introduced as a source of iron and protein, but in small quantities because of the high phenylalanine content. By 8 months, table foods are introduced at the same time as they would be for children without PKU. The quantity of Lofenalac is increased gradually but regularly to meet the increasing protein allowance during the first year of life, ie., approximately 1 tablespoon per month increase during the first year of life, so that the diet contains 18 to 20 tablespoons a day by 12 months of age. Lofenalac is diluted according to the fluid demand of the infant. The three-meal pattern of feeding established during infancy is continued for the older child and Lofenalac is provided at each meal and at bedtime (81).

Product 3229. Product 3229 is a recently developed preparation consisting of a mixture of L-amino acids with a higher protein content than Lofenalac (84). This L-amino acid mixture supplemented with vitamins, minerals, fat and carbohydrate is designed for feeding the older child with PKU (78). When reconstituted with water as directed, one pint of the product provides 400 kcal and contains the daily requirements of vitamins, minerals and amino acids, except phenylalanine, for a child 2 years of age or older. Additional calories and the required phenylalanine can be met

from conventional foods fed in prescribed amounts. The product has the characteristic bitter taste of L-amino acid mixtures, but it is palatable when flavored. A clinical trial with Product 3229 (84) revealed normal growth and development of PKU children with no adverse effects noted during a study of the various chemical and biochemical parameters. It is assumed that Product 3229 will be made more available for dietary treatment of PKU in the future.

Albumaid-XP (PKU-aid). Albumaid-XP is another phenylalanine-free product which has been tested and used for the dietary treatment of PKU (78,85). Albumaid-XP or PKU-aid as it is also called (78), is a hydrolysate of beef serum from which phenylalanine has been removed, leaving other essential amino acids in satisfactory amounts. Acid hydrolysis and subsequent treatment with ion-exchange resins removes phenylalanine and other aromatic amino acids (85). The neutral hydrolysate is supplemented with L-tryptophan, L-tyrosine, vitamins, minerals, and carbohydrate in the form of maize starch. The final product contains amino acids equivalent to approximately 40% by weight, carbohydrate amounts to 50%, with the remainder consisting of vitamins, minerals, and moisture.

Albumaid was sought as an alternate to the use of Lofenalac for older children with PKU (85). Satisfactory physical and mental development has been achieved with the use of Albumaid-XP or Lofenalac. Because Albumaid-XP is a

phenylalanine-free product, phenylalanine requirements can be met by introducing phenylalanine into the diet in the form of natural foods. In this respect Albumaid-XP is more advantagous than Lofenalac. The disadvantage of using Albumaid-XP in infancy is that more attention must be given to maintaining adequate caloric intake. Generally, Lofenalac is used for hungry children with large appetites, whereas Albumaid-XP is appropriate for picky eaters. During periods when appetites change, but protein needs remain constant, a high protein product (Albumaid-XP) and a high carbohydrate-fat product (Lofenalac) can be invaluable in maintaining an adequate regimen for the treatment of PKU (85).

Additional products. Several other special food products low in phenylalanine are used in the dietary management of individuals with PKU. Some of those products and their composition of nutrients are listed in Table 5 (84).

Yamashita et al. (86) experimented with an enzymatic process in which a fish protein concentrate and a soybean protein isolate were used to produce a low-phenylalanine, high-tyrosine food for use by PKU patients. Those two plasteins were found to be low in phenylalanine and adequately high in tyrosine and tryptophan. The plasteins are flat in taste because they do not contain any significant amounts of salt, free amino acids or very low molecular-weight peptides. For these reasons these fish and soybean plasteins can serve as acceptable dietetic food material for patients with PKU.

TABLE 5

Content of critical nutrients in some special products used in the diet of PKU individuals (84).

Product	Carbohydrates	Fat	Protein	Phenylalanine	Calcium	Phosphorus	Sodium	Potassium	Cal/100 g
	g/100 g				mg/100 g				
Aprotein									
Pasta	89.3	0.1	0.5	12	0.65	12	20	10	340
Rusk	86.2	8.5	1.0		7.0	51	30	40	420
Paygel									
Wheat starch	87.5	1.0	0.3	20	7.0	6	65	12	370
Baking mix	83.1	7.5	0.3	15	5.0	48	55	10	410
CAL power beverage									
Lemon	49.5	0	0	0	0.1	0	2.7	1.0	182
Grape	49.5	0	0	0	0.2	0	10.8		182
Prono gelled dessert									
Orange	97.6	0	0.06	1	170	0	21.0	394	390
Strawberry	97.6	0	0.05	1	165	0	32.0	647	390

TERMINATION OF DIETARY TREATMENT

Although there is a general agreement concerning the value of dietary treatment of children with PKU, there is little agreement pertaining to the optimal time, if any, for discontinuing the dietary treatment (67, 87-89). It has been estimated that by the age of 5 years the brain has developed to 90% of its adult size (90). By 10 years, 95% of the brain is fully developed, and by the age of 14 brain growth probably ceases. Since the developing brain appears to be most vulnerable to toxic effects of phenylalanine, those ages seem worthy of consideration for discontinuing treatment. Concerns for the discontinuation of diet therapy (89) have included possible adverse behavioral changes, decreases in functional intellectual capacity, lessening of general well-being, and subsequent pregnancies of phenylketonuric women, resulting in mentally retarded children. Arguments in support of discontinuation of the diet by 4 to 6 years of age (67,91-93) include such things as minimal central nervous system growth and maturation beyond that age, completion of myelination of neurons, and no evidence of deterioration of intellectual abilities after the diet has been discontinued.

Effects on intelligence. It is not known whether maintenance of low serum phenylalanine levels beyond 4 to 6 years of age in children with PKU is necessary to assure

continued and normal development (91). Holtzman et al. (91) observed no consistant difference in IQ and physical and neurologic status between a group of 4 year olds who had their low-phenylalanine diet terminated and those who remained on the phenylalanine restricted diet. The Solomons et al. (92) reported that 7 PKU children who had diets terminated from 6 to 9 years of age, showed no discontinuities of intellectual development in a 5 to 9 month postdiet period. Beckner et al. (89) collected data from 12 patients between 4 years 6 months, and 16 years 6 months, who were placed on a moderately controlled protein diet. Observations 2 years later of those PKU patients indicated no trend or significant change in IQ. Between the first and last comparable test, 4 subjects showed increases in IQ, 5 showed decreases, and 3 stayed essentially the same. Intellectual development of 26 PKU patients (75) (treated within the first 3 weeks of life) was observed when dietary treatment was discontinued. In 11 patients followed for 6 years and 15 followed for 2 to $3\frac{1}{2}$ years, deterioration was not observed. Those studies indicated that discontinuation of treatment in the individual child did not produce a drop in intelligence score.

Effects on behavior. The major reasons for variations of opinion concerning the duration of dietary treatment are the uncertainties of the consequences of early discontinuation and the expectation that discontinuation of treatment will have an adverse effect on the behavior of the PKU child

(87). Proponents for discontinuation of the phenylalanine-restricted diet in all children entering school point to the difficulties in maintaining an older child on this diet, to the limitations of "normal" socialization and peer relationships and to the secondary emotional consequences (67,94). Observations by Moen et al. (95) indicated that children with PKU have lower self-esteem for several "diet management" related reasons. The necessity of adhering to a special diet may contribute to lowered self-esteem. Out of 23 families, one-third reported family tension centered around the child with PKU and aggravated problems of parental feelings and attitudes attributable to the special diet. One study (88) indicated that PKU children on low-phenylalanine diets exhibited poor concentration and attention span and tended to be hyperactive, excitable and anxious. With the elimination of dietary restriction and the introduction of normal foods, the PKU children showed increased emotional maturity, independence and self-confidence. Studies (67,89,92, also indicated that discontinuation of dietary treatment resulted in relaxation of general family tension and meal planning because there was no longer conflict about the PKU child not being allowed foods the rest of the family ate. Early termination of the diet appeared to improve general behavior and school performance.

Psychological aspects. Although the question of when to terminate the phenylalanine-restricted diet is important,

other related issues such as the social and emotional impact on the child and the parents and their interrelationships during the dietary change, are of equal significance (67). A study of 23 families with classical PKU children, who were started on the diet within the first 2 weeks of life and who were terminated at the age of 5 was undertaken in order to study emotional responses and social behaviors after diet discontinuation. Ninty-one percent of the parents indicated that meal time became less stressful because they did not have to watch the child's food intake as closely. Parents (70%) reported that their children asked for more food, that total food intake increased significantly and monitoring of weight gain became necessary. Some children (70%) hesitated to try new foods, such as meat, eggs, and milk products. None of the children were enthusiastic about new foods. Two-thirds of the parents reported that their children appeared to miss the "old" diet and to prefer it because it was "special". The children appeared confused as to why they were no longer on their special diet and why previously forbidden foods could now be eaten. The study indicated that both parents and children need more preparatory discussion prior to diet discontinuation. Troubled relationships and stress situations could be prevented by on-going counseling. Increased support for the parents and age-appropriate educational efforts directed towards the child with PKU could reduce undue anxieties and avoid unnecessary conflicts.

MATERNAL PHENYLKETONURIA AND PREGNANCY

Only after successful treatment and prevention of mental retardation, did investigators become concerned about pregnancies in the phenylketonuric woman. In the past, women with untreated PKU infrequently became pregnant since they rarely had the capacity for marriage or for the bearing of children (98). However, the screening of infants for PKU in the late 50's produced a substantial change due to the fact that early treatment of phenylketonuria with a low-phenylalanine diet produced mentally normal women with the desire to bear children. The problems related to the effects of high maternal blood phenylalanine on the developing fetus are now of great concern.

Mental retardation and abnormalities in non-PKU offspring. It has been suggested that an increased phenylalanine concentration in the mother with phenylketonuria may interfere with normal central nervous system development of the fetus (84). A review of the literature reveals that offspring born to PKU mothers are often mentally retarded and many have congenital abnormalities.

In 1963 Denniston (99) reported on a mentally retarded institutionalized female with PKU. Upon examination of 5 of her offspring, all were reported as mentally retarded although none had been reported to have PKU. Mabry and associates (100,101) also reported on phenylketonuric

mothers, all of whose living children available for examination were retarded, but without PKU. Frankenburg et al. (102) described 8 non-PKU children born to 3 PKU mothers. All the children were mentally retarded with IQ's lower than those of the mothers. Brown and Waisman (103) observed a hyperphenylalaninemic female with plasma phenylalanine levels averaging 16 mg/100 ml who produced 4 educatable retarded offspring. Growth was not retarded in 3 out of the 4 children, although each child did have some sort of minor abnormality. In 1967, Stevenson and Huntley (104) reported on 2 phenylketonuric sisters on a non-restricted diet who had had 16 spontaneous abortions, 6 children died in infancy and only 4 children survived. All the infants of term gestation exhibited intrauterine growth retardation and microcephaly at birth. All live births to those females had varying combinations of premature birth weight, microcephaly, cardiac defects, mental and physical retardation, dislocated hips and strabismus. Each of those offspring were reported to be non-PKU. Later, Huntley and Stevenson (105) reported additional pregnancies in each of those 2 sisters. Both babies, one of whom died, exhibited microcephaly and heart disease. Those babies also had phenylalanine concentrations in the umbilical cord blood higher than the corresponding concentrations in the maternal blood. The concentration of phenylalanine in the blood of the infant who survived returned to normal promptly, which is typical of the nonphenylketonuric child. Kerr et al. (106) reported that administration of excess

L-phenylalanine to pregnant monkeys induced artificial hyperphenylalaninemia. Monkeys supplemented with phenylalanine throughout pregnancy delivered infant monkeys with low birth weights. Concentrations of phenylalanine and tyrosine in the umbilical cord were greater than corresponding concentrations in the maternal blood in 8 out of 9 animals. The infant monkeys demonstrated a significant reduction in learning abilities. The observation that phenylalanine concentrations in blood from the umbilical cord was greater than corresponding concentrations in the mother was in accord with the findings of Waisman (107), who reported that phenylalanine was transported actively across the placenta and concentration was twice as high in umbilical cord blood as in maternal blood. Evidence indicates that the high concentrations of phenylalanine or its metabolites in the mother with PKU contributes to the mental retardation often noted in offspring.

Normal development in offspring of PKU mothers. Occasionally, a child born to an untreated phenylketonuric mother develops normally. Goldstein et al. (108) reported on an untreated PKU Negro woman, with average intelligence, IQ 85, who gave birth to an apparently normal child. Although the mother's blood phenylalanine level was elevated to 23 mg/100 ml, that of the infant was normal. The infant experienced transient hyperphenylalaninemia until 6 months of age, but now has phenylalanine levels ranging between 2

to 4 mg/100 ml. Neurologic evaluation demonstrated no abnormalities of cranial nerve functions, postural reflexes, or deep tendon reflexes. Although the child registered performance within normal limits at 3 months, further testing at an older age will be necessary to determine future development of cognitive skills.

Pregnancy screening for increased phenylalanine levels. Since damage to the developing brain may occur before birth, preventative therapy must be instituted during gestation in phenylketonuric women (98). Since treatment of PKU girls is often stopped or relaxed at various prepubertal ages, pregnancy may occur in apparently normal girls who have high phenylalanine levels or PKU (109). It has been suggested that screening for increased phenylalanine levels be done in antenatal clinics to detect women with untreated PKU (109, 110). A routine phenistix urine test given in one clinic revealed an undiagnosed case of PKU in a pregnant woman. The woman was placed on a synthetic diet containing 30 mg/kg/day of phenylalanine to keep blood levels between 2 and 6 mg/100 ml. The mother gave birth to a baby girl with a low birth weight (1,910 gm) at 38 weeks gestation. Development was reported as normal although further testing will need to be done to check for normal intelligence. The low birth weight of the infant implied that restriction of the mother's diet might have led to fetal malnutrition, although low birth weight is also common in births of untreated PKU mothers.

Hanley et al. (111) reported that it may not be necessary to maintain concentrations of blood phenylalanine as low as 2 to 4 mg/100 ml in PKU mothers during pregnancy. They maintained that a concentration below 10 mg/100 ml is probably safe for normal fetal development. That level was based on evidence that maintenance of concentrations of phenylalanine in PKU children above 4 mg/100 ml but below 10 mg/100 ml was safe, while a concentration below the normal of 2 to 3 mg/100 ml was hazardous. A concentration above normal but below 8 mg/100 ml is a logical range of safety for a developing fetus. Findings of Farguhar (109) supported the assumption that mental retardation in the offspring can be prevented if PKU mothers are maintained on low phenylalanine diets during pregnancy. He observed an untreated mentally retarded PKU mother who was maintained on a strictly controlled, low phenylalanine diet beginning at the 20th week of gestation. Fetal development was reported to be normal and without threat of miscarriage or malformation. The child was shown not to have PKU or high phenylalanine levels and made normal progress during the first year of postnatal life without treatment. Because of the child's healthy development, it was suggested that during the first half of pregnancy the fetal brain is relatively unharmed by hyperphenylalaninemia or PKU. It is encouraging to find a case where institution of the diet delayed until mid-pregnancy prevented obvious harmful effects to the baby, since introduction or reintroduction of the diet to PKU

pregnant patients in the first trimester is very nauseating.

Dietary management of pregnant PKU women. Consumption of low-phenylalanine diets by PKU mothers during pregnancy has been shown to prevent development of mentally retarded offspring (108-110). Although reports are encouraging they do not prove that such treatment will always result in intellectually normal children (84). There is also no conclusive evidence that congenital abnormalities are prevented by initiation of the dietary therapy during the second half of pregnancy. Because of reports of the importance of maintaining low phenylalanine levels during gestation in pregnant women, nutritionally adequate diets low in phenylalanine should be prescribed for PKU pregnant women. As with any pregnancy, nutritional adequacy of the diet is important. The prescribed diet should meet the RDA for nutrients during pregnancy. Although the phenylalanine content of the diet may vary depending on several determinants, a daily phenylalanine intake of approximately 15 mg/kg can be used to calculate a hypothetical PKU diet during pregnancy. For an average woman (height 162 cm, weight 58 kg) the estimated daily phenylalanine intake would be 870 mg. Table 6 (84) shows the recommended dietary allowances for nutrients during pregnancy in comparison with diets using Lofenalac and Product 3229. In addition to the amino acid mixture, natural foods low in phenylalanine, such as certain vegetables and carbohydrates, are needed to supplement the

TABLE 6

Comparison of the Recommended Dietary Allowances in Pregnancy with Equivalent Nutrients in Hypothetical Diets using Lofenalac or Product 3229 (84).

				Fat-soluble Vitamins (IU)			Water-soluble vitamins							Minerals					
	Cal (avg)	Protein (g)	Phenyl-alanine (mg)	A	D	E	C (mg)	Folic acid (μg)	Niacin (mg)	B_1 (mg)	B_2 (mg)	B_6 (mg)	B_{12} (μg)	Calcium (mg)	Phos-phorus (mg)	Iodine (μg)	Iron (mg)	Mag-nes-ium (mg)	Zinc (mg)
Recommended Dietary Allowances in pregnancy	2,350[a]	76		5,000	400	15	60	800	15.5[a]	1.35[a]	1.6[a]	2.5	4.0	1,200	1,200	125	18	450	20
Lofenalac 450 g[a] (and 34 equivalents)[b]	2,058	67.2	360	4,500	1,200	15	150	150[c]	12	1.4	5.4	1.5	13.5	2,700	2,100	195	36	30	9
Product 3229 335 g[a] (and 58 equivalents)[b]	1,360	68.0	0	6,800	1,350	34	177	170[d]	27	2.0	3.4	1.7	8.5	2,120	1,700	221	40	680	14

[a] Nutrient analyses are for the specified amounts of Lofenalac and Product 3229. [b] Nutrients provided by natural food equivalents are in addition to those of the respective formulas. [c] Approximately 85 μg of folic acid are additionally provided in 34 equivalents. [d] Approximately 125 μg of folic acid are additionally provided in 58 equivalents. (It is of note that "pure forms" of folacin may be effective in doses less than one-fourth of the Recommended Dietary Allowances.

PKU diet (Table 7). The apparent advantage of Product 3229 over Lofenalac is demonstrated by contrasting sample diets (Table 8 and 9); a total of 58 equivalents of natural foods can be offered to women taking Product 3229, but only 34 equivalents are allowed with Lofenalac. Because large quantities of Lofenalac are needed to provide adequate protein intake and because relatively large quantities of Lofenalac together with the natural foods would increase caloric intake and lead to excessive weight gain Product 3229 appears to be the more suitable preparation in dietary management of pregnant PKU females.

Because of the difficulties in reintroducing amino acid preparations during pregnancy, some investigators have recommended continuing PKU females on a phenylalanine restricted diet during adolescence and childbearing years. If the diet was discontinued during the school years, the phenylalanine-restricted diet should be initiated preferably several months before conception because at that time such a diet would probably be better tolerated than if it were introduced in the beginning of pregnancy.

Other factors related to nutrition during pregnancy include: excessive energy expenditure, increased protein synthesis, altered fluid and electrolyte metabolism and other food related aspects such as nausea, vomiting, food craving, and weight gain. Furthermore, social, cultural, religious, and psychological influences need to be taken into account in the planning of a diet which is not only appropriate in

TABLE 7

Sample equivalents for natural foods included in PKU diets (84).

Food	1 Equivalent[1]
Vegetables	
Carrots	1/3 cup
Celery	2 small inner stalks
Cucumber	1/2 medium (pared)
Escarole	1 lg. or 9 sm. leaves
Green beans	1/3 cup
lettuce	2 large leaves
Tomato	1/2 small
Fruits	
Applesauce	2/3 cup
Banana	1/3 small
Peaches, canned	2 medium halves
Pears, canned	4 small halves
Pineapple, fresh, diced	3/4 cup
Strawberries, fresh	1/2 cup
Fruit Juices	
Apple juice	1 1/2 cups
Orange juice, frozen, diluted	3 tablespoons
Cereals-Ready to Eat	
General Mills, Cocoa Puffs	3 tablespoons
Quaker, Puffed Rice	6 tablespoons
Kelloggs, Rice Krispies	3 tablespoons
Nabisco, Wheat Honeys	2 tablespoons
General Foods, Alpha-Bits-Oat	2 tablespoons
Cooked Cereals	
Cream of wheat, cooked	1 tablespoon
Oatmeal, cooked	1 tablespoon
Cream of rice, cooked	2 tablespoons
Crackers and Cookies	
Sunshine-Applesauce Cookie	1 cookie
Nabisco, Premium Saltine Cracker	1 cracker
General Mills, Daisy's	9 daisy's
Popcorn (popped)	1/4 cup
Fats	
Mayonnaise	2 tablespoons
French dressing	4 tablespoons

[1]One food equivalent contains approximately 15 mg of phenylalanine.

TABLE 8

Sample menu for pregnant PKU woman using Product 3229 (84).

Meal	Menu	Equivalents	Phenylalanine (mg)	Protein (g)	Calories
Breakfast					
Fruit or juice	1/3 banana	1	15	0.36	28
Cereal	3/4 cup corn flakes	4	60	1.6	71
Coffee lightener	3 oz. Coffee Rich Liquid	1	15	0.3	138
Toast	1 slice toast	7	105	2.0	62
Butter or margarine	Butter, 1 teaspoon				36
Coffee	Coffee, 1 cup	1	15	0.3	5
		14	210	4.56	340
Product 3229				25.00	500
Lunch					
Soup	1/2 cup tomato soup	2	30	0.8	44
Sandwich	2 slices bread	14	210	4.0	124
	2 tablespoons tuna fish	14	210	5.76	40
	1 teaspoon mayonnaise			0.05	34
	1 stalk chopped celery	1/2	7.5	0.2	3
	2 canned pear halves	1/2	7.5	0.2	76
	Lettuce, 2 large leaves	1	15	0.45	7
		32	480	11.46	328
Product 3229				18.00	360
Dinner					
Hot vegetable plate	1 potato	6	90	2.1	76
	1/3 cup carrots	1	15	0.45	15
	1/3 cup green beans	1	15	0.6	10
	Butter, 1 teaspoon				36
Salad with French Dressing	Lettuce, 2 large leaves	1	15	0.45	7
	Escarole, 9 leaves	1	15	0.4	5
	Cucumber, 1/2 medium	1/2	7.5	0.3	7
	Tomato, 1/2 small	1	15	0.55	11
	French dressing, 1 Tbsp.			0.08	57
Fruit	Applesauce, 1/3 cup	1/2	7.5	0.02	91
		12	180	5.13	315
Product 3229				25	500
Total Diet		58	870	21.15	983
Total Product 3229				68	1360
Total		58	870	89.15	2343

TABLE 9

Sample menu for pregnant PKU woman using Lofenalac (84).

Meal	Meal	Equivalents	Phenylalanine (mg)	Protein (g)	Calories
Breakfast					
Fruit or juice	1/3 banana	1	15	0.36	28
Cereal	3/4 cup corn flakes	4	60	1.6	71
Coffee lightener	3 oz. Coffee Rich Liquid	1	15	0.3	138
Toast	1 slice toast	7	105	2.0	62
Butter or margarine	Butter, 1 teaspoon				36
Coffee	Coffee, 1 cup	1	15	0.3	5
		14	210	4.56	340
Lofenalac		8	120	22.4	686
Lunch					
Fruit plate with dressing	2 peach halves	1	15	0.4	78
	2 pear halves	1/2	7.5	0.2	76
	3/4 cup fresh pineapple	1	15	0.4	52
	1/2 cup strawberries	1	15	0.5	28
	1 tablespoon mayonnaise	1/2	7.5	0.15	101
Rusk	1 slice low-protein rusk			0.1	42
Dessert	1/2 cup orange sherbert	3	45	0.7	144
	2 vanilla wafers	1	15	0.28	36
		8	120	2.73	557
Lofenalac		8	120	22.4	686
Dinner					
Hot vegetable plate	1 potato	6	90	2.1	76
	1/3 cup carrots	1	15	0.45	15
	1/3 cup green beans	1	15	0.6	10
	Butter, 1 teaspoon				36
Salad with French dressing	Lettuce, 2 large leaves	1	15	0.45	7
	Escarole, 9 leaves	1	15	0.4	5
	Cucumber, 1/2 medium	1/2	7.5	0.3	7
	Tomato, 1/2 small	1	15	0.55	11
	French dressing, 1 Tbsp.			0.08	57
Fruit	Applesauce, 1/3 cup	1/2	7.5	0.2	91
		12	180	5.13	315
Lofenalac		8	120	22.4	686
Total diet		34	510	12.42	1212
Total Lofenalac		24	360	67.20	2058
Total		58	870	79.62	3270

its quantitive and qualitive components but also accepted and well tolerated. Those and other concerns call for an individual approach in the design of a diet for the pregnant phenylketonuric woman.

THE PKU COLLABORATIVE RESEARCH STUDY

Current investigations of phenylketonuria include the longitudinal study which was instituted in October of 1967 to test the effect of dietary restriction of phenylalanine in the treatment of PKU (112). Support for the study came from the Bureau of Community Health Services, Health Services Administration, U. S. Department of Health, Education and Welfare. The investigation began in 19 medical centers but at present is being conducted in 15 centers, located in 11 states (four clinics withdrew from the study subsequent to its initiation).

Plans for the study began to emerge in 1965, subsequent to the development of a reliable and inexpensive screening procedure for PKU. Within a short time, 40 states had passed laws requiring routine blood screening of newborns. These events enhanced prospects that a collaborative study would provide a sample sufficiently large and representative to permit the research findings to have general application in the treatment of PKU. The study was designed to evaluate the consequences of low-phenylalanine dietary therapy on cognitive, physical, and psychosocial development of children with PKU (73,112). The initial sample of 216 children presumed positive for PKU were assigned randomly to one of two test diets. Treatment group 1 consisted of patients whose serum phenylalanine level was targeted for control between 1.0 and 5.4 mg/100 ml. Treatment group 2 consisted

of patients with PKU whose serum phenylalanine level was targeted between 5.5 and 9.9 mg/100 ml. The control groups consisted of siblings with PKU who were untreated previously (control group 1) and siblings without PKU (control group 2). Despite attempts to maintain the PKU child in a designated group, clinic and parents were unable to maintain patients' serum phenylalanine in their designated "zones". Differences in the phenylalanine levels of the two treated groups were minimal throughout the study period and therefore the mean IQ performance at age 4 did not differ. It was observed (73) that the mean IQ of the first 111 4 year olds tested was within the normal range despite being below the general population of nonaffected children. Definitive conclusions regarding intellectual performance must await observations of school achievement and subsequent assessment of visual-perceptual development, linguistic skills, and psychosocial adjustment. The PKU collaborative project will continue to accumulate additional data and will focus on the issue of diet discontinuation.

SUMMARY

Phenylketonuria (PKU) is an inborn error of metabolism resulting in mental retardation when untreated. The patient with "classical" PKU lacks the liver enzyme, phenylalanine hydroxylase, and therefore is unable to convert the essential amino acid, phenylalanine, to tyrosine. This results in the accumulation of phenylalanine and its metabolites in the blood. An early symptom of the disease is dermatitis, such as atopic eczema and seborrheic dermatitis. Pigmentation of the child is generally lighter than normal due to altered conversion of tyrosine to the pigment, melanin. The child may also exhibit abnormal electroencephalograms, behavior, and body movements. Many theories, such as impaired transport of essential amino acids into the brain, glutamine deficiency and abnormal synthesis of structural proteolipids, have been suggested in an attempt to explain the mental defect.

Evidence indicates that variant forms of PKU may exist in which other components of the phenylalanine hydroxylating system besides phenylalanine hydroxylase are missing. These variant forms may result in neurological deterioration despite strict dietary controls of blood phenylalanine levels. Screening programs have been designed to detect the various forms of hyperphenylalaninemia, so that diagnosis and institution of treatment can be carried out within the first 4 weeks of life. The preferred PKU screening methods

currently in use include the Guthrie bacterial inhibition assay, the McCaman and Robins fluorometric assay, and chromatographic procedures. Despite large scale screening programs, it has been observed that 5 to 10% of infants ultimately proven to have PKU are not detected by screening. Early testing before the fourth day of life and low protein formulas may prevent detection. Those infants who are detected by screening are started on low-phenylanine diets in order to prevent the severe mental retardation associated with untreated PKU.

The nutritional requirements for children with PKU can be met by providing a semisynthetic diet derived either from a modified protein hydrolysate or from a mixture of L-amino acids, so that the diet contains an extremely low amount of phenylalanine or is free of it. The most popular dietary formula currently in use to treat PKU is Lofenalac. Lofenalac is an enzymatic hydrolysate of casein, containing carbohydrate, fat, minerals, and vitamins, which can be reconstituted with water to make a milk substitute. Product 3229 is a recently developed product designed to feed the older PKU child. It consists of a mixture of L-amino acids, supplemented with vitamins, minerals, fat and carbohydrate. Albumaid-XP is a phenylalanine free product made from a hydrolysate of beef serum. Early dietary management using those and other food products has been shown to prevent severe mental impairment of the PKU child.

The question of when to discontinue dietary treatment

of children with PKU is still controversial. Concerns about discontinuing diet therapy have included possible adverse behavioral changes, lessening of general well being, and subsequent pregnancies of phenylketonuric women who give birth to mentally retarded children. Evidence indicates that dietary restriction of phenylalanine in pregnant PKU women can prevent abnormalities and mental retardation of offspring. Arguments in support of discontinuing the diet at 4 to 6 years of age suggest no impairment of intelligence after diet discontinuation and general improvement of behavior and family relations due to elimination of dietary restriction. More knowledge, concerning treatment and management of phenylketonuria, will be gained with the continuation of the Collaborative Study of Children Treated for Phenylketonuria.

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PHENYLKETONURIA
AN INBORN ERROR OF METABOLISM

by

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Phenylketonuria (PKU) is an inborn error of metabolism resulting in mental retardation when untreated. The patient with "classical" PKU lacks the liver enzyme, phenylalanine hydroxylase, and therefore is unable to convert the essential amino acid, phenylalanine, to tyrosine. This results in the accumulation of phenylalanine and its metabolites in the blood. An early symptom of the disease is dermatitis, such as atopic eczema and seborrheic dermatitis. Pigmentation of the child is generally lighter than normal due to altered conversion of tyrosine to the pigment, melanin. The child may also exhibit abnormal electroencephalograms, behavior, and body movements.

Evidence indicates that variant forms of PKU may exist in which other components of the phenylalanine hydroxylating system besides phenylalanine hydroxylase are missing. Screening programs have been designed to detect the various forms of hyperphenylalaninemia, so that diagnosis and institution of treatment can be carried out within the first 4 weeks of life. The preferred PKU screening methods currently in use include the Guthrie bacterial inhibition assay, the McCaman and Robin fluorometric assay, and chromatographic procedures. Despite large scale screening programs, it has been observed that 5 to 10% of infants ultimately proven to have PKU are not detected by screening. Those infants who are detected b screening are started on low-phenylalanine diets in or to prevent the severe mental retardation associated w untreated PKU.

The nutritional requirements for children with PKU can be met by providing a semisynthetic diet derived either from a modified protein hydrolysate or from a mixture of L-amino acids, so that the diet contains an extremely low amount of phenylalanine or is free of it. The most popular dietary formula currently in use to treat PKU is Lofenalac. Lofenalac is an enzymatic hydrolysate of casein, containing carbohydrate, fat, minerals, and vitamins, which can be reconstituted with water to make a milk substitute. Product 3229 is a recently developed product designed to feed the older PKU child. It consists of a mixture of L-amino acids, supplemented with vitamins, minerals, fat and carbohydrate. Albumaid-XP is a phenylalanine free product made from a hydrolysate of beef serum. Early dietary management using these and other food products has been shown to prevent severe mental impairment of the PKU child.

The question of when to discontinue dietary treatment of children with PKU is still controversial. Concerns about discontinuing diet therapy have included possible adverse behavioral changes, lessening of general well being, and subsequent pregnancies of PKU women who give birth to mentally retarded children. Arguments in support of discontinuing the diet at 4 to 6 years of age suggest no impairment of intelligence after diet discontinuation and general improvement of behavior and family relations due to elimination of dietary restriction. More knowledge, concerning treatment and management of PKU, will be gained with the continuation of the Collaborative Study of Children Treated for Phenylketonuria.