Medical Progress

Phenylketonuria and Its Variations

A Review of Recent Developments

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SENSITIVE METHODS FOR SCREENING newborn infants for elevation of blood phenylalanine have been widely used in the past six years. This screening has caused a revolution in concepts about phenylketonuria (PKU). The discovery of hyperphenylalaninemic conditions which to a variable degree biochemically can mimic true PKU has demanded increasing knowledge of the pathophysiologic features of PKU and has necessitated changing methods of diagnosis and management. An enormous expenditure in research time and effort has produced much data, not all of it significant. This article is intended to review some of these facts and concept changes and to put them in historical context.

Historical

The concept of PKU was static until screening programs based on the method of Guthrie and Susi[1] for detection of elevated serum phenylalanine levels came into use. As recently as the early 1960's, it was generally believed that any prolonged elevation in serum phenylalanine level (hyperphenylalaninemia) invariably led to mental retardation. Historically, this belief was not unreasonable, but it reflected the bias that results when screening programs focus on special or selected populations. The clinical and biochemical features of PKU were first defined in severely retarded children.[2]

The first biochemical aberration found in these children was the urinary excretion of phenylpyruvic acid (PPYA).[2] This substance caused their urine to turn green when a 10 percent ferric chloride solution was added. When Fölling[2] screened the urine of institutionalized retarded patients with ferric chloride, he found other subjects with similar biochemical patterns and clinical courses. Fölling demonstrated that these patients had greatly elevated serum phenylalanine levels and defects in phenylalanine metabolism. His findings were later substantiated by Jervis,[3] whose studies suggested greatly reduced activity of the hydroxylation enzyme which converts phenylalanine to tyrosine. An elevated serum phenylalanine level, urinary excretion of phenylalanine metabolites, especially PPYA, and retardation became synonymous with PKU in the minds of most clinicians.

Armstrong et al[4] showed that often a considerable time after birth had to pass before PPYA could be detected in the urine of newborn PKU patients, and that its detection was unlikely if the

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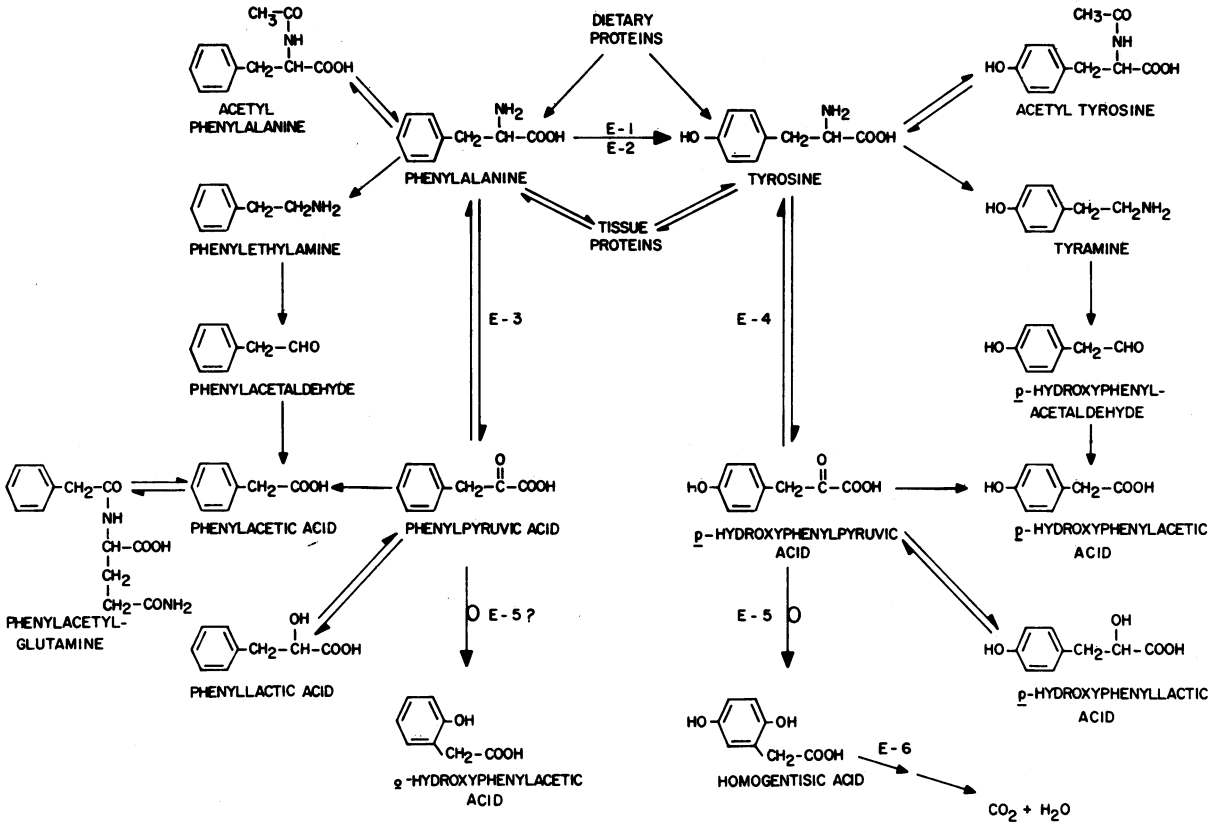

Chart 1.—Some pathways of phenylalanine and tyrosine metabolism. Enzymes: *E-1*=Phenylalanine hydroxylase: defective in classical PKU; *E-2*=Dihydropteridine reductase: normal in classical PKU; *E-3*=Phenylalanine-alanine aminotransferase: defective in one variant of hyperphenylalaninemia; *E-4*=Tyrosine-glutamic aminotransferase; *E-5*=*p*-Hydroxyphenylpyruvic oxidase: inhibited in transient tyrosinemia; defective in tyrosinosis; *E-6*=Homogentisic oxidase: defective in alcaptonuria. (From Koch R, Shaw KNF, Acosta PB, et al: An approach to management of phenylketonuria. J Pediat 76:815-828, 1970. Reproduced with permission.)

serum phenylalanine level was less than 15 mg per 100 ml. Obviously, patients screened too early or those with only minor elevations in serum phenylalanine would be missed by urinary screening.[5] If PPYA were present in urine at low concentrations and not tested for promptly, it also could be missed, because it is unstable and easily oxidized.[6]

Early screening programs were based upon ferric chloride testing for PPYA in urine. Hence in clinically normal persons who were not excreting PPYA, moderate elevations of serum phenylalanine levels went undetected. Conceptually, phenylpyruvic acid, therefore, was linked with increased serum phenylalanine in one direction and mental retardation in another. The illogical conclusion that increased serum phenylalanine inevitably results in mental retardation was perpetuated as long as urine testing was the sole method for mass screening.

Some early investigators[7,8,9,10] were aware, however, that elevated serum phenylalanine levels were not always associated with retardation. Older "atypical" PKU or intellectually normal children who manifested the biochemical abnormalities of PKU were detected when families were screened after identification of a PKU infant. Knox[11] reported only 30 known "high grade" PKU patients up to 1963, with IQ's between 60 and 107. The last ten patients were recognized from 1960 to 1963 after some centers had begun screening for increases in blood phenylalanine.

A positive motivating force for newborn screening was the report by Bickel et al[12] that phenylalanine restriction was associated with improved developmental and behavioral performance in a phenylketonuric child. They suggested that retardation might be prevented if dietary treatment were started in infancy. This report provided an incentive to develop a more reliable screening

method than the ferric chloride test. The Guthrie bacterial inhibition assay[13] accomplished this by measuring serum phenylalanine directly.

Shortly after the inception of blood screening programs, it became evident that there was a need to re-define the criteria for diagnosis of PKU. This was because of the method of screening and the age and population sampled. Most patients identified by ferric chloride testing before the Guthrie method of screening was used were older and retarded. In contrast, Guthrie screening focused on clinically normal newborns. An unexpected consequence of the screening programs was that many more infants were identified with elevated serum phenylalanine levels than would have been predicted on the basis of previous statistical data from retarded populations. It was difficult to reconcile these differences at the time. With further screening it was evident that some hyperphenylalaninemic infants and children without concomitant retardation (variants) accounted for a portion of the discrepancies.[14] A minor upheaval in the concepts about PKU was caused by the discovery of relatively large numbers of these variants. A similar sequence of events has occurred with other amino acid disorders. Biochemical abnormalities in homocystinuria, cystathioninuria and histidinemia were first detected in patients who were retarded or appeared retarded; but more extensive screening revealed that persons with obviously normal intelligence possessed the same biochemical aberrations.[15,16,17] With time, increasing numbers of hyperphenylalaninemic but clinically normal infants and children are being recognized who have intermittently positive ferric chloride tests and variably increased excretions of the "characteristic" urinary metabolites of PKU; PPYA and ortho-hydroxyphenylacetic acid (*o*-HPAA). These variant patients, who at times biochemically simulate PKU patients, are an enigma and the cause of great confusion. Variants have made the definition of PKU arbitrary to clinicians and other investigators. Guidelines for the treatment of hyperphenylalaninemia are now based upon diagnostic criteria for PKU which are reasonably reliable although not absolute. (See section on treatment and Table 1.) At present patients with persistently elevated serum phenylalanine levels (hyperphenylalaninemia) can be divided into two groups: PKU and variants. We define as phenylketonuric, patients with serum phenylalanine levels persistently greater than 25 mg per 100 ml and with the expected urinary metabolites of PKU while ingesting an ordinary diet. All other patients with abnormal elevations of blood phenylalanine but without elevated blood tyrosine are classified as variants.

Screening

Following development of the Guthrie test, screening of newborn infants on a large scale spread throughout this country, Europe and other areas of the world. Laws requiring screening were enacted in the mid 1960's in most states in the United States, and in most states not having mandatory screening, widespread voluntary screening programs were organized.

The incidence of PKU in 1963, based on data from retarded populations, was estimated by Knox[18] to be between 1:20,000 and 1:25,000 among patients from mixed European populations. Results of mass screening of newborns in Massachusetts between 1959 and 1963 by the Guthrie method suggested that the incidence of PKU was 1:7933.[19] The incidence of hyperphenylalaninemia from the same center based on later newborn screening data was approximately 1:10,000 live births.[20] The incidence figures did not include patients with hyperphenylalaninemia associated with transient tyrosinemia, which has been shown to be the most frequent cause of transient mild elevations in serum phenylalanine.[21]

The incidence figures for PKU reported by screening and treatment centers, which were high shortly after initiation of the newborn blood screening programs, now are decreasing and approaching the figures calculated from the screening of retarded populations. The incidence of persistent hyperphenylalaninemia without tryosinemia, which includes PKU and the variant disorders, has remained relatively constant in the United States at about 1:13,000 live births.[22] The incidence of the variant disorder was estimated by Berman et al[22] in 1969 to be 1:50,000. Hanley et al[23] showed the incidence of variants in Canada was 1:54,000. Subsequently, Hanley[24] suggested that the ratio between variants and true PKU is 1:2. A more recent report from Australia, however, suggests that the ratio is 1:1.[25]

It is difficult to make comparisons of the incidence figures in the United States with figures from other countries. Reports have not always been specific about the diagnostic criteria for PKU. The conditions described may be different and comparisons of frequencies, therefore, may not al-

TABLE 1.—*Course of Untreated Phenylketonuria*

Clinical	*Laboratory*
Birth	
Physical: Normal Neurological: Normal Development: Normal	Serum: ±elevated serum phe. normal serum tyr. Urine: ±elevated phe metabolites EEG: normal
15 days to 3 months	
Physical: Eczema may be present, musty or vinegary odor of PAC Neurological: normal to variable increased irritability or lethargy, seizures ± Development: normal to questionable delay in motor milestones	Serum: Phe. >25 mg % Tyr. −normal or decreased Urine: elevated phe, PPYA, *o*-HPAA +$FeCl_3$, +DNPH EEG: may be abnormal, i.e.: hypsarrhythmia, petit mal variant, multifocal spiking
3 months to 6 months	
Physical: Eczema may be present, odor present, 30 percent microcephaly Neurological: 50 percent with seizures, increased irritability or lethargy Development: delays usually present and becoming more obvious	Serum: Phe. 30-100 mg % Tyr. normal or decreased Urine: elevated phe, PPYA, *o*-HPAA +$FeCl_3$, +DNPH EEG: 90-95 percent with abnormal pattern, hypsarrhythmia, petit mal variant, multifocal spiking
6 months to 1 year	
Physical: Eczema frequently present, odor present, 30 percent mild microcephaly Neurological: 50 percent with seizures, increased irritability, rarely apathy and lethargy Development: obviously delayed motor milestones	Serum: Phe. 30-100 mg % Tyr. normal or decreased Urine: elevated phe, PPYA, *o*-HPAA +$FeCl_3$, +DNPH EEG: 90-95 percent with abnormal pattern, hypsarrhythmia, petit mal variant, slow waves, multifocal spiking
1 year to 3 years	
Physical: Eczema may be present, odor present, skin, hair and iris may be lighter than family, 30 percent mild to moderate microcephaly Neurological: 30-50 percent with seizures, increased tremors and irritability Development: moderate to severe M.R., may have destructive and autistic behavior	Serum: Phe. 30-50 mg % Tyr. normal or decreased Urine: elevated phe, PPYA, *o*-HPAA +$FeCl_3$, +DNPH EEG: 90 percent moderately to severely abnormal hypsarrhythmia, petit mal variant, slow waves, multifocal spiking
3 years to 6 years	
Physical: Eczema uncommon, odor present, skin, hair and iris lighter than family, 30 percent mild to moderate microcephaly Neurological: 30-50 percent with seizures, increased tremors, irritability Development: moderate to severe M.R., may have destructive and autistic behavior	Serum: Phe. usually >20 mg % Tyr. normal Urine: elevated phe, PPYA, *o*-HPAA +$FeCl_3$, +DNPH EEG: 30-50 percent abnormal, petit mal variant, slow waves, multifocal spiking
6 years to adult	
Physical: Eczema uncommon, odor present, skin, hair and iris lighter than family, 30 percent mild to moderate microcephaly Neurological: 10-20 percent with seizures, increased tremors, irritability Development: moderate to severe M.R. may have destructive and autistic behavior	Serum: Phe. usually >20 mg % Tyr. normal Urine: elevated phe, PPYA, *o*-HPAA +$FeCl_3$, +DNPH EEG: 10-20 percent abnormal, petit mal variant, multifocal spiking

Phe.= phenylalanine, Tyr.= tyrosine, PPYA = phenylpyruvic acid, *o*-HPAA = orthohydroxyphenylacetic acid, PAC = phenylacetic acid, $FeCl_3$ = Ferric chloride, M.R. = mental retardation.

ways be valid. Incidence figures reported and geographic variations are indicated in Table 2.

When centers reporting incidence figures are unable to make a firm diagnosis of a variant disorder, natural inclination would be to diagnose PKU, and to treat, because of the poor prognosis in untreated PKU. Patients with serum phenylalanine levels of 10 to 15 mg per 100 ml were reported as having PKU, even without the presence of urinary metabolites characteristic of PKU. Many of these non-PKU patients were treated with restricted phenylalanine diets, and as a result diet-deficiency syndromes and even deaths were reported.[42] Screening programs have been criticized because of this.[43]

The consequences of misdiagnosis cannot be

TABLE 2.—*Incidence Rates of* PKU

Place	*Reference*	*Method*	*Period*	*Number Tested*	*Cases of* PKU*	*Approximate Incidence*
Massachusetts	26	Guthrie	1962–1964	217,752	27	1:8,000
Georgia	27	Guthrie	1/67–6/68	57,494	2	1:29,000
North Carolina	28	Fluorometric	1966–1967	151,734	6	1:25,000
Elkland County, Indiana	29	Phenistix with mail return	1961–1967	13,169	4	1:3,300
New York State	30	Guthrie	1965–1968	958,346	77	1:12,000
California	31, 32, 33, 34	Various newborn blood	1966–1968	620,000	39	1:16,000
Oregon	35	Urine Guthrie	1963–1965 1965–1966	30,000	3	1:10,000
Ontario, Canada	23	Guthrie	1965–1968	392,098	26 (+5 Variants)	1:15,000 (1:13,000 including variants)
Israel	36	Guthrie	—	27,000	3	1:9,000
	37	Guthrie	—	178,174	7 PKU (+9 Variants)	1:25,000 (1:11,000 including variants)
Denmark	38	Phenistix by home visit or physician at approximately 5 weeks	4/67–10/68	320,000	15 (8 diagnosed after 5 weeks—4 were false negatives at 5 weeks)	1:21,000
		Guthrie	9/64–10/68	60,400	4 PKU (+2 Variants)	1:15,000 (1:10,000 including variants)
Ireland	39	Guthrie	2/66–5/67	62,856	14	1:4,500
France	40	Guthrie	—	79,730	9	1:9,000
Belgium	41	Guthrie	1965–1967	22,471	6	1:3,700

*Criteria for diagnosis not necessarily consistent between reports. Some reports of PKU are surely variants. See text.

minimized. In our opinion, however, the positive aspects have justified the screening programs. Screening programs have forced unproven concepts to be re-evaluated and some to be rejected. Increasing numbers of children and some adults who are clinically normal but biochemically abnormal have now been identified. Some infants who do not need dietary restriction have been found, and treatment has been stopped. Another important direct and beneficial consequence of the PKU screening programs has been the very early detection of infants with PKU. The average age of PKU patients at the time of detection has fallen dramatically from eight years to less than six months in one large treatment center.[44] It is commonly believed that the prognosis for intellectual development is best when dietary treatment is started very early in infancy.[45]

From the Collaborative Study for the treatment of PKU, based at Children's Hospital of Los Angeles, have come recent reports of a 2:1 ratio of males to females in the first 90 PKU infants identified by newborn screening.[46,47] In an early report by Jervis[48] of 50 cases of phenylketonuria a ratio of three females to two males was found. This seeming discrepancy has raised the question of whether female PKU infants are being missed by the present blood phenylalanine screening of newborns within the first three days of life. It is possible that a metabolic difference between infant males and

females accounts for a slower neonatal rise in blood phenylalanine levels in the female. The discrepancy may be an artifact of small sample size. Other possible explanations for a discrepancy have been suggested and further study is needed.[49] At present, we recommend that especially for females the PKU blood specimens be taken at the latest possible time before discharge from the newborn nursery. A urine specimen should also be checked with a Phenistix® at the first well baby visit. If developmental delay in any child is observed, a serum phenylalanine determination may be warranted regardless of whether or not phenylalanine was determined previously or what the results.

Variant Disorders

The unexpected finding of relatively large numbers of hyperphenylalaninemic children who are not retarded (variants) has spurred investigators to find a method for distinguishing them from children with PKU. Early studies to identify these variants were based on their ability to excrete or metabolize a standard "load" (amount) of phenylalanine. A four-hour oral tolerance test with L-phenylalanine 100 mg per kg of body weight had been used previously by Hsia et al[50] in genetic studies of parents of PKU patients to determine the heterozygous or carrier state. The disappearance rate of blood phenylalanine following the administration of L-phenylalanine was determined. This four-hour test did not differentiate the variant forms of hyperphenylalaninemia from PKU.[22] However, extending the monitoring period to over 24 hours led to the recognition of at least two distinct patient populations.[51] When phenylalanine was administered in the form of a balanced protein supplement such as whole or evaporated milk, findings were similar.[52] Although most patients could readily be separated into two groups, PKU and variant, a few patients could not immediately be classified solely on the basis of loading studies. There appears to be a spectrum within the group of variant disorders.

Reports indirectly support the concept of at least two hyperphenylalaninemic patient populations; however, the evidence is scanty. Shortly after the Massachusetts mass screening of newborns was started, Kennedy[53] reported a series of atypical hyperphenylalaninemic patients whose blood phenylalanine levels were moderately high, but who were mentally normal or only mildly retarded, despite lack of dietary restriction. The ethnic background of this group of patients was largely Italian, in contrast to the northern European background most frequently associated with PKU. Rosenblatt and Scriver[54] reported hyperphenylalaninemia in clinically normal patients of Mediterranean ancestry, and suggested that they represented a genetically distinct disorder from PKU. Another observation which may be pertinent is that PKU is common only to non-Ashkanazi Jews. In the Ashkanazi Jews PKU has not been detected although variants have been identified.[36,37] Woolf[55] suggested that the variant disorders were a manifestation of the third allele for the PKU gene, but Hsia et al[14] concluded that the variant disorder could be explained best by differences in modifier genes. Justice and coworkers[56] reported decreased liver phenylalanine hydroxylase activity in hyperphenylalaninemic patients (variants) and no activity in PKU patients. The latter findings suggest that the variant patients represent a "milder" form of PKU. Bessman,[57] however, has described an "isozyme" with the ability to metabolize phenylalanine. Woolf[58] has confirmed Bessman's findings of two separate protein fractions with phenylalanine hydroxylase activity in rat liver. Kaufman and Fisher[59] have published detailed findings of phenylalanine hydroxylase "isozymes." Kaufman[60] believes the decrease in phenylalanine hydroxylase activity in hyperphenylalaninemia (variants) is genetically determined. From these findings one could speculate that the variants have an isozyme which allows them to metabolize phenylalanine to an appreciable but lesser degree than normal.

A picture of the variant patient is beginning to emerge. Clinically, the majority are entirely normal. They have no distinguishing characteristic features. They do not, as a rule, have seizures, nor do they have abnormal electroencephalographic (EEG) patterns.[61] This is decidedly different from untreated PKU patients, where as many as 75 to 95 percent of infants and children are reported to have abnormal EEG patterns.[62,63] Biochemically, at times, the variants can be mistaken for PKU patients. Their serum phenylalanine levels may climb to as high as 75 mg per 100 ml[56] and often attain levels in the 30 to 40 mg per 100 ml range on an ordinary diet, but generally will not exceed 20 to 25 mg per 100 ml. In most instances the blood phenylalanine levels are less than 20 mg per 100 ml. The levels of urinary metabolites generally

are proportional to the elevation of the serum phenylalanine.[22] Variant patients may excrete large quantities of PPYA; however, it is usually undetectable.[22] Excretion of *o*-HPAA is usually increased only moderately—20 to 40 times normal—on an ordinary diet. However, it also can be present in greatly increased amounts.[64] One of the most striking characteristics of the variant is the fluctuation in the serum phenylalanine levels during sustained high intake of phenylalanine. The oscillations in the serum phenylalanine levels in addition to the responses to various kinds of loading studies suggest that the variants have some adaptive mechanism, possibly an inducible enzyme, or a modified enzyme.[65]

Intellectually, the variant group seems to follow the distribution curve for the normal population. One of the authors (M.E.B.) is personally following 35 variant patients. All are developing in an entirely normal manner with the exception of two patients for whom another specific cause for retardation is known. Biochemically the phenylalanine tolerance tests of these infants and children are comparable to those determined on adult variant patients who also are intellectually normal in every respect.[66]

Mental Retardation in PKU

As a consequence of the detection and recognition of variant patients, clinicians and investigators have been stimulated to look for causes other than elevated levels of phenylalanine to explain retardation in PKU. It is extremely difficult to ignore the experimental and neuropathological findings (see section on neuropathology) which are reported to be associated with high levels of blood phenylalanine. On the other hand, there are reports of patients who have been spared retardation despite very high blood levels of phenylalanine and significantly elevated levels of the urinary metabolites of PKU.[67-69] Perry et al[70] described two siblings with identically elevated blood phenylalanine levels and characteristic urinary metabolites of PKU. One child was intellectually normal, the other profoundly retarded. Perry postulated that the decreased plasma glutamine level in the retarded child may have been the factor responsible for the retardation, since the concentrations of other metabolites and amino acids were comparable. There are reports which are in conflict with this hypothesis,[71] and also reports which document abnormal levels of other plasma amino acids in untreated severely retarded PKU patients.[72,73] The possibility that some factor other than elevated blood phenylalanine is responsible for the retardation has long been considered. Fuller and Schuman[74] indicated that the ultimate intelligence in treated and untreated PKU is trimodal in distribution, which suggests that intelligence in PKU patients may be unrelated to phenylalanine levels. Retarded patients with low phenylalanine levels—less than 20 mg per 100 ml—have been reported.[75] Retardation in such cases could be due to causes other than PKU.

Wooley and Van der Hoeven[76] implied that the mental defect of phenylketonuria might be prevented by administering serotonin precursors. This contention is supported by the finding that blood serotonin levels are normal in variant patients but low in untreated patients with PKU.[77]

Neuropathology

Poser and van Bogaert[78] in 1959, Malamud[79] in his report of eight cases in 1966, and more recently Crome and Pare[80] in their review and case reports of 1969 established the histologically variable and non-specific findings characteristic of PKU. Other investigators[81-84] have confirmed these variable findings, especially those of demyelination or defective development of myelin and the spongy lesions of the white matter as described by Malamud. Usually there is some decrease in brain weight. Most of these reports have emphasized the increasing degrees of myelin defect and spongy changes with increasing age in untreated patients. Evidence has been offered that dietary treatment may prevent these neuropathologic changes.[81,85] The lack of findings in the treated patient reported by Salguero et al[81] may have been because the patient was too young to show neuropathological changes and not because of treatment.

The structural alterations of the brain which increase with advancing age in untreated PKU are evidence of permanent damage. Likewise, with advancing age the mental defect associated with PKU becomes totally irreversible. In our experience other neurological manifestations of the disease, such as irritability, EEG changes and hyperactive behavior, are frequently reversible following dietary treatment even in older persons. Therefore, phenylalanine or one or more of its metabolites appears to have both reversible and irreversible effects.

Alterations in brain lipid metabolism in PKU which worsen with advancing age have been described recently in conjunction with permanent myelin and spongy changes. These lipid changes may be either the cause or the effect of the permanent neuropathologic damage. Analysis of brain tissue by Menkes,[86,87] Gerstl et al[88] and Cumings et al[89] have shown reductions in total lipids, cholesterol, and certain proteolipids as well as alterations in composition and amounts of cerebrosides and sulfatides. Lipid alterations have been variable but in most patients increase with increasing severity of histopathologic changes.

In the brain of a retarded child born of a phenylketonuric mother, Menkes and Aeberhard[90] found alterations in lipids and sulfatides similar to those found in PKU. The findings suggest that elevations of blood phenylalanine or its metabolites are toxic to the brain or interfere with normal brain development.

Neuropathologic studies of human material ordinarily present the late and static stages of PKU. Animal models of PKU have offered a research tool for investigation of the developmental and dynamic aspects of the neuropathologic features of this disease, and of the associated metabolic processes. Unfortunately, an animal model with a block in phenylalanine hydroxylation comparable to the metabolic error seen with human PKU does not exist. In neuropathologic and metabolic studies hyperphenylalaninemia is artificially produced by giving the animal large amounts of phenylalanine in the diet or by injection. The phenylalanine to tyrosine pathway is intact in these animals, and therefore the models not only have an artificially unbalanced hyperphenylalaninemia but also tyrosinemia. All studies have suffered from the fact that some of the changes seen may be reflections of imbalances of amino acids other than phenylalanine. Nevertheless, Chase and O'Brien[91] have duplicated many of the pathologic findings of human PKU in infant rats that were made phenylketonuric experimentally. The brains of these rats were lighter than normal and the lipid and sulfatide contents were decreased. Of special interest in these studies was the finding that the first eight days of life was the most critical for normal brain development. The comparable period in humans occurs during the fetal stage. Winick's[92] observation of the critical fetal period of brain growth partially explains the great risk of brain damage to infants born of phenylketonuric mothers. Experiments have shown that in the developing animal high phenylalanine concentrations and deviations from an optimum ratio of essential amino acids results in impairment of protein synthesis.[93] Appel[94] has shown *in vitro* that phenylalanine can inhibit protein formation. Peterson and McKean[95] using brain homogenates demonstrated the same inhibition. Swaiman et al,[96] Aoki and Siegel,[97] Chase and O'Brien,[91] and Longenecker et al[98] also have shown impaired protein synthesis in developing animals as a consequence of amino acid imbalances.

Decreased brain weight has been a common neuropathologic finding in human PKU. The finding by Chase and O'Brien[91] of decreased amount of dioxyribonucleic acid (DNA) in developing brains of experimental PKU rats indicates impairment of cell division. Since the increase in the number of brain neuronal cells ceases at an early age in both the rat and man, permanent deficiency of cell number is a likely consequence of untreated PKU.

Dermatoglyphics

In 1965 Hirsch[99] reported abnormalities in the palm prints of PKU patients compared with controls. Alter[100] in 1967, in describing the dermatoglyphics of 100 PKU patients and 100 controls, did not confirm Hirsch's findings. He did find decreased frequency of whorls on fingers and an increase in the mean atd angle in affected females compared with control females. In attempting to reconcile the different findings of Hirsch and Alter, the differences in the frequency of palm print abnormalities seem more a difference between their control groups than between their PKU groups.

The questionable validity of Hirsch's controls, and the fact that differences in Alter's studies were limited to females, makes the findings of questionable significance. Certainly dermatoglyphic analysis would be of no assistance in diagnosing or screening for PKU.

Maternal PKU

Variables influencing the outcome in PKU are the concentration of phenylalanine and the age of the child when exposed to it. Subjects with temporary hyperphenylalaninemia and variants with blood levels of phenylalanine below 20 mg per 100 ml do not appear to be at great risk of intellectual deterioration.[22] Patients with PKU may not have

TABLE 3.—*Phenylketonuric Mothers and Their Children*

Report	Date of Report	Mother Age	Mother I.Q.	Status of Children: Not Certain or Unreported	Normal	Retarded Non-PKU	PKU
Jervis (48)	1937	34	49	2			2
Jervis (48)	1937	50	50	1			
Jervis (107)	1939	27	60	1			
Jervis (108)	1954	—	High Grade	2			
Partington (111)	1962	36	102		1		2
Mabry et al (101)	1963	70	49			5	
Mabry et al (101)	1963	77	82	7		1	
Mabry et al (101)	1963	49	27			1	
Richards (124)	1964	70	50			3	
Coffelt (125)	1964	26	96*			2	
Forbes et al (126)	1966		*			1	
Allen (127)	1966	35	110			4	
Fisch et al (117)	1966	32	88			1	
Perry & Tishler (128)	1966	29	90			1	
Mabry et al (106)	1966	35	97			4	
Mabry et al (106)	1966	40	92			4	
Mabry et al (106)	1966	58	52	1		2	
De Menibus et al (129)	1967	—	—				
De Menibus et al (129)	1967	—	—	1		9	1
Colombo (130)	1967	35	90				3
Stevenson & Huntley (118)	1967	33	99	4		1	
Stevenson & Huntley (118)	1967	31	76	3		2	
Williams (120)	1968	—	62			4	
Waisman (110)	1968	—	94		1	3	
Allan & Brown (109)	1968	27	66		1	3	
Frankenburg et al (116)	1968	39	54			2	
Frankenburg et al (116)	1968	29	77			2	
Frankenburg et al (116)	1968	35	74			4	
Yu & O'Halloran (121)	1970	29	Borderline-dull normal			3 (2 PKU variants)	
Yu & O'Halloran (121)	1970	47	Mild-moderately retarded			6	
Totals	29			22	3	68	8

*Same mother.

significant intellectual deterioration even though allowed to have high blood levels after the age of six years. Probably little cerebral damage occurs with high serum phenylalanine levels even after three to four years. As indicated earlier, infancy appears to be a critical period for damage to occur from high phenylalanine levels. We could postulate that a fetus would be at even greater risk from excess phenylalanine exposure. Such an observation was supported by the report of Mabry et al[101] which described seven non-PKU retarded children of three PKU mothers.

The fetus of the PKU mother may be exposed to blood phenylalanine levels higher than those in the blood circulating in the mother. Crumpler et al[102] postulated and then showed that there is an active placental mechanism for maintaining higher fetal than maternal levels of amino acids. The mechanism apparently continues to operate even with considerably elevated, artificially induced, phenylalanine levels in pregnant rhesus monkeys.[103,104] Bessman[105] speculated that these findings are not due to an active placental mechanism, but rather due to an equilibration phenomenon—a rapid drop in the mother's levels and slow placental clearance.

In 1966 Mabry et al[106] reported three additional PKU mothers with 11 children, and summarized the known cases of PKU mothers at that time. In Table 3 Mabry's summary of cases has been brought up to date. The reports of normal children of PKU mothers by Jervis[48,107,108] (listed by Mabry[106]) did not describe the results of any formal psychometric evaluations or descriptions of the children's ability to cope. These cases therefore are listed as of uncertain status. Cases of maternal PKU published subsequent to Mabry's report have been added to give a total of 29 mothers with 101 chil-

dren. Eight of these children had PKU, and 22 are of uncertain status because the reports are incomplete or the child died in early infancy before evaluation could be done. This leaves 71 non-PKU children of PKU mothers, of which at the most three were normal and 68 were affected. The mother reported by Allan and Brown,[109] who gave birth to a normal infant, was treated with a low-phenylalanine diet after the fifth month of pregnancy. The status of two of the reported PKU mothers having the other reported normal children is questionable. In Waisman's[110] case the mother's IQ was borderline—83. The mother in the family reported upon by Partington[111] was not a typical phenylketonuric. Her IQ was 102, and her fasting urine was negative by ferric chloride test and only faintly positive to Phenistix.® Her urine tests were strongly positive following a phenylalanine load; however, this may occur in PKU variants. Fasting phenylalanine blood levels on two occasions were 20.6 and 20.8 mg per 100 ml. Within a population of phenylketonurics such levels are low.[5] The blood phenylalanine level one hour after loading was 37.2 mg per 100 ml on one occasion and 38.0 mg on another. Unfortunately, phenylalanine was not measured beyond four hours at either time. Therefore, a critical determinant of the variant disorder is missing (see earlier section, Variant Disorders).

Reports of retarded non-PKU children of PKU mothers frequently mention microcephaly, muscle hypertonicity, and hyperreflexia. Intrauterine growth retardation also is part of the syndrome.[116-118] In addition to these findings and the intellectual defects, reports of anomalies in some of these children indicate that they may have a higher than normal risk of congenital defects of organ systems other than the central nervous system. These defects include a variety of congenital heart lesions,[109,118-120] strabismus,[118,121] clefts of upper eyelids,[116] lipodermoids of cornea,[116] hemivertebrae,[116] spinal fusion in lumbar region,[117] club foot,[116] atrophic fingernails,[116] congenital cataracts,[109] missing distal phalanx and bilateral incurved fifth fingers,[116] dislocated hips,[118] hydrocele,[118] hypertelorism,[121] epicanthic folds,[109] and mongoloid facies with normal chromosomes.[121]

There is no question that the risk of mental retardation or congenital anomalies in children born of mothers with PKU is extremely high and may even be 100 percent.

In contrast, infants born of variant mothers do not appear to be in great risk of being retarded. The two mothers reported by Woolf et al[112] were, in light of current knowledge, probably variants and not phenylketonuric. Both mothers had phenylalanine levels below 10 mg per 100 ml. The six children of these two mothers were all normal. The mother reported by Onisawa et al[113] was also a variant with blood phenylalanine levels ranging between 6 and 15 mg per 100 ml. She had a PKU child who was not treated with diet until 16 months of age. His severe mental retardation can be attributed to late initiation of dietary treatment. A second child, who had a blood phenylalanine level of 12 mg per 100 ml and a negative ferric chloride test, and therefore is probably a variant, received dietary treatment and is not retarded. A third child, reported as heterozygous for PKU, had normal intelligence. Lund and Ovalbøl[114] also reported a variant mother who had one PKU child and one normal child. No formal psychometric testing of the normal child was reported, but development was normal during the first year of life. The mother had normal intelligence and blood phenylalanine levels ranging between 6 and 12 mg per 100 ml and up to 31 mg per 100 ml four hours after a phenylalanine load. Her urine was negative to ferric chloride except after loading. Among the cases reported by Kang and Paine[115] was that of a mother with persistent hyperphenylalaninemia who appeared to be a variant. Although psychological test results were not reported, the child was said to be normal. The mother reported by Partington[111] also may have been a variant, and she also had a normal child.

We conclude from the reports of these few mothers with phenylalanine levels ranging up to 15 and 20 mg per 100 ml that variant mothers have only a slight risk of producing retarded infants.

Some PKU females treated from early infancy are now reaching childbearing age. Many of these girls have not received any dietary treatment for several years but have normal or near normal intelligence. They may now find a restricted diet—Lofenalac®—unpalatable; however, several of our teenaged PKU patients still prefer Lofenalac® to milk even though dietary restrictions were stopped several years ago.

Dietary treatment during pregnancy, even with ideal control, is associated with some risk of nutritional deficiency that could damage the fetus. We believe that, in general, PKU women should be advised against pregnancy. If they desire children,

then pregnancy should be planned so that dietary control is established before conception and extended throughout pregnancy. From our current knowledge of the favorable outcome of pregnancy in mothers with the variant disorder, it appears that it is probably safe for the blood phenylalanine levels to range between 10 and 15 mg per 100 ml. Allowing moderately elevated blood phenylalanine levels would decrease the risk of some unknown nutritional defect. To date, two PKU mothers have been treated successfully during pregnancy and have given birth to normal children.[109,122] One of the mothers had blood phenylalanine levels up to 12.5 mg per 100 ml but repeatedly had positive reaction to tests for urinary PPYA.

Fisch et al[123] showed that mothers heterozygous for the PKU gene excreted greater than normal amounts of phenylalanine and its metabolites in breast milk. Postulating that PKU mothers (homozygous) would excrete even greater quantities, these investigators thought it inadvisable for such mothers to nurse their infants.

Behavioral Changes with PKU

Next to intellectual deficit, behavioral disturbance has been described as the most characteristic symptom of PKU. Hyperactivity, irritability, destructiveness, rage and fear reactions, and autistic behavior have been described repeatedly.[108,131-134] In our experience, however, these behavioral abnormalities are frequent among mentally retarded populations in general, especially among those subjected to institutionalization, and are not specific for phenylketonurics.

More recently, Siegel et al[135] compared a group of PKU children with non-PKU matched controls on a school behavior rating scale. They found the PKU children more clumsy, talkative, and hypersensitive, but did not find that they differed significantly in hyperactivity. However, Johnson[136] in a study comparing a much larger group of PKU residents in institutions with a control group found the phenylketonurics significantly more hyperactive. He also found the PKU subjects rated higher than controls on the following behaviors: "self-destructive," "destroys clothing," "upsets furniture," "aggressive," "destroys ward property," "runs and paces," "attacks residents," "breaks windows," and "bangs doors when secluded."

The attention span of phenylketonurics, even those with mild mental retardation or normal intelligence, may be less than in persons without PKU.[137]

From these reports and our own experience, we believe that retarded PKU patients, and even non-retarded or "borderline" PKU patients, not on a special diet, often have behavioral characteristics which make them a problem for parents and teachers. Hyperactive and destructive behavior is sometimes an indication for continuing dietary treatment beyond the age when no further intellectual deterioration is expected.

Some of the behavioral changes in PKU patients may be iatrogenic and not a result of the metabolic abnormality. Physicians must use caution and avoid being the cause of unnecessary psychological sequelae as a result of their investigations with these children. Sibinga et al[138] studied 30 PKU children, 16 of whom had experienced immobilization and sensory restriction during the first three years of life. Fourteen of these 16 had been in hospital during the first three years of life, whereas, only three of the 14 non-restricted PKU children had been. There was a significantly increased incidence of serious behavioral pathology as well as some decrease in intellectual level in the children experiencing immobilization and sensory restriction. Other variables between these PKU children appear to have been controlled. Many of the restricted children had been in hospital and restrained for metabolic studies, nine of them for three days or longer.

Wood et al[139] pointed out that learning in PKU children was curtailed by prohibitions against free explorations and the opportunity to make meaningful discriminations. These restrictions occurred because of the necessity of dietary control and the parents' reaction to the situation.

Psychopathologic Change in Relatives of Phenylketonurics

An increased incidence of psychopathologic conditions among parents and relatives of phenylketonurics has been reported in the older literature. More recent reports do not support this belief. Perry et al[140] surveyed a large number of relatives of PKU patients and a similar number of relatives of patients with Down's syndrome. No significant differences were found between the groups in incidence of psychosis, admissions to mental hospitals, personality disorders, chronic alcoholism, suicide, or criminal behavior. Larson

and Nyman[141] compared the mental hospitalization and suicide rate of relatives of PKU patients with these rates in the general population and did not find significant differences. Blumenthal[142] in a well-controlled study compared the parents of 108 phenylketonurics with the parents of 102 non-PKU retarded and of 121 children with cystic fibrosis. The parents of the PKU children did not differ from the other two groups when a standardized interview schedule was used to assess their mental health.

Dietary Treatment

The serum phenylalanine level at which treatment is warranted is speculative. Berman et al[22] suggested that if the serum phenylalanine level remains at less than 20 mg per 100 ml on an ordinary diet, dietary treatment may not be needed. However, from the screening data provided by Guthrie,[75] there are retarded patients with serum phenylalanine levels below 20 mg per 100 ml. It is possible that their retardation is not related to hyperphenylalaninemia. It is also possible that their protein intake—they are in institutions—is low and the low serum phenylalanine values reflect this feature.

Such inconsistencies make it difficult to reconcile levels of phenylalanine and retardation. For this reason, many clinicians empirically treat all patients who have persistent hyperphenylalaninemia from any cause. Until there is a method for absolute diagnosis, perhaps at the enzyme level, or until it can be shown unequivocally that dietary treatment is not associated with improved development or more normal intelligence, most clinicians will continue to treat patients having significant hyperphenylalaninemia. Thirty-five years of intensive research has not determined the cause or causes of the retardation in PKU, and the precise reason for the efficacy of dietary treatment is yet to be found.

The increased detection of non-retarded persons with elevated phenylalanine levels has brought about reconsideration of the evidence for the effectiveness of treatment with low-phenylalanine diets. Despite severe criticisms of the research designs and interpretation of the data supporting the efficacy of dietary treatment,[43] most clinicians remain convinced, from empirical observations, that phenylalanine restriction is of value, especially in patients treated from early infancy.

Criticism of the evidence favoring dietary treatment has included these points:

- Comparisons made between treated and untreated groups included a selection bias. Untreated patients were first identified because of mental retardation, whereas most successfully treated patients were identified because of biochemical screening programs of newborns or as siblings of known phenylketonurics.
- Environmental factors have not been controlled. Some studies have included a disproportionately greater number of institutionalized retardates in untreated groups as compared with treated groups. Analyses of data have not recognized that patients receiving dietary treatment may receive a greater amount of parental time, stimulation, and attention than untreated patients and there is a placebo effect from the diet. Parents who maintain good dietary control compared with those who do not may be parents who are of higher intelligence and more capable of stimulating their children.
- Reports of treatment starting in the newborn period have included patients with hyperphenylalaninemia who would not have become mentally retarded in the natural course of their disorder.
- Invalid comparisons have been made between developmental tests in infancy and intelligence tests at older ages.
- "Blind" studies have not been done. The psychologists making developmental and intelligence tests knew which individuals were in diet-treated and control groups at the time of test administration.
- The restricted diet may be dangerous in some cases and lead to decreased intelligence through phenylalanine and protein deficiency.

A further difficulty in assessing the value of dietary treatment is that no center has had a sufficient number of cases to make statistically valid comparisons of the great number of variables leading to developmental differences. A Collaborative Study of Children Treated for Phenylketonuria, involving 18 centers distributed across the United States, was initiated by Dr. Richard Koch of Childrens Hospital of Los Angeles in October, 1967, to answer many of these questions while considering the above objections.

Before any dietary treatment is begun, a diagnosis of PKU should be fairly firmly established. Hyperphenylalaninemia due to transient tyrosinemia should not be treated by phenylalanine re-

striction. Most investigators would not treat the variant disorders of hyperphenylalaninemia when the levels of serum phenylalanine remain consistently below 15 mg per 100 ml while the patient is receiving an ordinary diet. Many clinicians would not treat patients whose levels remained consistently below 20 mg per 100 ml and especially if the urinary metabolites of classical PKU were absent. 'If, however, the serum phenylalanine levels are persistently greater than 20 mg per 100 ml while the patient is ingesting an ordinary diet, it would be prudent to initiate dietary therapy. Periodically the patients should be restudied and "challenged" by tolerance tests to confirm the diagnosis. We suggest that this be done at four months, nine months, eighteen months and also at two to three years unless the diagnosis has been consistently reaffirmed by previous challenges.

Certain precautions must be observed when treating PKU. Practically all protein foods provide too much phenylalanine to meet both the protein needs and at the same time lower the serum phenylalanine levels in PKU patients. Specially prepared commercial products meet the above requirements. They are usually protein hydrolysates which have been modified and which can be obtained only with a prescription. In prescribing such a diet, the aim should be to meet the basic protein and caloric requirements and to give as much phenylalanine as can be tolerated while the blood phenylalanine level is maintained in the desired range. During infancy it is often difficult to conform to these requirements and at the same time give natural foods, but as many natural foods should be included as possible. This problem may soon be overcome. Bickel et al[143] have reported the successful treatment of PKU patients with a product prepared entirely from individual amino acids to which were added fats and vitamins. Because the mixture was entirely devoid of phenylalanine, many more natural foods could be given.

Clinicians at most treatment centers follow the Nutrition Research Council recommendations for proteins and calories[144] and attempt to maintain serum phenylalanine levels between 4 and 8 mg per 100 ml. The patient's blood phenylalanine levels must be monitored frequently to avoid subnormal levels. If the maximum amount of tolerated phenylalanine is given, there is little likelihood that a phenylalanine deficiency will occur. However, since the aim of treatment is to lower the serum phenylalanine level, a critical point of phenylalanine restriction will be reached at which patients may go from an anabolic to a catabolic state. The levels of phenylalanine will decrease and then increase again as a reflection of these states and breakdown of tissue proteins.[145,146] If one does not monitor frequently enough, or monitors blood phenylalanine levels only, there is a considerable likelihood that dietary treatment will not be optimal.

Initially, after the diagnosis is made and the serum phenylalanine level is being reduced by dietary restriction, serum phenylalanine levels should be monitored at least twice weekly. When the serum levels decrease to the recommended range, the levels should be checked weekly and ideally never at intervals longer than two weeks. If monitoring is done less frequently it is impossible to evaluate the quality of dietary control. Urine should be checked at three-month intervals for the metabolites of PKU. The urine reflects the quality of dietary control and also may show signs of a deficiency state. Often a catabolic state will be reflected by a mild to moderate generalized aminoaciduria. If the deficiency state is severe and persists for any length of time, especially in a young patient, growth and even intelligence may suffer. Not only must the phenylalanine needs be met, but also those for proteins, calories, fats, vitamins, and minerals.

The complications associated with dietary treatment have been reviewed by Hanley et al.[147] Signs associated with a deficiency state include: anorexia, diarrhea, anemia, hypoproteinemia, bone demineralization, rashes, hypoglycemia, seizures, and even death.

Length of necessary dietary and phenylalanine restriction is uncertain. Initially it was thought to be for the life time of the patient. We believe it is probably unnecessary beyond the age of six years for the prevention of retardation. Dietary restriction after six years may help prevent hyperactive behavior. Brain growth, as reflected by head circumference, approaches 90 percent of adult size by six years and myelinization is also nearly completed by that time. Allen[148] routinely discontinues dietary restrictions by three years of age and has noted no deterioration in performance, EEG tracings or behavior in any of his patients. Ancillary to this are the reports of Fisch et al[149] who suggest that blood phenylalanine levels in the 10 to 12 mg per 100 ml range after

one year of age have little effect on the eventual intellectual outcome. This further supports Winick's[92] data which suggests that the permanent number of brain cells is affected critically in the first six months after birth.

One must in the end make judgments about diet discontinuation for the individual patient. Seizures, hyperactivity, and deterioration in performance have been reported as guidelines in particular cases.[150,151] Clayton et al[152] suggested that EEG abnormalities provoked by an L-phenylalanine load are an indication for continuing dietary treatment. This may not be a useful criterion since EEG abnormalities have been provoked in control patients as a consequence of L-phenylalanine loading.[153] The question of diet discontinuation is much simpler for males than for females because of the problems of fetal malformations and retardation in untreated maternal PKU. It has been shown that PKU patients with normal IQ and with little evidence of damage are less likely to suffer further intellectual deterioration on reintroduction of normal diet than are patients who are damaged severely.[154]

California Crippled Children's Service Program

Compulsory screening for PKU was implemented in California in 1966. The law and subsequent State Health Department regulations established that screening tests would be performed by regular licensed clinical laboratories and not in central state-owned or operated laboratories as is mandated in some states of the United States.[33] By the end of 1967, 179 laboratories distributed widely over the state had been approved for PKU screening tests.[155] Quality control in these laboratories is maintained by means of control specimens submitted to them regularly by the State Health Department.

A "fail-safe" system for follow-up of specimens found positive by screening is maintained through local health departments and the Bureau of Maternal and Child Health in the State Health Department. If a newborn's blood is positive (4 mg per 100 ml or greater) on screening, a second specimen is requested. After a second positive test, the infant should probably be referred for prompt diagnostic evaluation to a center approved by the Crippled Children's Service (CCS).

These centers provide initial treatment, follow-up treatment, and consultations with physicians.[156] As a condition of approval these centers are required to provide the services of a multidisciplinary team, including a pediatrician expert in these problems, a psychologist, a social worker, a nutritionist, and a nurse. Complete laboratory capability for differential diagnosis of obscure metabolic diseases and their variations is also expected. All of these centers can when necessary call on a variety of other specialists such as geneticists, neurologists, child development experts, and experts in speech and hearing. As of the beginning of 1970, the following centers were approved for rendering these services to CCS: Childrens Hospital of Los Angeles, Orange County Medical Center, Los Angeles County-University of Southern California Medical Center, Children's Health Center at San Diego and Children's Hospital at San Francisco. Before 1970, certain individual pediatric specialists were approved for rendering diagnostic and management services under CCS support. These specialists continue to render some services to CCS-supported PKU patients and are in addition to the approved centers with complete teams.

In addition to original diagnostic evaluation for all patients needing such services, CCS will support continuing patient management, the required diet (Lofenalac®) and authorized consultations and re-evaluations for those patients determined to be in need of such support after financial screening. Continuing patient management may be supplied by physicians authorized to render such services in the local community with consultation or periodic re-evaluation being supplied by one of the approved centers. During the 1968-69 fiscal year, 107 children with the diagnosis of PKU were on the California CCS caseload.[157]

The center concept has proven itself in California and elsewhere as being a very necessary corollary to mass screening programs. Clinicians at these centers usually can separate the severe from the mild forms of metabolic diseases and thus distinguish those variants not in need of treatment. Patients not in need of treatment thereby avoid expensive and at times dangerous dietary manipulations. The teams in these centers see enough patients to develop the experience needed to render the very carefully formulated counseling that the parents of these children always need. Support of a special diet for PKU patients through the CCS program is justified economically. The cost is small compared with the cost of years of

state hospitalization when severe brain damage is allowed to occur. From a humane point of view and family morale, if just one patient is kept from deteriorating, the state program is well justified.

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