proceedings of

INTERNATIONAL
CONFERENCE ON
INBORN ERRORS
OF METABOLISM

May 30 - June 3, 1966 • Dubrovnik, Yugoslavia

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Thirty-seven specialists in phenylketonuria and other inborn errors of metabolism which may cause mental retardation attended an International Conference on Inborn Errors of Metabolism in Dubrovnik, Yugoslavia, May 30–June 3, 1966. This conference was sponsored by the Federal Institute of Public Health of Yugoslavia and the Children's Bureau, Welfare Administration, United States Department of Health, Education, and Welfare. The participants were from Belgium, Germany, Greece, India, Ireland, Israel, Mexico, Pakistan, Poland, Scotland, Sweden, Yugoslavia, and the United States of America.

The participants reported on their countries' efforts to detect inborn errors of metabolism and to develop treatment and management programs. Discussion was open and informal. Many new ideas were presented, new techniques of exploring problems of inborn errors of metabolism considered, and gaps in knowledge noted.

The participants set a number of goals for themselves. Two of these goals were to determine how many untreated persons with phenylketonuria grow up "normally," and under what conditions dietary management is essential, for how long, and with what result.

Co-chairmen were Vukan Ćupić, M.D., Director of the Mother and Child Health Institute of the Republic of Serbia, Yugoslavia, and Mitchell I. Rubin, M.D., Chairman of the Department of Pediatrics, State University of New York, Buffalo, New York, United States of America.

This conference was the first organized and supported by the Children's Bureau, using United States-owned foreign currency under its program for interchange of experts.

It was not possible to give each participant of the conference an opportunity to review this manuscript. Hopefully, no statement has been misconstrued.
CONTENTS

Clinical Aspects of Phenylketonuria and Allied Conditions

George Jervis, M.D. .................................................. 1
Discussion ......................................................... 4

Laboratory Screening and Diagnosis

Robert Guthrie, M.D. ............................................. 17
Discussion ......................................................... 22

A Proposed Classification for the Hyperphenylalaninemas

John H. Menkes, M.D. ........................................... 29
Discussion ......................................................... 33

Screening Tests for Other Congenital Abnormalities

Donough O'Brien, M.D., F.R.C.P. ................................. 34
Discussion ......................................................... 35

Phenylketonuria--A Public Health Responsibility in Maryland

Benjamin D. White, M.D., M.P.H. ............................... 39
Discussion ......................................................... 48

Dietary Management

Werner Grüter, M.D. ................................................. 51
Discussion ......................................................... 52

Suggested Followup ................................................ 62

Participants ........................................................ 63

Special Consultants ............................................... 67
This presentation is a brief survey of the clinical manifestations of phenylketonuria and allied conditions.

Phenylketonuria is characterized by near normal physical growth. Physical abnormalities, such as distorted facial features and cranial anomalies, although occasionally observed, are less common than in other forms of severe mental retardation. Retarded intellectual development is the most striking clinical feature. Data derived from institutional population show that between 50 and 60 percent of adults with phenylketonuria have IQ's below 20; some 30 percent have IQ's between 20 and 50. The remaining group (amounting to no more than 10 percent) have IQ's between 50 and 70.

Patients with normal intelligence have been observed, and about 2 dozen cases have been reported in literature. It is possible that the incidence of these cases is higher than hitherto reported, but no large survey of normal adult population has been made.

According to recent data obtained by the Guthrie test in newborns, when mental status obviously cannot be determined, the incidence of phenylketonuria is 1:10,000. Data obtained from surveys among retarded individuals would suggest that the incidence in the general population is about 1:20,000. The discrepancy between these two figures is not clear. One of the explanations may be that a higher-than-expected number of patients with phenylketonuria will never develop mental retardation. This problem is of basic significance for the evaluation of the dietary treatment of phenylketonuria.

Intellectual deterioration is said to be a characteristic feature of phenylketonuria. A certain number of patients show progressive decline in IQ during the first 5 to 6 years of life. It is assumed that all babies with phenylketonuria are born with normal intelligence, but it would be difficult to prove this assumption because of methodological difficulties in assessing "intelligence" during the first weeks or months of life. At any event, it is a matter of common clinical observation that the extent and the tempo of intellectual deterioration varies considerably from patient to patient.

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for reasons unknown. Moreover, the age at which an intellectual plateau is reached is still unknown and probably varies. Elucidation of all these problems concerning intellectual deterioration is necessary in order to plan and assess properly dietary treatment of the disease.

Psychotic manifestations are not rare in the disease. It is not uncommon to see phenylketonuric children with the diagnosis of childhood schizophrenia. However, nothing is characteristic in the behavior pattern of the patients. Extremely disturbed behavior may, in fact, be observed in some children and quiet, passive behavior in others.

Neurological abnormalities are common, particularly in the very young. High percentages (70-90 percent) of abnormal EEG records have been reported, the most frequent pattern being that of hypsarhythmia. Hypertonicity of muscles, abnormalities of reflexes, and other minor signs of cerebral involvement are frequently present in the PKU infant, but are less frequent in the adult.

Defect of pigmentation is a well known feature of the disease. In a high percentage of patients, the hair is blond and the skin light. Examples of blond, lightly pigmented patients in darkly pigmented families of Spanish or Italian descent are on record. Japanese patients with hair much lighter than the normal members of the family have been observed. However, the defect in pigmentation is not a constant feature; fullblooded Negro patients with dark pigmentation have been reported.

Dermatitis, mostly in the form of eczema or of atopic dermatitis, occurs in 20-40 percent of infants and children with phenylketonuria.

The clinical picture thus far sketched is not sufficiently characteristic to justify a diagnosis of phenylketonuria on purely clinical observations. The diagnosis is ultimately assured by the demonstration of a high blood level of phenylalanine and of urinary output of phenylpyruvic, o-hydroxyphenylacetic acids, and other derivations of phenylalanine.

Others in this symposium will discuss authoritatively the various aspects of the biochemical diagnosis of phenylketonuria. It has been said, not without some justification, that unfortunately today phenylketonuria has been equated with hyperphenylalaninemia, thus losing its clinical characteristics. It may be timely at this symposium to attempt a provisional classification of conditions accompanied by increased blood levels of phenylalanine:

**Classical Form of Phenylketonuria** - This is characterized by the presence of the mental manifestations and the neurological abnormalities mentioned above, together with the biochemical findings in blood and urine. The absence of mental retardation does not rule out the diagnosis.

**Hyperphenylalaninemia** - There is no involvement of the central nervous system and no mental retardation. The blood level of phenylalanine is usually between 5 and 15 mg. percent. No phenylpyruvic acid is present in the blood or the urine. Blood tyrosine level is usually high. No cases of
classical phenylketonuria are present in the family, nor are relatives heterozygous for the PKU gene. Some instances of this condition are temporary; the findings revert to normal within a few weeks or months after birth. In others, the biochemical abnormality persists to adolescence and beyond.

"High" PKU Heterozygotes - The blood level of phenylalanine is usually between 5 and 15 mg. percent, but blood tyrosine is normal. Phenylketone may occasionally appear in the urine, particularly under a stress condition such as acute infectious disease. There is no central nervous system involvement and no mental retardation. The carrier test for the PKU gene is positive, and there may be classical cases of PKU in relatives.

Hyperphenylalaninemia With Deficiency of Transaminase - Only two cases have been reported. There is very high blood level of phenylalanine with no conversion to phenylpyruvic acid.

It is probable that other conditions accompanied by increased blood levels of phenylalanine will be identified in the future.

The clinical aspects of other genetically determined metabolic diseases involving the central nervous system are as follows:

Homocystinuria - There are characteristic features which usually make it possible to recognize the disease clinically. Dolichostenomelia is often present, similar to that which is seen in Marfan's syndrome. There may be, in addition, dislocation of the lenses. Mental retardation is not always present. Abnormalities of the EEG are common. There is often evidence of multiple venous or arterial thromboses. Final diagnosis rests on the demonstration of homocystine in the urine and increased blood level of methionine.

Histidinemia - The clinical picture is not characteristic. Some delay in physical growth may be present. Mental retardation, when present, is not severe. Speech defects are common. Convulsive manifestations have been observed.

Maple Syrup Urine Disease - A syndrome of severe failure to thrive and progressive mental deterioration with evidence of brain damage are present. The peculiar odor of the urine makes it possible to recognize the disease promptly.

Few cases of other rare forms of amino acid disorders have been reported, but clinical symptoms and signs are still not well known. The friable hair may be a clue for clinical diagnosis in argininosuccinicaciduria; renal involvement may be for hyperprolinemia.

2/ Auerbach, V. H. Personal communication to George Jervis.

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Carbohydrate disorders which are characterized by mental retardation include galactosemia, gargoylism, and Pompe's disease.

**Gargoylism** - At present, several types varying in clinical manifestations and biochemical findings are recognized. In all, osseous changes are present. It should be noted, however, that bone changes are inconspicuous in the Sanfilippo syndrome, in which mental retardation is severe.

**Pompe's Disease, a Variety of Glycogenosis** - Cardiomegaly is one of the main signs.

Disorders of lipid metabolism involving the central nervous system show a large variety of symptoms and signs, few of which, however, are characteristic. Among those which are characteristic are the "cherry red spot" in the eyeground of Tay-Sachs disease and the splenomegaly of Gaucher's and Niemann-Pick diseases.

Wilson's disease is a metabolic disorder in which the clinical picture is quite characteristic and the diagnosis possible on clinical examination.

With rare exceptions, the clinical examination of patients affected by forms of genetically determined mental retardation with alterations of metabolism is not conclusive. However, the astute clinician is often able to uncover diagnostic clues which are of considerable help in the selection of the proper biochemical tests. The diagnosis rests ultimately on these tests.

**DISCUSSION**

O'BRIEN (United States): Phenylketonuria is the easiest inborn error of metabolism to diagnose and treat. Other inborn errors are rare, and some of these are discussed in *Rare Inborn Errors of Metabolism in Children With Mental Retardation*, published by the Children's Bureau, U. S. Department of Health, Education, and Welfare, 1965.

MENKES (United States): Apparently tyrosine does not damage the brain. Cerebroside changes may be secondary to myelin damage and not primary. EEG changes may be reversible even after many years. Protein under-nutrition per se may alter myelin--thus, the danger of a diet very low in phenylalanine. Some possible mechanisms of mental retardation associated with phenylketonuria are:

1. Impaired serotonin synthesis.
2. Impaired gamma-aminobutyric acid synthesis.
3. Impaired epinephrine synthesis.
4. Impaired synthesis of myelin protein.
5. Interference with cerebroside formation.
6. Interference with amino acid transport mechanism.
7. Toxic effects of elevated ammonia.

ZETTERSTROM (Sweden): Some patients with tyrosinemia may be mildly retarded. There is need for much more information as to the basis for brain damage in phenylketonuria. Sweden has four or five cases of tyrosinemia similar to the first seven described.

GRÜTER (Germany): Cerebral deterioration may continue to 13 or 14 years with continued dysmyelinization.

CAHALANE (Ireland): There are probably 40 cases of homocystinuria in Ireland. Some have intestinal malabsorption.

VIS (Belgium): The amino acid distribution of kwashiorkor is shown on page 6. In kwashiorkor, there is impairment in the degradation of phenylalanine, tyrosine, and histidine. The ratio of the excretion of hydroxyproline to creatinine serves as a useful determinant of malnutrition. Other blood and urinary free amino acid levels for children are shown in the tables and charts on pages 7-15.
TYPICAL EXAMPLES OF PATHOLOGICAL PATTERNS
OF URINARY FREE AMINO ACIDS IN DEFICIENCY DISEASES

- Glycine
- Histidine
- Glutamine & Glutamic acid
- Alanine
- Serine
- Lysine
- Threonine
- Tyrosine
- Phenylalanine
- Leucine
- Isoleucine
- Proline

o Kw. D176
v Rickets
• Kwashiorkor
x Scurvy
* Normal values
TYPICAL EXAMPLES OF PATHOLOGICAL PATTERNS
OF URINARY OR PLASMA FREE AMINO ACIDS
IN PHENYLKETONURIA FOR CHILDREN 9 MONTHS - 2 YEARS

μM/1000 mL.
<table>
<thead>
<tr>
<th>Free Amino Acids</th>
<th>9 Months-2 Years</th>
<th>4-6 Years</th>
<th>6-10 Years</th>
<th>8-10 Years</th>
<th>10-11 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine b/</td>
<td>370</td>
<td>143-561</td>
<td>655</td>
<td>284-941</td>
<td>1061</td>
</tr>
<tr>
<td>Histidine</td>
<td>300</td>
<td>99-533</td>
<td>572</td>
<td>355-381</td>
<td>816</td>
</tr>
<tr>
<td>Glutamine c/</td>
<td>170</td>
<td>64-164</td>
<td>328</td>
<td>237-338</td>
<td>381</td>
</tr>
<tr>
<td>D-alanine</td>
<td>107</td>
<td>65-120</td>
<td>322</td>
<td>121-264</td>
<td>322</td>
</tr>
<tr>
<td>Serine</td>
<td>95</td>
<td>6-16</td>
<td>118</td>
<td>72-167</td>
<td>160</td>
</tr>
<tr>
<td>Lysine</td>
<td>70</td>
<td>10-137</td>
<td>162</td>
<td>74-210</td>
<td>160</td>
</tr>
<tr>
<td>Threonine</td>
<td>52</td>
<td>0-250</td>
<td>232</td>
<td>62-374</td>
<td>162</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>46</td>
<td>0-93</td>
<td>79</td>
<td>64-217</td>
<td>168</td>
</tr>
<tr>
<td>Phenylalanine d/</td>
<td>25</td>
<td>6-50</td>
<td>114</td>
<td>41-170</td>
<td>100</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>23</td>
<td>5-47</td>
<td>114</td>
<td>41-170</td>
<td>100</td>
</tr>
<tr>
<td>Leucine</td>
<td>22</td>
<td>0-50</td>
<td>9</td>
<td>41-170</td>
<td>100</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>21</td>
<td>3-42</td>
<td>5</td>
<td>24-53</td>
<td>55</td>
</tr>
<tr>
<td>Methionine</td>
<td>17</td>
<td>3-42</td>
<td>5</td>
<td>24-53</td>
<td>55</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>12</td>
<td>0-38</td>
<td>2</td>
<td>0-3</td>
<td>2</td>
</tr>
</tbody>
</table>

*Values given in µM./24 h.*

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<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Infant 1</th>
<th>Infant 2</th>
<th>Infant 3</th>
<th>Infant 4</th>
<th>Infant 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>7 0-60</td>
<td>7 0-39</td>
<td>7 0-29</td>
<td>13 0-46</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>6 0-42</td>
<td>16 0-25</td>
<td>33 6-51</td>
<td>29 2-48</td>
<td></td>
</tr>
<tr>
<td>3-aminobutyric</td>
<td>-- --</td>
<td>5 0-18</td>
<td>-- --</td>
<td>20 0-44</td>
<td></td>
</tr>
<tr>
<td>3-aminobutyric</td>
<td>2 0-15</td>
<td>0 0</td>
<td>1 0-25</td>
<td>9 0-47</td>
<td></td>
</tr>
<tr>
<td>3-alanine</td>
<td>1 0-11</td>
<td>7 0-14</td>
<td>-- --</td>
<td>9 0-29</td>
<td></td>
</tr>
<tr>
<td>3-amino adipic</td>
<td>1 0-9</td>
<td>0 0</td>
<td>0 0</td>
<td>5 0-34</td>
<td></td>
</tr>
<tr>
<td>3-cystine</td>
<td>1 0-70</td>
<td>68 49-82</td>
<td>100 34-258</td>
<td>90 4-220</td>
<td></td>
</tr>
</tbody>
</table>

a/ The amino acids are classified in order of decreasing importance of their average excretion in infants between the ages of 9 months and 2 years. Variations may be very important for a single amino acid.

b/ There may be traces of citrulline in the urine. This amino acid is then titrated with glycine. The results are expressed in μM of glycine.

c/ The peaks of the glutamine and asparagine appear in the same place. The results are expressed in μM of glutamine.

d/ The peak of 1-methylhistidine may contain anserine and carnosine. The results are expressed in μM of 1-methylhistidine.
### MEAN AND RANGE OF VALUES OF PLASMA AMINO ACIDS a/

(Values given in $\mu$M. per liter of plasma)

<table>
<thead>
<tr>
<th>Plasma Amino Acids</th>
<th>Children (20 Cases)</th>
<th>Adults (Soupart)</th>
<th>Adults (Stein and Moore) b/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 Months-2 Years</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>219</td>
<td>99-313</td>
<td>300-380</td>
</tr>
<tr>
<td>Glycine</td>
<td>170 c/</td>
<td>56-308 c/</td>
<td>232-283</td>
</tr>
<tr>
<td>Glutamine</td>
<td>135 d/</td>
<td>46-290 d/</td>
<td>140-370</td>
</tr>
<tr>
<td>Valine</td>
<td>127</td>
<td>57-262</td>
<td>168-216</td>
</tr>
<tr>
<td>Proline</td>
<td>115</td>
<td>51-185</td>
<td>103-210</td>
</tr>
<tr>
<td>Serine</td>
<td>92</td>
<td>24-172</td>
<td>115-164</td>
</tr>
<tr>
<td>Lysine</td>
<td>87</td>
<td>45-144</td>
<td>105-155</td>
</tr>
<tr>
<td>Leucine</td>
<td>75</td>
<td>45-155</td>
<td>78-115</td>
</tr>
<tr>
<td>Histidine</td>
<td>64</td>
<td>24-112</td>
<td>32-92</td>
</tr>
<tr>
<td>Threonine</td>
<td>60</td>
<td>33-128</td>
<td>137-194</td>
</tr>
<tr>
<td>Taurine</td>
<td>49</td>
<td>19-91</td>
<td>80-100</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>45</td>
<td>11-122</td>
<td>22-58</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>44</td>
<td>26-94</td>
<td>40-63</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>40</td>
<td>23-69</td>
<td>38-67</td>
</tr>
<tr>
<td>Ornithine</td>
<td>40</td>
<td>10-107</td>
<td>30-64</td>
</tr>
<tr>
<td>Arginine</td>
<td>31</td>
<td>11-65</td>
<td>40-102</td>
</tr>
<tr>
<td>Methionine</td>
<td>21</td>
<td>3-29</td>
<td>11-26</td>
</tr>
</tbody>
</table>
\( \alpha \)-aminobutyric & 5 & 0-17 & 14-21 & 21-34 \\
\( \frac{1}{2} \)-cystine \( c / \) & 4 & 0-40 & 70-100 & 90-108 \\
Aspartic acid & 2 & 0-9 & 2-8 & 1-5

\( a / \) The variations may be important between different individuals.
\( c / \) There may be traces of citrulline. This amino acid is then titrated with glycine. The results are expressed in \( \mu \)M. of glycine.
\( d / \) The peaks of the glutamine and asparagine appear in the same place. The results are expressed in \( \mu \)M. of glutamine.
\( e / \) The \( \frac{1}{2} \)-cystine is absent in all children except two, whereas important quantities are found regularly in adult plasma.
ZETTERSROM (Sweden): Breast milk has a lower protein content than whole milk. Could this account for the lower incidence in Sweden of positives for phenylketonuria via the Guthrie testing method?

SCOTT (Scotland): Scotland has three infants with hyperphenylalaninemia who have high tyrosine levels; in one case, the tyrosine level remained elevated for 9 months.

General Comments: Behavior disturbance with normal IQ is occasionally seen with classical phenylketonuria alterations. Prolonged high protein feeding in premature infants may result in positive ferric chloride urine tests (p-hydroxyphenylpyruvic acid); the positive results disappear when the protein in the diet is lowered. On the high protein diet, both the blood phenylalanine and tyrosine may be elevated; when the diet is reduced to 1.5 grams percent, the tyrosine may remain moderately elevated at the time when the blood phenylalanine has returned to normal. These elevated levels in premature infants usually have returned to normal within 6 weeks. The blood level of phenylalanine rises in all women in their third trimester of pregnancy.
LABORATORY SCREENING AND DIAGNOSIS

ROBERT GUTHRIE, M.D.
United States

Methods of Screening

There are probably only three methods used to screen young infants for phenylketonuria:

1. Ferric Chloride Testing of Urines - Twenty-seven county health departments in California tested 240,000 infants by 650,000 diaper tests and found only 8 cases of PKU (1:30,000). The youngest case was 4 months old, and four cases were 1-year-olds. This program in California was carried out during the 1962-63 period when the trial of the bacterial inhibition assay detected 39 cases among 405,000 newborns in 29 other States. Thus, it would appear that diaper testing misses more than one-half of the cases as well as detects them too late.

Urine-impregnated filter paper collected for ferric chloride testing by a central laboratory appears to give better results. Dr. Brian Turner,1/ Sydney, Australia, has detected six cases of PKU among 100,000 infants by this method.

2. Fluorometric Testing - The McCaman-Robins 2/ fluorometric method, using dried blood spots, has been automated by Drs. Summer and Hill 3/ (North Carolina). However, Dr. Irwin 4/ (Sumerlin Memorial Laboratory, San Diego, California) claims better and more efficient results by placing each punched-out disc in distilled water in a small test tube, shaking the tube

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1/ Turner, Brian. Private communication to Robert Guthrie.
for 30 minutes, and then following with the usual McCaman-Robins procedure.

3. **Paper Chromatography** - Dr. Scriver 5/ (Montreal) uses serum from newborn babies for one-dimensional paper chromatography as his primary method for detecting PKU, in addition to looking for other aminoacidemias. No results are available for PKU, but he has detected other abnormalities, especially tyrosinemas. Dr. Mary Efron 6/ (Boston) does not recommend that her paper chromatographic method be substituted for the bacterial inhibition assay or the fluorometric methods for PKU screening.

Comments on Use of Bacterial Inhibition Assay for PKU Screening

A correction should be made in our publication.7/ Spores being prepared as an inoculum should be "washed" by centrifuging in distilled water, not 0.9 percent NaCl, after pasteurization. This will remove the dead vegetative cells and cellular debris, giving a more stable spore preparation.

The automatic device manufactured by the Fundamental Products Company of Los Angeles eliminates handwork in punching out the blood discs and placing them on the agar trays. Such a machine makes possible other microbiological tests for maple syrup urine disease, galactosemia, and histidinemia with little or no additional effort.

**Laboratory Confirmatory Procedures**

Chromatography is the best and simplest laboratory procedure to confirm the identity of phenylalanine, as well as to exclude an associated tyrosine elevation, which is now known to be an important clue to a hyperphenylalaninemia not caused by PKU.

We have always advocated that those laboratories already using the Guthrie screening test should prepare the type of chromatogram described in Children's Bureau Publication 419.8/ Strips are cut from the chromatogram and placed on the agar screening plate. Such chromatograms can be prepared from dried blood spots or dried urine-impregnated paper. They will reveal by

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8/ Guthrie, Robert, and Whitney, Stewart, op. cit.
growth zones the Rf values of the active substance or substances, as phenylalanine in blood or phenylalanine plus phenylpyruvic acid in urine from infants old enough to permit excretion of the acid.

Quantitative Use of Bacterial Inhibition Assay

We developed the test originally in Buffalo in 1957 to measure the levels of blood phenylalanine in children with PKU placed on diet treatment. We impregnated each of three ½-inch paper discs with 0.01 ml. of serum and compared the growth zones with those produced by serum controls. Four years later, when we attempted to use the procedure for screening, we simply substituted the punched-out disc from a dried spot of blood for use of measured aliquots of serum. Fortunately, by using a suitable paper with a high absorptive quality and care in proper spotting of blood, the amount of dried blood in the disc punched from the central region turns out to be surprisingly uniform, apparently with as little as 5 percent variation (this has not been studied carefully).

Further, by adjusting the concentration of the inhibitor, β-2 thienylalanine, we can adjust the sensitivity of the assay to that range of concentrations most critical. In PKU, that range is 1 to 10 mg. percent. Within this range, it has now been demonstrated by several laboratories that the McCaman-Robins method for serum analysis and the bacterial inhibition assay of dried blood spots from the same series of blood specimens give results which are not significantly different.

For this reason, many treatment programs in the United States and other countries have used the bacterial inhibition assay exclusively for monitoring their children on diet treatment. The parents have been taught to collect the blood spots for the laboratory, and determinations are now made routinely as frequently as once per week. This permits more satisfactory control of the patient.

A summary of results as of December 31, 1965, from 40 States in the United States shows a total of 203 confirmed cases found among 2,205,006 infants screened—or a frequency of 1 per 10,862. (See the following tables.)

It should be noted that in 13 States an effort was made to retest every infant between 4 and 6 weeks by a second blood test. Out of approximately 350,000 such infants, 6 of the 35 cases were missed with the first test and detected only by the second test. So the single test is still missing cases of phenylketonuria in newborns, presumably because of the early testing prior to discharge of infants from hospitals.

PKU DETECTION IN NEWBORN INFANTS IN UNITED STATES OF AMERICA a/
(As of December 31, 1965)

<table>
<thead>
<tr>
<th>State (or Territory)</th>
<th>Number Newborns Tested</th>
<th>Number Cases Found</th>
<th>State (or Territory)</th>
<th>Number Newborns Tested</th>
<th>Number Cases Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>21,752</td>
<td>3</td>
<td>New Jersey</td>
<td>107,410</td>
<td>1</td>
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<tr>
<td>California</td>
<td>60,169</td>
<td>7</td>
<td>New Mexico</td>
<td>13,977</td>
<td>1</td>
</tr>
<tr>
<td>Colorado</td>
<td>12,000</td>
<td>0</td>
<td>New York</td>
<td>173,238</td>
<td>13</td>
</tr>
<tr>
<td>Connecticut</td>
<td>115,108</td>
<td>11</td>
<td>North Dakota</td>
<td>35,000</td>
<td>3</td>
</tr>
<tr>
<td>Delaware</td>
<td>29,115</td>
<td>1</td>
<td>Ohio</td>
<td>129,160</td>
<td>13</td>
</tr>
<tr>
<td>Georgia</td>
<td>31,800</td>
<td>4</td>
<td>Oklahoma</td>
<td>13,035</td>
<td>3</td>
</tr>
<tr>
<td>Idaho</td>
<td>22,000</td>
<td>1</td>
<td>Oregon</td>
<td>66,000</td>
<td>6</td>
</tr>
<tr>
<td>Illinois</td>
<td>55,141</td>
<td>4</td>
<td>Pennsylvania</td>
<td>260,037</td>
<td>14</td>
</tr>
<tr>
<td>Iowa</td>
<td>316</td>
<td>0</td>
<td>Puerto Rico</td>
<td>2,153</td>
<td>0</td>
</tr>
<tr>
<td>Louisiana</td>
<td>49,000</td>
<td>3</td>
<td>Rhode Island</td>
<td>27,020</td>
<td>8</td>
</tr>
<tr>
<td>Maine</td>
<td>39,000</td>
<td>2</td>
<td>South Carolina</td>
<td>34,307</td>
<td>2</td>
</tr>
<tr>
<td>Maryland</td>
<td>30,400</td>
<td>11</td>
<td>South Dakota</td>
<td>5,746</td>
<td>0</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>338,726</td>
<td>35</td>
<td>Tennessee</td>
<td>16,932</td>
<td>9</td>
</tr>
<tr>
<td>Michigan</td>
<td>95,500</td>
<td>4</td>
<td>Texas</td>
<td>85,000</td>
<td>8</td>
</tr>
<tr>
<td>Minnesota</td>
<td>129,533</td>
<td>9</td>
<td>Vermont</td>
<td>5,577</td>
<td>1</td>
</tr>
<tr>
<td>Missouri</td>
<td>40,226</td>
<td>4</td>
<td>Virgin Islands</td>
<td>565</td>
<td>1</td>
</tr>
<tr>
<td>Montana</td>
<td>19,289</td>
<td>2</td>
<td>Virginia</td>
<td>21,112</td>
<td>3</td>
</tr>
<tr>
<td>Nebraska</td>
<td>6,990</td>
<td>2</td>
<td>Washington</td>
<td>17,345</td>
<td>6</td>
</tr>
<tr>
<td>Nevada</td>
<td>21,000</td>
<td>5</td>
<td>West Virginia</td>
<td>9,411</td>
<td>0</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>28,000</td>
<td>1</td>
<td>Wisconsin</td>
<td>36,916</td>
<td>4</td>
</tr>
</tbody>
</table>

Total Number Newborns Tested - 2,205,006
Total Number Cases Found - 203
Frequency - 1:10,862

a/ Using the "bacterial inhibition" assay technique to test dried spots of blood. From data compiled as a result of replies to questionnaires mailed to all 50 States in February 1966.
PKU DETECTION IN NEWBORN INFANTS IN FOREIGN COUNTRIES a/  
(As of December 31, 1965)

<table>
<thead>
<tr>
<th>Country</th>
<th>Number Newborns Tested</th>
<th>Number Cases Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>479</td>
<td>0</td>
</tr>
<tr>
<td>Belgium</td>
<td>7,608</td>
<td>5</td>
</tr>
<tr>
<td>Canada</td>
<td>65,898</td>
<td>6</td>
</tr>
<tr>
<td>Denmark</td>
<td>12,500</td>
<td>0</td>
</tr>
<tr>
<td>Germany</td>
<td>62,479</td>
<td>8</td>
</tr>
<tr>
<td>Ireland</td>
<td>15,000</td>
<td>2</td>
</tr>
<tr>
<td>Israel</td>
<td>65,158</td>
<td>5</td>
</tr>
<tr>
<td>New Zealand</td>
<td>1,840</td>
<td>0</td>
</tr>
<tr>
<td>Pakistan b/</td>
<td>5,481</td>
<td>0</td>
</tr>
<tr>
<td>Poland</td>
<td>130,912</td>
<td>21</td>
</tr>
<tr>
<td>Scotland</td>
<td>7,277</td>
<td>0</td>
</tr>
<tr>
<td>Sweden</td>
<td>21,505</td>
<td>1</td>
</tr>
<tr>
<td>Switzerland</td>
<td>4,721</td>
<td>0</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>23,635</td>
<td>3</td>
</tr>
<tr>
<td>Totals</td>
<td>424,493</td>
<td>51</td>
</tr>
</tbody>
</table>

Frequency - 1:8,323

a/ Using the "bacterial inhibition" assay technique to test dried spots of blood. From data compiled as a result of replies to a letter mailed to the various laboratories in April 1966.
b/ Added May 1966.
DISCUSSION

SZEINBERG (Israel): The phenylketonuria screening program in Israel is summarized on the following charts. No phenylketonuria has been found among the European Jews in Israel.

SUMMARY OF THE PHENYLKETONURIA SCREENING PROGRAM AMONG NEWBORNS IN ISRAEL

<table>
<thead>
<tr>
<th></th>
<th>First Year 1964-65</th>
<th>Second Year 1965-66</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of live births</td>
<td>63,788</td>
<td>66,685</td>
</tr>
<tr>
<td>Infants screened at age of 3-5 days</td>
<td>27,113</td>
<td>38,045</td>
</tr>
<tr>
<td>Screening coverage</td>
<td>43%</td>
<td>57%</td>
</tr>
<tr>
<td>Positive cases detected</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Frequency of PKU among newborns</td>
<td>1:9,038</td>
<td>1:19,023</td>
</tr>
<tr>
<td>&quot;Presumed&quot; positive results negative on second test</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Frequency of &quot;presumed&quot; positives</td>
<td>1:4,519</td>
<td>1:5,435</td>
</tr>
<tr>
<td>Repeated examinations of newborns at 10-20 days</td>
<td>1,413</td>
<td>-</td>
</tr>
</tbody>
</table>
### ETHNIC DISTRIBUTION OF NEWBORNS SCREENED IN ISRAEL

(According to country of birth of parents)

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>First Year 1964-65</th>
<th>Second Year 1965-66</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jews Ashkenazi</td>
<td>5,951</td>
<td>7,788</td>
</tr>
<tr>
<td>Jews non-Ashkenazi (total)</td>
<td>11,233</td>
<td>18,254</td>
</tr>
<tr>
<td>Yemen</td>
<td>1,292</td>
<td>2,494</td>
</tr>
<tr>
<td>Iraq-Iran</td>
<td>1,653</td>
<td>3,216</td>
</tr>
<tr>
<td>Turkey</td>
<td>165</td>
<td>803</td>
</tr>
<tr>
<td>North Africa</td>
<td>1,906</td>
<td>5,981</td>
</tr>
<tr>
<td>Bulgaria-Greece</td>
<td>82</td>
<td>285</td>
</tr>
<tr>
<td>Syria-Lebanon</td>
<td>77</td>
<td>222</td>
</tr>
<tr>
<td>Afghanistan</td>
<td>25</td>
<td>55</td>
</tr>
<tr>
<td>India-Pakistan</td>
<td>85</td>
<td>139</td>
</tr>
<tr>
<td>Buchara</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>Nonspecified Jews</td>
<td>5,922</td>
<td>5,039</td>
</tr>
<tr>
<td>Nonspecified Arabs</td>
<td>7,033</td>
<td>7,013</td>
</tr>
<tr>
<td>Druzes</td>
<td>2,858</td>
<td>4,896</td>
</tr>
<tr>
<td>Circassians</td>
<td>32</td>
<td>75</td>
</tr>
<tr>
<td>Armenians</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Samaritans</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>27,113</td>
<td>38,045</td>
</tr>
</tbody>
</table>

### ETHNIC DISTRIBUTION OF MENTALLY DEFICIENT SCREENED IN ISRAEL IN 1965-66

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Jews Ashkenazi</td>
<td>173</td>
</tr>
<tr>
<td>Jews non-Ashkenazi (total)</td>
<td>432</td>
</tr>
<tr>
<td>Yemen</td>
<td>70</td>
</tr>
<tr>
<td>Iraq-Iran</td>
<td>172</td>
</tr>
<tr>
<td>Turkey</td>
<td>16</td>
</tr>
<tr>
<td>North Africa</td>
<td>139</td>
</tr>
<tr>
<td>Bulgaria-Greece</td>
<td>7</td>
</tr>
<tr>
<td>Syria-Lebanon</td>
<td>4</td>
</tr>
<tr>
<td>India</td>
<td>1</td>
</tr>
<tr>
<td>Nonspecified Jews</td>
<td>23</td>
</tr>
<tr>
<td>Nonspecified Arabs</td>
<td>167</td>
</tr>
<tr>
<td>Arabs</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>775</td>
</tr>
</tbody>
</table>
ETHNIC DISTRIBUTION OF PKU CASES IN ISRAEL

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Jews Ashkenazi</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jews non-Ashkenazi</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Yemen and Aden</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>North Africa</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Iran-Iraq</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arabs</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

SCOTT (Scotland): The level of 6 mg. percent is considered the upper limit of normal blood phenylalanine in Scotland because 330 "false positives" with levels of 6-8 mg. percent were found in 15,000 infants. This gives a "false-positive" rate of approximately 1:45.

CARALSKA (Poland): Poland began screening newborns for phenylketonuria in January 1965. By April 1966, a total of 130,912 newborn babies had been screened.

PHENYLALANINE LEVELS IN BLOOD IN 130,912 NEWBORN BABIES SCREENED FOR PHENYLKETONURIA IN POLAND

<table>
<thead>
<tr>
<th>Phenylalanine Level</th>
<th>Number Screened</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 2</td>
<td>65,717</td>
<td>50.20</td>
</tr>
<tr>
<td>2</td>
<td>55,888</td>
<td>42.69</td>
</tr>
<tr>
<td>Over 2</td>
<td>7,789</td>
<td>5.95</td>
</tr>
<tr>
<td>Under 4</td>
<td>1,372</td>
<td>1.05</td>
</tr>
<tr>
<td>4</td>
<td>87</td>
<td>0.07</td>
</tr>
<tr>
<td>Over 4</td>
<td>59</td>
<td>0.04</td>
</tr>
<tr>
<td>Totals</td>
<td>130,912</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Follow-up test confirmed phenylketonuria for 21 of the 59 infants. The incidence for Poland, therefore, is 1:6,234.

The newborns were screened by the Guthrie inhibition assay method between

- 24 -
the 5th and 7th day of life when the mother and baby were discharged from
the hospital. The second Guthrie test was made when the infants were 3
weeks old, and the levels of phenylalanine were higher generally on the
second test. The Berry quantitative chromatographic method 1/ and the com-
bined chromatography-inhibition Guthrie test confirmed phenylketonuria for
21 of the 59 infants with presumed positive results. These 21 babies were
also tested by the ferric chloride method between the 6th and 8th weeks of
the infants' lives; about half the infants had negative results from the
ferric chloride test.

In January 1966, Poland began to use the enzymatic spectrophotometric LaDu
method 2/3/ for quantitative estimations of phenylalanine and tyrosine in
blood and quantitative estimations of phenylalanine in urine and started
determinations of orthohydroxyphenylacetic acid in urine by the chromato-
graphic method. (See page 56 for dietary treatment of these infants.)

KIAN (Pakistan): The total number of infants screened in Pakistan is
5,481. No cases of PKU were found. We did find four cases among the 417
mentally defectives tested.

VIS (Belgium): The screening program has just begun in Belgium. Only
7,600 infants have been screened, but five cases of PKU have been detected.

PUNEKAR (India): The All-India Institute of Mental Health in Bangalore
routinely carries out clinical and biochemical investigations as a part of
its study of phenylketonuria. In 1963, the Institute reported the first
case of phenylketonuria in India (many authors erroneously cite Bhaskaran's
study 1/ in 1952 as the earliest report of phenylketonuria in India). To
date five cases have been reported; three came from the general population,
and two from homes for the mentally retarded.

The Institute screened 165 mentally defective children during 1959-61. Only
one child gave positive urinary reaction to ferric chloride and dinitro-
phenylhydrazine tests. The incidence was 1:165. During 1962-65, 499 men-
tally defective children were screened, and no phenylketonuria was detected.
The incidence then dropped to 1:664. These figures, of course, do not give
a true figure of incidence. The only way to arrive at such a figure would

1/ Berry, H. K.: Paper Chromatographic Method for Estimation of Phenyl-
alanine. PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE,
1957, 95, 71-73.
2/ LaDu, B. N., and Michael, P. J.: An Enzymatic Spectrophotometric Method
for the Determination of Phenylalanine in Blood. JOURNAL OF THE LABORATORY
AND CLINICAL MEDICINE, 1960, 55, 491-496.
3/ LaDu, B. N., and others: A Quantitative Micromethod for the Determina-
tion of Phenylalanine and Tyrosine in Blood and Its Application in the
1/ Bhaskaran, R.: Phenyl Pyruvic Oligophrenia--A Short Review With the
be to carry out a mass screening of newborns and mentally defective children through the Guthrie test.

The first child with phenylketonuria in India was a male Hindu aged 9 months, weighing 6.5 kg., from Bangalore, Mysore. The child was referred to the All-India Institute of Mental Health in 1963 because of recent convulsions, inability to sit, and a vague feeling on the part of the mother that the child was not normal. The boy had a fair complexion, lightly pigmented skin, flaxen hair, and brown eyes. The flexures of arms and legs were covered by an eczematous rash. Detailed questioning revealed that the child had been a slow feeder since birth and had acquired neck control at 7½ months. Teeth had not appeared, and the child was unable to recognize his mother.

Neurological examination revealed opisthotonos with hyperkinesis, cranial nerve palsies, and no abnormality in sight, hearing, and fundus. The cardiovascular, respiratory, and gastrointestinal systems were noncontributory. The roentgenogram of the skull revealed no abnormality. The Wassermann reaction was negative. There were no abnormalities in the hematological and biochemical, cerebrospinal fluid, and urine constituents of the child.

Ferric chloride and dinitrophenylhydrazine tests had positive urinary reactions. The urine samples were tested further for the presence of other ketones and acetoacetic acids by performing Rothera's and Gerhardt's tests; the reactions were negative.

The blood serum of the child gave quantitative analysis of a phenylalanine concentration of 35 mg. per 100 ml.

The Institute made a presumptive diagnosis of phenylketonuria and began arrangements for a low phenylalanine diet with added tyrosine. Unfortunately the child died suddenly at home of a respiratory infection before the diet was started. A postmortem was not possible.

The father of the child was the mother's maternal uncle. Both parents appeared to be of normal intelligence. Two siblings, a female aged 5 years and a male aged 2 months (the male was born after the death of the child with phenylketonuria), appeared to be normal.

Centerwall and Kapur 2/ in 1964 described an 8-year-old Muslim girl from Kolar in Mysore State, South India. She had an IQ of 25, and her serum phenylalanine was 11 mg. percent. No family history was obtained.

Centerwall 3/ in 1965 discovered two more children with phenylketonuric characteristics. One was an 11-year-old Hindu girl from Delhi, North India; the other, a 10-year-old Hindu boy, also from Delhi but unrelated to the

3/ Centerwall, W. R. Personal communication to B. D. Punekar.

Provided by the Maternal and Child Health Library, Georgetown University
The girl had an IQ of 25 and a serum phenylalanine level of 19 mg. percent. Her parents were first cousins.

The boy had an IQ of 25 and a serum phenylalanine level of 20 mg. percent. His parents were third cousins. The younger sister of this Hindu boy was predicted to have PKU on the basis of the description given by the family of her retarded development, characteristic odor, history of convulsions, and relative fairness of coloring. No confirmation of her case has been made.

Four of the five children with phenylketonuria belonged to Hindu families. All the children were below 11 years of age. The serum phenylalanine levels for all the children were high. The IQ's were below 25. Three of the four sets of parents having children with phenylketonuria had consanguineous marriages. Three of the five children with phenylketonuria came from Mysore. The question of whether consanguineous marriages are more common in Mysore than in other parts of India needs further investigation.

It has been suggested that India has a low incidence of phenylketonuria as compared with Western countries. Some factors may be:

1. Infant mortality is still high in India.

2. In India, the incidence of common childhood diseases such as kwashiorkor, anemia, and tuberculous meningitis is high. As a result, clinicians who are overburdened in treating these common diseases often are unable to diagnose and, therefore, treat phenylketonuria.

3. Protein malnutrition is a serious problem in India for a large section of the population. The results of the screening tests for phenylketonuria are, therefore, sometimes misleading.

4. No one has yet undertaken an intensive study of the incidence of phenylketonuria in the rural areas. The predominantly village life of India lessens the burden of mentally handicapped children on society because the slow, noncompetitive rural conditions do not call attention, as urban life does, to weak intelligence and social inadaptability.

5. Mental retardation is considered a stigma in India. A large section of the people will not, therefore, take advantage of the schools, homes, and institutions meant for mentally handicapped children.

ZAVALA (Mexico): Three cases of phenylketonuria were detected in Mexico in the screening of 1,500 mentally retarded children. These were immigrants and not Mexicans.

CAHALANE (Ireland): In Ireland the present incidence of positive results from the Guthrie test is 1:314. Two of the cases were tested on the 3rd day of life, and levels of blood phenylalanine were higher than 20 mg. percent. There is no followup testing in Ireland, so theoretically some cases of phenylketonuria may be missed.
PANTELAKIS (Greece): The generalized screening programs are just getting underway in Greece.

ANDREJEVIĆ (Yugoslavia): During the period October 5, 1965, to May 23, 1966, the Mother and Child Health Institute of the Republic of Serbia screened 23,635 newborn infants for PKU. Three infants had higher than normal concentrations of phenylalanine. The incidence, as a result of this testing, was 1:7,878.

Case 1 - This infant was 18 days old when his blood was tested; he had concentrations of blood phenylalanine of 10 mg. percent. When the infant was admitted to the hospital 38 days later, his blood phenylalanine level was 45 mg. percent, urine was aromatic, and the ferric chloride test was positive. The baby had eczema, was restless and hypertonic, and had a slight tremor of the fingers. This infant had a 7-year-old sister who was mentally retarded; testing showed that she also had phenylketonuria.

Case 2 - This infant was 5 days old when blood was taken; the phenylalanine level was 7.4 mg. percent. He was admitted to the hospital 38 days later with a blood phenylalanine level of 16 mg. percent. His urine was not aromatic, and the ferric chloride test was negative.

Case 3 - This infant was tested when he was 7 days old, and he had a blood phenylalanine level of 17 mg. percent. Twenty-five days later he was admitted to the hospital with a blood phenylalanine level of 25 mg. percent.

The Institute tested these three infants three ways: by bacterial inhibition assay; one-dimensional chromatography, using the Helen Berry method;1/ and combined chromatography and inhibition assay, using the Guthrie method. One change was made in the Guthrie method: the filter strip with the dried blood spot was not immersed into distilled water.

The inhibition assay method appears to be a very sensitive and reliable screening method for detection of phenylketonuria in the hospital nursery. The ferric chloride method is not as sensitive: in the testing at the Institute, the ferric chloride test was positive while the blood phenylalanine level was 15 mg. percent or higher and negative while blood phenylalanine was 20 mg. percent. (See page 57 for the dietary treatment of these infants.)

General Comments: There should be an exchange of specimens between various laboratories to verify slightly elevated blood phenylalanine levels. The geographic differences in so-called normal levels might be related to dietary and other factors which produce the apparent differences in blood phenylalanine levels in normal infants. Certain quinone derivatives (as in vitamin K) might inhibit phenylalanine hydroxylation activity (this may be seen particularly in mature infants receiving vitamin K).

1/ Berry, H. K.: Paper Chromatographic Method for Estimation of Phenylalanine. PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 1957, 95, 71-73. - 28 -

Provided by the Maternal and Child Health Library, Georgetown University
As a consequence to the increasingly widespread use of screening programs designed to detect individuals with abnormalities in serum phenylalanine levels, a number of patients with "atypical phenylketonuria" have been encountered.1/2/3/4/

In order to facilitate the diagnosis of these "atypical" cases, we wish to propose a system of nomenclature and diagnostic criteria for patients with elevated phenylalanine levels:

**Type 1--Phenylketonuria**

1. Blood phenylalanine levels are over 20 mg. percent.

2. Ferric chloride test is positive after 2 to 30 days of age.

3. Excretion of phenylpyruvic and o-hydroxyphenylacetic acid is appropriate to the blood phenylalanine concentration.

4. Phenylalanine tolerance is very low, and blood phenylalanine rises rapidly if patient is fed much more than the daily phenylalanine requirement.

5. Biochemical abnormalities remain unchanged for the duration of the patient's life.

Type 2--Phenylalaninemia 5/

1. Blood phenylalanine levels are over 20 mg. percent.

2. Ferric chloride test is negative or only slightly positive during loading tests.

3. Excretion of phenylpyruvic and o-hydroxyphenylacetic acid is inappropriately low for phenylalanine concentration.

4. Rise in blood phenylalanine is slow following birth, requiring up to 4 weeks to reach maximum levels.

5. Phenylalanine tolerance is that of a homozygote with phenylketonuria (Type 1).

6. Phenylalanine tolerance increases slightly with age.

Type 3 6/7/

1. Blood levels during neonatal period are 20 mg. percent or higher.

2. Ferric chloride test is positive.

3. Excretion of phenylpyruvic acid and o-hydroxyphenylacetic acid is appropriate to the blood phenylalanine concentrations.

4. Biochemical abnormality decreases steadily with age, and this type may be undetectable after 2 to 3 years by measurement of fasting blood levels.

5. On phenylalanine loading test (100 mg. or 150 mg./kg.; orally), no rise in tyrosine is observed; phenylalanine is poorly metabolized (ratio of 4 hours/2 hours levels 1.0 or greater).

6. Phenylalanine tolerance is abnormal in one parent.

Type 4 8/9/10/11/

1. Fasting blood phenylalanine levels are less than 20 mg. percent.

---

5/ Kang, E. S., et al., op. cit.
7/ Efron, Mary, and Kennedy, J. Personal communication to John H. Menkes.
8/ Woolf, L. I., et al., op. cit.
11/ Menkes, John H. Unpublished data.

- 30 -
2. Ferric chloride test is negative but becomes positive during loading tests.11/

3. Excretion of phenylpyruvic and o-hydroxyphenylacetic acid is appropriate to blood phenylalanine levels.

4. Biochemical abnormality remains constant with age.

5. Phenylalanine loading test is abnormal in one parent.9/10/11/

Type 5--Tyrosyluria 12/

1. Phenylalanine levels are usually below 20 mg. percent.

2. Blood tyrosine is elevated more markedly than phenylalanine.

3. Ferric chloride test is positive.

4. Tyrosyluria is seen in premature infants and occasionally in full-term infants.

5. Phenylalanine elevation is transient; tyrosine elevation may persist for several months.

6. Phenylalanine loading tests are normal when blood level has returned to normal.

Type 6--Transient Hyperphenylalaninemia

1. Phenylalanine levels are usually below 8 mg. percent and return to normal within a few weeks.

2. Tyrosine levels are normal.

3. Subsequent phenylalanine loading tests are normal.

Type 7 13/

1. Phenylalanine and tyrosine levels are transiently high.

2. Ferric chloride test is negative.

Fasting phenylalanine levels for phenylketonuric heterozygotes (Type 1) are


almost always below 3.5 mg. percent. However, during the last trimester of pregnancy, female Type 1 heterozygotes may have hyperphenylalaninemia, but the blood level of the amino acid usually remains below 15 mg. percent.

When given a phenylalanine tolerance test, individuals heterozygous for phenylketonuria show an elevation of blood phenylalanine which is usually greater and more prolonged than normal individuals.

The incidence of the various types of hyperphenylalaninemia among 55,216 patients tested in Maryland is as follows:

<table>
<thead>
<tr>
<th>Type</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1--Phenylketonuria</td>
<td>6 (or 1:9,200)</td>
</tr>
<tr>
<td>Type 2--Phenylalaninemia</td>
<td>1</td>
</tr>
<tr>
<td>Type 3</td>
<td>2</td>
</tr>
<tr>
<td>Type 4</td>
<td>2</td>
</tr>
<tr>
<td>Type 5--Tyrosyluria</td>
<td>25 percent of all premature infants</td>
</tr>
<tr>
<td>Type 6--Transient Hyperphenylalaninemia</td>
<td>26</td>
</tr>
<tr>
<td>Type 7</td>
<td>0</td>
</tr>
</tbody>
</table>

While it is likely that Type 5 represents a defect in the cofactor responsible for conversion of phenylalanine to tyrosine, the enzymatic lesion for Types 2, 3, 4, 6, and 7 is unknown. It has been postulated that infants of Types 3 and 4 represent atypical heterozygotes for phenylketonuria (Type 1). In part, this assumption is based on the observation of the normal loading test in one of the two parents. Since it is equally likely that these forms represent a dominantly transmitted disorder, complete genetic evaluation will be required to settle the uncertainty.

17/ Menkes, J. H., and Avery, M. E., op. cit.
DISCUSSION

SZEINBERG (Israel): Type 3 patients could probably go on a regular diet at 3 years of age even with an abnormal tolerance test.

ZETTERSTROM (Sweden): One patient was given 150 mg. per kilo per day of phenylalanine, and there were no phenylketones in the urine. When the phenylalanine intake was raised to 175 mg. per kilo per day, the phenylketones appeared.

GUTHRIE (United States): Such variations in the blood levels of phenylalanine are not really remarkable; one would expect to find such a spectrum in a disease like phenylketonuria. The phenylalanine requirements vary for individuals as each handles the phenylalanine differently.

General Comments: There are questions about the reliability of testing functional development of the brain in the infant. Some broad comprehensive type of testing is necessary for adequate evaluation of the child.
The objectives of screening programs need first of all to be related to the groups involved. For the newborn, it now seems justifiable to test for high blood phenylalanine levels and probably for galactosemia and homocystinuria. Whether there are advantages in multiple screening techniques for these groups remains to be demonstrated—the Denver screening of 1,500 newborns noted only evanescent elevations of tyrosine and branched-chain amino acids.

Among other risk groups, that is, among the retarded, the emotionally disturbed, and young children with any ill-defined illness, more elaborate screening programs are required. These should include: one-dimensional paper chromatograms of serum and urine, the nitroprusside cyanide test for cystine and homocystine in urine, serum copper oxidase determination, the fluorescent galactosemic screening test, some form of ferric chloride test, and a precipitation test using CTAB (cetyltrimethylammoniumbromide) or cetylpyridinium chloride for the acid mucopolysaccharidoses.

A single experience in screening some 300 institutionalized children discovered a number with undiagnosed phenylketonuria, about one for every two known. One case of histidinemia and one case of homocystinuria were noted also. In addition, there were some apparently new conditions, including two with glycinuria, prolinuria, and hydroxyprolinuria who had normal blood levels for these amino acids. An unexpected finding was six cases of cystinuria, in four of which there was also evidence of renal tubular rejection of histidine.
GUTHRIE (United States): The inhibition assay principle 1/ has been used to develop a number of convenient agar-diffusion microbial tests. These tests have been applied to the detection of metabolites in human blood and urine as associated with various diseases or with human development. The screening test for phenylketonuria, employing Bacillus subtilis inhibited by \( \beta \)-thienylalanine, has been described. 2/

Four criteria for routine screening tests for such diseases are:

1. The test must be applicable to all newborn infants.

2. The test should be applied early, before the infant leaves the hospital nursery.

3. The method must be inexpensive, rapid, and sensitive.

4. There must be a reliable confirmation method available.

The bacterial inhibition assay method meets these criteria.

Four inborn errors of metabolism, for which early detection is desirable, are PKU, maple syrup urine disease, galactosemia, and histidinemia.

The blood specimens for all these tests are collected carefully on a special filter paper with each 7/16-inch (11 mm.) circle filled with blood from a heel puncture. These filter papers are sent to the laboratory where punched-out paper discs are tested upon a solid agar medium inoculated with the test organism. Before testing, the filter papers are subjected to a brief period in an autoclave to coagulate the blood pigments so that they will not diffuse out into the agar medium and interfere with the reading of test results. These test materials are very inexpensive, and the agar culture medium is easy to prepare. The melted agar medium is inoculated with Bacillus subtilis spores, poured into a large, flat dish, and allowed to harden. Rows of


- 35 -
blood discs are placed upon the agar surface along with blood discs of known amounts of the appropriate amino acids to serve as controls.

In PKU, blood phenylalanine is detected by inhibiting Bacillus subtilis with $\beta$-2 thienylalanine as a phenylalanine antagonist at $1.5 \times 10^{-5}$M. A growth zone will then appear around the paper disc in response to any phenylalanine present. This test already has received extensive trial.

In maple syrup urine disease, the branched-chain amino acid, leucine, is detected by using 2-methylleucine as an antagonist to inhibit Bacillus subtilis. After experiments, 2-methylleucine was selected with a dozen different chemical analogs of the branched-chain amino acids and with several species of Bacillus. In a concentration of $10^{-5}$M, 2-methylleucine is used in the same minimal culture medium used in the PKU test.

For histidinemia, Bacillus subtilis is inhibited by azaserine, a histidine-antagonist in this organism, by using $2 \times 10^{-5}$M concentration. Azaserine is rather unstable, and the concentration range that produces a satisfactory result is rather restricted. Nevertheless, this is the only inhibitor found so far that permits a satisfactory inhibition assay for histidine with this microorganism.

As with the PKU test, in these tests for maple syrup urine disease and histidinemia, levels of the amino acids can be estimated over a range of 1-20 mg. percent.

For all three tests, dried Bacillus subtilis spores ("instant bacteria") was employed as the inoculum, thus avoiding the problem of maintaining bacterial stock cultures. None of the usual sterile precautions are required during the procedure. These features permit laboratories unfamiliar with bacteriological techniques to easily use these tests.

The test for galactosemia is based upon a different principle. This is designated a "metabolite-inhibition assay" because the substance tested for--galactose--specifically inhibits the test organism. Dr. Kenneth Paigen of Roswell Park Memorial Institute suggested the use of this "galactosemic" mutant of Escherichia coli, strain W-3101 (isolated by Dr. E. Lederberg). This mutant has a specific deficiency in the same enzyme, uridyl transferase, as is the case in the human galactosemic mutant for which we test. Its growth is inhibited when galactose enters the bacterial cell, is converted by galactokinase to galactose-1-phosphate, which then accumulates. This is the same phenomenon that occurs in the human galactosemic where the freely diffusible galactose molecules are converted to galactose-1-phosphate and the latter nondiffusible substance is trapped inside the cells. Because of these properties of galactose-1-phosphate, the dried blood spots are not autoclaved as has been the previous practice with the other tests. Instead, they are treated with glacial acetic acid vapor to split any accumulated galactose-1-phosphate to free galactose. Such treatment also fixes the blood pigments.

The cells of this Escherichia coli strain can be dried also by "freeze drying," or by a more simple procedure with "instant bacteria," which offers the
same convenience as dried bacterial spores. In this test, the blood disc is surrounded by an inhibition zone proportional to the combined blood galactose and galactose-1-phosphate.

Current investigation is directed toward a "multiple test" for a number of rare inherited conditions associated with elevation in a blood constituent, such as methionine, glycine, proline, hydroxyproline, tyrosine, tryptophan, citrulline, and sarcosine. A mixed inoculum, prepared from several amino acid-requiring mutant auxotrophs, would be used as a multiple screening test requiring but a single spot of blood from each infant. Such a test would be designed to show a positive response to a single blood disc from an infant with any one of these rare conditions. This procedure may make practical the screening of all newborns for many inborn errors, although each condition alone would be too rare for a separate procedure. Use of mutants, each with a genetic block early on the biosynthetic chain, will expand the test responses to include other possible human inborn errors not yet actually discovered.

SZEINBERG (Israel): The nitroprusside cyanide test may be a personal hazard in the laboratory; perhaps some safer alternative can be devised.

Because of streaking problems, Tel-Hashomer had modified the original Efron technique.1/ Samples are applied in the usual way, using S&S 903 paper for collection and Whatman 3 mm. 20 cm. x 20 cm. for running. Chromatograms are run, 7 samples per sheet and 12 sheets per tank in the Shandon-type tank containing a rack for the papers. The first run is in isopropanol:water, 3:1. The papers are then dried and rerun 24 hours later using the conventional butanol:acetic acid:water, 12:3:5. Papers are stained initially with ninydrin and secondarily with diazotized sulfanilic acid for histidine and Ehrlich's for citrulline. A second paper is stained with isatin for the imino acids.

Routine chromatography is done for indoles, but the findings in 400 samples have been variable. Almost half the children had increased urinary indican, indole acetic, and indole lactic acids. This was attributed to dietary conditions.

Of special interest was the finding of indole acrylyl glycine, which was not thought to be specific. One-dimensional and, if necessary, two-dimensional chromatography of phenolic compounds is also routine for urine samples; experience has been too limited to evaluate the usefulness of the procedures.

VIS AND THIRIAR (Belgium): The availability of column chromatography of amino acids is important as a final diagnostic technique.

PANTELAKIS (Greece): Glucose-6-phosphate dehydrogenase deficiency is indirectly an important cause of kernicterus and, therefore, mental retardation.

General Comments: One should be cautious in testing dilute urine samples because the clinical tests would then be weaker than one would anticipate.
Phenylketonuria lends itself to the philosophy and practice of public health perhaps more than any other condition, disorder, or disease. With phenylketonuria, the principles of preventive medicine can be applied fully through early casefinding, treatment, followup services, genetic counseling, and basic health education.

Interest in phenylketonuria, coupled with vigorous research, has led to simplified methods of detection and treatment. First came the urine-screening method, which demonstrated that, when 10 percent ferric chloride was dropped on the urine-saturated diaper of an infant with phenylketonuria, a definitive blue-green color appeared. The urine-screening test was refined further when a commercial laboratory developed a test tape impregnated with dried ferric chloride.1/ However, it was found that, until the infant reaches 4 to 6 weeks of age, these two procedures were not reliable. Therefore, brain damage may have already occurred to some degree before treatment could be started.

In recent years, Dr. Guthrie has developed the bioassay screening procedure which semiquantitatively determines the blood level of phenylalanine.2/3/ This procedure, which can be performed with a drop of blood from the heel of the infant, may be used with reliable results when an infant is as young as 2 or 3 days of age.

Obviously, this method is highly desirable—if we believe in the premise that the earlier the treatment of phenylketonuria can be initiated, the greater the possibility that mental retardation will be prevented.4/

Since phenylketonuria is such a rare condition, the question has been raised frequently as to whether or not mass screening programs are truly justified. It is important to remember, however, that in the past 10 years the incidence of "true" phenylketonuria and/or hyperphenylalaninemia has increased from an estimated 1 in 40,000 live births to an estimated 1 in 10,000 live births at the present time. Perhaps the incidence will prove to be higher.

With the simplicity and relatively low cost of mass screening programs, early casefinding must be recognized as the humanitarian approach and, basically, a public health responsibility. Mass screening among newborn infants has already pointed out the variations in serum phenylalanine levels which heretofore were not recognized. Certainly this recognition is quite important since it has precipitated more careful evaluation before treatment is begun and has led to more careful followup.

Screening with the Guthrie test now has become a routine procedure in many of our hospitals in the United States; but, because of the rarity of phenylketonuria, the importance of the procedure often is not reinforced by the actual finding of a case. Therefore, the need to motivate hospital personnel to carry out the procedure adequately and responsibly becomes a continuing process. Public health agencies can provide this stimulus to hospital personnel through the use of films and actual demonstrations on the most effective timesaving methods. In Maryland, we have found that periodic communications, such as a monthly newsletter on the overall phenylketonuria screening program, is an effective motivation device also.

Many other countries have begun screening programs for phenylketonuria or are planning to do so. Since this is a relatively new public health responsibility, it would be extremely helpful if we could share our experiences, including problems specific to laboratory procedures, casefinding, treatment, and followup. The comments made in this paper relate to the State of Maryland. The problems faced are, nevertheless, common to most other places.

Maryland is a fairly small State, covering approximately 10,577 square miles. Eighty-one percent of its 3,465,640 population is centered in the metropolitan Baltimore and Washington areas. Of the total number of residents, 16.7 percent are nonwhite. Ninety-nine percent of all births in the State take place in one of the 49 general hospitals which are inspected and licensed by the Maryland State Department of Health.

In February 1964, the State Department of Health initiated a program of screening for phenylketonuria by introducing the Guthrie test on a pilot

4/ LaDu, Bert N.: The Importance of Early Diagnosis and Treatment of Phenylketonuria. ANNALS OF INTERNAL MEDICINE, 1959, 51, 1427-1433.
basis in four hospitals in Baltimore. In January 1965, the State Department of Health offered the procedure at no charge to all 49 hospitals in the State. As of March 1966, 44 hospitals were participating in this screening program. Each of the other five hospitals was either performing the Guthrie test or having it done by a commercial laboratory approved by the State Department of Health.

Basic Problems

The mass screening program for phenylketonuria has presented a number of problems, most of which have been resolved during the pilot period through conscientious efforts on the parts of physicians, the medical centers, and the State Department of Health. In the early stages of the program, an insufficient quantity of blood for the specimen in the Guthrie collection unit represented a major problem. One reason for this was the use of unsuitable lancets for making adequate heel punctures. This problem was solved by changing from lancets with very fine points to those with broad points.

Another problem has centered around developing adequate followup procedures to be certain that a repeat specimen is received from each infant whose initial specimen had an insufficient quantity of blood or had a phenylalanine content of 4 mg. percent or higher. As recommended by Guthrie, 4 mg. percent or higher is considered to be a presumptive positive. As the program has grown, communications have improved and these problems are decreasing.

In the early stages of the program, there was a tendency among the small hospitals whose maternity services were limited to retain blood specimens until a large number had been accumulated. This problem has been alleviated by requiring all hospitals to mail specimens daily, regardless of number.

The problems specific to the laboratory have been minor but nevertheless, bothersome. In the initial stage of the program, one of these problems was the tendency to "over-read" the Guthrie test results. Proper lighting and background for reading have reduced the day-to-day variations in test results. The laboratory also had problems with media in dehydrated form because they absorbed moisture from the atmosphere during prolonged storage. This led to difficulty in interpretation of results. This has been corrected now.

Procedures

It is very necessary to adopt sound procedures in the laboratory if the screening program is to be carried out optimally. The Maryland State Department of Health Laboratory has developed a stringent routine for routing and registering specimens for PKU testing and reporting results. This routine is outlined on page 47.
Recommended Staffing Pattern for Laboratory

The accuracy of the Guthrie test is dependent upon personnel who are sufficiently trained in the technique and who receive proper supervision. After 15 months of operation, the Maryland State Department of Health Laboratory has found that the program can be carried out optimally if the supervision and final responsibility rest with a microbiologist, preferably with experience in microbiologic assay procedures. Knowledge of the problems associated with biological assays is essential.

One laboratory assistant works under the supervision of the microbiologist. The assistant is responsible for preparing media and reagents, setting up specimens, and reading and reporting results under the supervision of the microbiologist. Experience has shown that one qualified laboratory assistant can handle approximately 300 specimens a day along with the incidental paperwork. If a clerk is provided to handle the paperwork, the laboratory assistant can process 500 specimens a day. Generally speaking, one laboratory assistant and one clerk should be able to handle a screening and followup program involving 100,000 specimens a year. Experience has shown that the laboratory assistant needs only a minimal educational background and that persons with more sophisticated educational backgrounds (for example, a college degree) tend to become bored very quickly with the routine. As a consequence, the accuracy of the test is jeopardized.

Analysis of the Maryland Program

Analysis of data obtained during the 15 months of the Maryland statewide screening program revealed that the State Department of Health Laboratory processed 55,216 specimens for the Guthrie test. This means that 80 percent of the 68,789 infants born in the State between January 1, 1965, and March 31, 1966, were tested. As previously noted, five hospitals are not participating in the statewide program, and 12,361 births took place in them during this period.

Of the 55,216 specimens processed by the State Department of Health, 1,694 initial specimens (or 306 per 10,000 specimens received) were unsatisfactory; repeat tests were made. As expected, most (or 9,989 per 10,000 specimens) had phenylalanine levels in the range of 2 mg. percent or less. Forty specimens (or 7 per 10,000 specimens) were in the 4-mg.-percent range; repeat tests had results in the normal range for all 40. Twenty-four specimens (or 4 per 10,000 specimens) had readings of 6 mg. percent or higher.
ANALYSIS OF SERUM PHENYLALANINE AND TYROSINE FOLLOWUP
ON 24 INFANTS WHOSE INITIAL GUTHRIE TEST RESULTS WERE
6 MG. PERCENT OR HIGHER

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Guthrie Test Results</th>
<th>LaDu Serum Followup</th>
<th>Dietary</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Normal a/</td>
<td>Normal a/</td>
</tr>
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<td>6</td>
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<td>Normal a/</td>
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<td>Normal a/</td>
<td>Normal a/</td>
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<td>Normal a/</td>
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<td>6</td>
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<td>Normal a/</td>
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<td>Greater than 20 b/</td>
<td>Normal a/</td>
</tr>
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<td>11</td>
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<td>Normal a/</td>
<td>31.2 c/</td>
</tr>
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<td>12</td>
<td>6-10</td>
<td>Normal a/</td>
<td>31.1 c/</td>
</tr>
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<td>6-10</td>
<td>Normal a/</td>
<td>5.5</td>
</tr>
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<td>14</td>
<td>6-10</td>
<td>Greater than 10 d/</td>
<td>Normal a/</td>
</tr>
<tr>
<td>15</td>
<td>6-10</td>
<td>Normal a/</td>
<td>Normal a/</td>
</tr>
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<td>6-10</td>
<td>Normal a/</td>
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<tr>
<td>24</td>
<td>Greater than 20</td>
<td>Greater than 20</td>
<td>Normal a/</td>
</tr>
</tbody>
</table>

a/ Normal range is less than 4 mg. percent.
b/ Serum phenylalanine remained at 6-8 mg. percent with patient on normal diet at 8 months of age.
c/ Tyrosine levels returned to normal within 1-4 weeks.
d/ Serum phenylalanine remained at 6-mg.-percent level without treatment.
The table on the preceding page shows an analysis of the results of a serum phenylalanine followup on 24 infants whose initial Guthrie test results were 6 mg. percent or higher. Cases 1 through 10 had initial Guthrie results of 6 mg. percent. Results of subsequent serum phenylalanine analyses by the LaDu method were in the normal range for eight of the infants and above 20 mg. percent for two. These two infants were placed on treatment. The treatment was discontinued for one of them at the age of 8 months; when he was hospitalized for reevaluation and placed on a normal diet, his phenylalanine level remained between 6 and 8 mg. percent.

Cases 11 through 16 had initial Guthrie results of 6-10 mg. percent. Serum analyses revealed elevated tyrosine levels but normal phenylalanine levels in three cases. The tyrosine levels returned to normal within a few weeks. The other three cases had normal phenylalanine and tyrosine levels by the LaDu method.

Cases 17 through 24 had initial Guthrie test results greater than 20 mg. percent, and the serum analysis for phenylalanine was above 20 mg. percent for all. These infants were placed on treatment where they have remained.

In summary, a total of nine infants are now being treated. One of these had an initial Guthrie result of 6 mg. percent, and eight had initial Guthrie test results of greater than 20 mg. percent. Of interest is the fact that three of these infants are Negroes; determinations about white ancestry have not been completed.

### RESULTS OF PSYCHOLOGICAL EVALUATIONS OF FIVE INFANTS WITH PHENYLKETONURIA AT 8 MONTHS OF AGE

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Psychometric Results</th>
<th>Age Treatment Started</th>
<th>Control</th>
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</thead>
<tbody>
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<td>1</td>
<td>Suspect</td>
<td>1 month</td>
<td>Poor</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
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<td>Good a/</td>
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<tr>
<td>3</td>
<td>Normal</td>
<td>1 month</td>
<td>Good a/</td>
</tr>
<tr>
<td>4</td>
<td>Suspect to Normal</td>
<td>1 week</td>
<td>Good a/</td>
</tr>
<tr>
<td>5</td>
<td>Normal</td>
<td>21 days</td>
<td>Good a/</td>
</tr>
</tbody>
</table>

a/ Good control is maintenance of phenylalanine level of 2-6 mg. percent.

The above table shows the results of psychological followup evaluation for five of the nine infants who had diagnoses of "true" phenylketonuria and are being treated. A psychologist experienced in infant testing made the
evaluations when all the infants were 8 months of age. The results were inconclusive but encouraging in that none of the babies showed marked retardation.

Three infants were considered to be well within the normal range of performance for their age. Dietary treatment was begun for these three at 16 days, 21 days, and 1 month, respectively. The control was considered good, with serum phenylalanine levels staying in the range of 2-6 mg. percent. One infant was performing in the "suspect" range at 6 months, and his dietary control was considered poor. The fifth infant was functioning in the "suspect" to normal range; the dietary control was considered good for this infant. It should be noted that the fifth baby was being cared for in an adoption institution, and some of the "suspect" performance may relate to this fact.

Public Health Responsibility

Public health agencies must go beyond simple casefinding provided through mass screening programs. To this end, participation in the preventive aspects of total case management is imperative.

In most instances, it is impossible for medical centers to offer the necessary followup services in the homes of patients with phenylketonuria. Yet it is the dietary control of the infant in his own home that is the true test of the effectiveness of the treatment. Experience has shown that most mothers of young infants with phenylketonuria are frightened and bewildered. In order to overcome these obstacles to proper management, support for the mother is needed. This support is rarely provided through a weekly or even monthly visit to a clinic or hospital. The occasional pediatrician in private practice who chooses to treat his own cases can rarely give this continuing support which is needed to assure successful treatment.

Public health agencies must be accepted as an important part of a multidiscipline approach. They can provide effectively the continuing support to the family through broad services which are not limited to the indigent. Whether these services are successful, however, depends upon an intensive education program for professional public health personnel as to the nature of phenylketonuria and how they can give assistance to the physician and medical center, as well as to the parent in the home. In most instances, the public health nurse, with proper orientation, can give guidance to the parents of children with phenylketonuria. She can very effectively maintain a continuing liaison with the medical center and practicing physician, keeping them informed of specific problems, illnesses, family crises, or any other deterrents to the effective treatment and individual case management.

Public health agencies should be prepared also to provide continuing nutritional consultation services through the medical center or physician managing the case. Such consultation may be in the form of direct service to the family of the patient or to the public health nurse who will make frequent visits to the home.

- 45 -

Provided by the Maternal and Child Health Library, Georgetown University
To go beyond mass screening of infants, further casefinding can be provided through family studies; it is in this area that public health agencies can make a major contribution. However, it is difficult, if not impossible, for medical centers and private physicians to reach beyond the immediate family of the patient for further screening for phenylketonuria. Public health agencies are better equipped for this kind of surveying. Because of its genetic pattern, the high-risk group includes not only the parents and siblings, but relatives as well.5/

The survey brought out another important aspect which must receive emphasis in program planning—the need for health education. A questionnaire prepared to find out just how much the parents of children with confirmed phenylketonuria knew about the condition revealed that:

1. Sixty-one percent did not know that the disorder was inherited.

2. Fifty-eight percent did not understand the importance of early diagnosis.

3. Fifty-six percent had never discussed the condition with a professional person.

4. Fifty-six percent did not know that the condition can be treated with a special diet.

This point was demonstrated by the Maryland State Department of Health 2 years ago when it undertook a survey within the State of families and close relatives of persons known to have phenylketonuria. Parents, siblings, first and second cousins, and, in a number of instances, aunts and uncles were screened, using the ferric chloride urine test. After screening 258 individuals in this high-risk population, 16 additional cases of phenylketonuria were confirmed.6/ Six of these cases had not been diagnosed previously. All six were children under 12 years of age, and each was severely mentally retarded. It was difficult to understand why a simple ferric chloride screening procedure had never been carried out on any of these children. In any case, the results of this survey underscored the fact that screening of relatives of patients with phenylketonuria should be an integral part of public health phenylketonuria detection programs.

Casefinding through screening procedures should be expanded further by physicians and public health agencies to include all patients with neurological

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difficulties, such as convulsive disorders. It has been stated that 25 percent of patients with phenylketonuria have seizures. Hence, the diagnosis of epilepsy may result in overlooking the possibility of phenylketonuria. An abnormal electroencephalogram should not lead to ruling out phenylketonuria because 79 percent of individuals with phenylketonuria have abnormal EEG's.

Finally, public health agencies should assume the responsibility for screening all mentally retarded children in the special classes of public and private schools. Such a practice would uncover other high-risk populations, many of whom are in the childbearing age and might benefit from genetic counseling, along with health education.

While this paper concerns itself with casefinding as it relates to phenylketonuria, it is not intended to overlook the possibilities for other metabolic diseases. The public health application of broader screening procedures awaits only the proven results of research. The future of this program represents a true challenge. Only through a close cooperative relationship among research centers, medical centers, and practicing physicians can a public health department contribute to its fullest potential. Its success will be dependent upon an open line of communication among all concerned. With this achieved, we can hope to fulfill our obligation to humanity through the prevention of diseases and handicapping conditions.

Procedures of the Maryland State Department of Health Laboratory for Routing and Registering Specimens for PKU Testing and Reporting Results

A. Receipt

1. All specimens received for PKU testing are sent immediately to the Bioassay Laboratory for numbering and processing.

2. In the Bioassay Laboratory, the specimens are numbered consecutively (with duplicate numbers on the history form and the filter strip).

3. The numbered history forms are separated from their filter strips.

   a. The history forms are sent to the Registration Office, where a report sheet on which the specimens are listed in numerical order is prepared for each hospital.

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b. The Bioassay Laboratory retains the filter paper strips for further processing.

B. Reporting

1. Results of the PKU tests are recorded on report sheets, which are then signed and dated by the person reading the results. The report sheets, with the appropriate history forms, are returned promptly to the Registration Office so that the results can be transmitted to the hospitals.

2. One copy of each report sheet is retained in the Bioassay Laboratory and is filed under the name of the hospital. For specimens received from private physicians, county health departments, or sources other than hospitals, the history forms are filed in the Bioassay Laboratory in alphabetical order by the patients' names.

C. Followup

1. Any specimen found "Unsatisfactory" for any reason is reported on a Blood Phenylalanine Followup Form. A copy of the form is sent to the hospital or person submitting the specimen and to the health department in the county where the mother resides. A copy is filed in the Incomplete File until a satisfactory specimen is received. If a specimen is not received within 2 weeks, appropriate action is taken by the State Department of Health.

2. For specimens found to have phenylalanine levels of 4 mg. percent by the Guthrie technique, a request for a second blood filter paper card is made on a special form. Copies are sent to the hospital or person submitting the specimen and to the health department of the county where the mother resides. A copy of this report is filed in the Incomplete File until a followup specimen is received. If the followup specimen is not received within 2 weeks, appropriate action is taken.

3. For specimens found to have phenylalanine levels of 6 mg. percent or higher by the Guthrie technique, a request for a clotted-blood specimen is made by the same procedure as described in Step 2 above. A copy of this request is filed in the Incomplete File until a followup specimen is received.

DISCUSSION

ČUPIĆ (Yugoslavia): Forty percent of the infants in Yugoslavia are born in the home; this, therefore, presents a difficulty in screening the newborns for phenylketonuria. Perhaps specimens for screening this group could be obtained when the health teams went into the homes to vaccinate with BCG.
SCOTT (Scotland): There has been a general tendency in Scotland for physicians not to appreciate the significance of the results of the Guthrie testing. Another problem is that in Great Britain technicians must have 6 years' training; this requirement makes it difficult to recruit personnel to perform a monotonous routine procedure. Eighty percent of the births occur in hospitals, where infants remain until 6-8 days of age.

FARQUHAR (Scotland): Fifteen percent of the infants are born at home in Edinburgh. The chart on the next page shows the arrangement to insure the screening of infants born at home.
PROCEDURE USED IN SOUTHEAST SCOTLAND FOR GUTHRIE TEST

To medical health officer

Notification of birth register

MEDICAL HEALTH OFFICER

Register of at-risk families

TEST PAPERS

top copies should include place-name of domicile

STOBHILL HOSPITAL BACTERIOLOGIST

Blood specimens and patient information

PKU cases diagnosed later

MATERNITY UNITS
day-6 discharges and earlier ones after 48 hours

HOME deliveries and 48-hour discharges

More risk of families with PKU cases
diagnosed later

Provided by the Maternal and Child Health Library, Georgetown University
Sixty-eight cases of phenylketonuria were registered at Marburg between 1960 and March 1966. Forty-five of these patients are on a diet at the present time. The dietary treatment of the oldest 13 children was stopped for a variety of reasons. Ten children had severe cerebral damage and, therefore, were not treated.

The phenylalanine requirements of normally developing children were determined for various age groups up to 6 years by means of a column chromatography and microbiologic determinations. For an infant, the requirements were determined to be 30-50 mg. phenylalanine per kg. per day; for the 2- and 3-year-olds, 20-30 mg. phenylalanine per kg. per day; and for those 4-6 years old, 10-20 mg. phenylalanine per kg. per day.

When diet is started in the first 3 months of life, the child with phenylketonuria ultimately obtains a normal IQ. When diet is started between the 4th and 6th months, the IQ either remains stable or improves slightly.

No child at Marburg obtained a completely normal developmental quotient. When the diet was started between the 7th and 12th months, the children already had severe developmental retardation. Improvement was possible occasionally. Only one child became worse; the conditions of his biochemical control were, however, very poor.

If dietary treatment is started in the 2nd year of life, the developmental quotient may rise 40 points or more. The average improvement at Marburg was 20 points; final developmental quotients ranged between 23 and 88.

If treatment is started at 3 years of age or later, good results are possible but rare.

If the diet is started after 7 years of age, it is possible to prevent the slow, progressive deterioration of the untreated course of the disease. We believe the deterioration continues until puberty. In contrast to the mental retardation, one can, however, influence a few reversible or directly toxic symptoms, even in the adult. These are, for instance, cerebral disturbances of affect and behavior, extrapyramidal hyperkinesia, electroencephalographic changes, seizures, skin and hair color and eczema.
With good biochemical control (0.8 to 4.0 mg. phenylalanine), the amino acid pattern in the blood remains normal under the diet. In overtreated cases (phenylalanine levels at less than 0.5 mg. percent), one obtains a generalized aminoacidemia and aminoaciduria. With phenylalanine levels at greater than 8 mg. percent, an isolated phenylalaninemia is observed. Osteodystrophy may occur. The growth in terms of height and weight is normal in untreated children, but remains somewhat below average in children on a diet. The electroencephalographic findings frequently improve with treatment but, however, do not parallel intellectual development.

Every child with phenylketonuria diagnosed in his 1st or 2nd year should be treated. "Formes frustes" with blood levels above 8 mg. percent also should be treated so long as there is no agreement on the "danger level" of phenylalanine or other metabolites for the brain. Levels below 8 mg. percent should be followed carefully but not treated. Three- and four-year-old patients should be treated for a trial period of 1 year. Children 5 years and older should be treated only when their IQ's are above 40, and then for a trial period of 1 year.

The age at which diet can be terminated is uncertain. Perhaps it is possible to place 7- or 8-year-old children with PKU on a low-protein diet if they are followed closely.

Biochemical control should aim at phenylalanine levels of 1-3 mg. percent, with a maximum of 4 mg. percent. During fever and inflammatory diseases, a supplement of 30-50 grams of milk per day is necessary. Overtreated patients can be better recognized by column chromatographic analysis of blood and urine than by blood phenylalanine estimations.

**DISCUSSION**

COHEN (Israel): The Tel-Hashomer Unit has treated a total of 19 children with phenylketonuria (see p. 22 for discussion of laboratory screening). Three infants have been on treatment only 3 months and are not included in this discussion. Eight infants died of infection. The remainder of the 49 were considered too old or too retarded to benefit from therapy.

The charts on pages 53-54 show the ethnic groups with which Israel has had to work.
## ETHNIC DISTRIBUTION OF INFANTS WITH PHENYLKETONURIA DIAGNOSED BY THE TEL-HASHOMER UNIT

<table>
<thead>
<tr>
<th>Origin</th>
<th>Number Sibships</th>
<th>Number Children</th>
<th>Number Consanguineous Families</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Affected</td>
<td>Affected</td>
<td></td>
</tr>
<tr>
<td>Yemenite</td>
<td>15</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>Iranian and Afghan</td>
<td>7</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Iraqi</td>
<td>4</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>North African</td>
<td>6</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Arab</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ashkenazi</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>33</strong></td>
<td><strong>49</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>

## CHILDREN WITH PHENYLKETONURIA IN ISRAEL

<table>
<thead>
<tr>
<th>Origin</th>
<th>Number PKU Cases Diagnosed</th>
<th>Number Probable PKU Cases Died, Not Examined</th>
<th>Total Number PKU Cases</th>
<th>Number Independent Genetic Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yemenite</td>
<td>23</td>
<td>5</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>Iranian and Afghan</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Iraqi</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>North African</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Arab</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>51</strong></td>
<td><strong>6</strong></td>
<td><strong>57</strong></td>
<td><strong>30</strong></td>
</tr>
</tbody>
</table>
FAMILIAL STUDIES IN ISRAEL

<table>
<thead>
<tr>
<th>Number Infants With Phenylketonuria</th>
<th>Number Normal Siblings Examined by Guthrie Test</th>
<th>Number Examined</th>
<th>Number Other Normal Family Members Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probands</td>
<td>Secondary Cases</td>
<td>Total</td>
<td>Examed</td>
</tr>
<tr>
<td>Pedigree known</td>
<td>32</td>
<td>20</td>
<td>52</td>
</tr>
<tr>
<td>Pedigree unknown</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Totals</td>
<td>36</td>
<td>20</td>
<td>56</td>
</tr>
</tbody>
</table>

Tel Hashomer treated only children under 3 years of age at the onset of the program but recently decided to try therapy for older children also.

The usual practice was to hospitalize these cases; put the infants on a normal diet; and carry out within 2-4 days after admission general and neurological examinations, urine examination for phenylpyruvic and orthohydroxyphenylacetic acid, chromatography of urines for amino acids and indoles, blood test for phenylalanine levels, and other routine hematological and biochemical investigations. An EEG was obtained on the first psychological examination.

The children were then started on low phenylalanine diets by accepted procedures using Lofenalac® and discharged as soon as the blood phenylalanine levels were stabilized at 2-6 mg. percent. This usually takes 2-4 weeks.

Followup consisted of weekly Guthrie testing of specimens sent by mail and monthly attendance at the hospital for physical and psychological examinations until the child was 12 months of age. The children were examined every 3 months up to 2 years and then at 3-6 month intervals; the Guthrie testing of specimens sent by mail were continued on a monthly basis.

The main problems in Israel were:

1. **Defective Physical Development and Lack of Weight Gain** - This may have been caused by unduly strict control with blood phenylalanine levels below 2 mg. percent. Tel-Hashomer now aims at 2-6 mg. percent on Guthrie testing.

2. **Food Stealing** - This became especially marked after the infants were 2 years old, walking well, and socially independent. Solids, except for fruits and vegetables low in phenylalanine, were difficult to supply.
There was no suitable bread substitute. The low-phenylalanine bread mix available from the University of Toronto in Canada was very expensive--$1.50 a loaf--and, because of inadequate refrigerated storage in the homes, became inedible within 1-2 days. A low-protein biscuit from Liga Foods in Holland was acceptable but rather expensive. Baking bread locally proved unsuccessful.

3. Vomiting - This has been troublesome and on occasion has been associated with low phenylalanine levels. Usually no explanation was found. The vomiting has been severe enough occasionally for hospitalization and intravenous therapy. Anti-emetic drugs were of no value.

4. Social Contact - In some cases, children were kept out of kindergarten to avoid dietary indiscretions; this possibly resulted in a limiting effect on their development. Tel-Hashomer tried to impress on the parents the importance of normal social contact for the children with phenylketonuria.

5. Hyperkineticity - This was not as troublesome as reported elsewhere. Weaker children were particularly affected but only mildly, and drug therapy was not needed. The specific ethnic or cultural background may explain this; this is being investigated further.

RESULTS OF PSYCHOLOGICAL TESTING OF INFANTS WITH PHENYLKETONURIA TREATED WITH DIET AT THE TEL-HASHOMER UNIT

<table>
<thead>
<tr>
<th>Age at Start of Treatment</th>
<th>Present Age</th>
<th>Mean DQ or IQ Before Treatment</th>
<th>Present Mean DQ or IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I - Diet started before infants were 3 months of age.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>2 months</td>
<td>13 months</td>
<td>116</td>
</tr>
<tr>
<td>Case 2</td>
<td>17 days</td>
<td>15 months</td>
<td>108</td>
</tr>
<tr>
<td>Case 3</td>
<td>16 days</td>
<td>20 months</td>
<td>90</td>
</tr>
<tr>
<td>Case 4</td>
<td>17 days</td>
<td>19 months</td>
<td>80</td>
</tr>
<tr>
<td>Case 5</td>
<td>10 days</td>
<td>30 months</td>
<td>83</td>
</tr>
<tr>
<td>Case 6</td>
<td>12 days</td>
<td>25 months</td>
<td>50</td>
</tr>
<tr>
<td>Case 7</td>
<td>2(\frac{1}{2}) months</td>
<td>5 years a/</td>
<td>50</td>
</tr>
</tbody>
</table>

Provided by the Maternal and Child Health Library, Georgetown University
<table>
<thead>
<tr>
<th>Age at Start of Treatment</th>
<th>Present Age</th>
<th>Mean DQ Before Treatment</th>
<th>Present Mean DQ or IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group IIA - Diet started when children were between 3-24 months of age; children responded to treatment.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 8</td>
<td>9 months</td>
<td>5 years, 7 months</td>
<td>60</td>
</tr>
<tr>
<td>Case 9</td>
<td>16 months</td>
<td>3 years, 3 months</td>
<td>40</td>
</tr>
<tr>
<td>Case 10</td>
<td>3 months</td>
<td>3 years, 9 months</td>
<td>62</td>
</tr>
<tr>
<td>Case 11</td>
<td>2 years</td>
<td>3 years, 9 months</td>
<td>30</td>
</tr>
<tr>
<td>Case 12</td>
<td>9 months</td>
<td>2 years, 3 months</td>
<td>50</td>
</tr>
<tr>
<td>Case 13</td>
<td>7 months</td>
<td>5 years, 6 months</td>
<td>30</td>
</tr>
</tbody>
</table>

Group IIB - Diet started when children were between 3-24 months of age; children did not respond to treatment.

Case 14 | 1 year, 7 months | 3 years, 3 months | 28 | 34 |
Case 15 | 1 year, 7 months | 4 years | 20 | 20 |
Case 16 | 1 year, 6 months | 6 years | 15 | 30 |

a/ Diet terminated at 4½ years.

The IQ's of the children treated at the Tel-Hashomer Unit were lower than for children at other centers. The unsuitability of tests for the ethnic groups in Israel may be the reason. Normal siblings in families in Israel also tested out at these levels.

Tel-Hashomer stressed the use of a dietitian in controlling the child's diet. Visits to the homes by a dietitian and psychologist during this last year have improved markedly the level of control. Spot checks on the families were most useful. The dietitian also collected blood specimens for Guthrie testing on his visits. The services of the dietitian and psychologist were made available to other doctors and hospitals wanting to treat their patients with phenylketonuria. Maintenance of interest in the screening program by hospitals and doctors was regarded as essential.

CABALSKA (Poland): Each baby found to have phenylketonuria began his dietary treatment in the clinic (see page 24 for a discussion of laboratory screening and diagnosis).
The stay in the hospital was generally 4-6 weeks. When phenylalanine and its metabolites disappeared from urine, when the blood phenylalanine level decreased to normal values, when the baby was eating well and gaining in weight, he was discharged from the clinic. The dietary treatment at home was systematically controlled by the Guthrie inhibition assay and ferric chloride urine tests.

The development of all babies was evaluated by:

1. General pediatric examination; measurements of weight, height, circumference of head and chest.
2. Neurological control.
3. Psychological control.
4. Electroencephalogram.

The oldest group of babies with phenylketonuria seem to have normal somatic and psychic development.

VULOVIĆ (Yugoslavia): The Mother and Child Health Institute of the Republic of Serbia hospitalized the three infants with phenylketonuria (see page 28 for a discussion of the laboratory screening and diagnosis).

The amino acid blood levels of these infants are shown in the following chart.

All three infants at the age of 6-8 weeks were placed on a low phenylalanine diet of Lofenalac® or Cymogran®. In order to attain an optimal concentration of phenylalanine in the blood, Lofenalac® or Cymogran® was supplemented with powdered milk, vegetables, eggs, cereals, and juices.
AMINO ACID BLOOD LEVELS OF INFANTS WITH PHENYLKETONURIA IN YUGOSLAVIA

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Case 1 Mn./l</th>
<th>Case 1 Mg./100 ml.</th>
<th>Case 2 Mn./l</th>
<th>Case 2 Mg./100 ml.</th>
<th>Case 3 Mn./l</th>
<th>Case 3 Mg./100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine + Ornithine</td>
<td>236.5</td>
<td>3.5</td>
<td>400.0</td>
<td>5.8</td>
<td>250.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Histidine</td>
<td>109.8</td>
<td>1.8</td>
<td>84.8</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>46.4</td>
<td>0.8</td>
<td>80.0</td>
<td>1.4</td>
<td>108.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>10.3</td>
<td>0.14</td>
<td>11.2</td>
<td>0.15</td>
<td>5.25</td>
<td>0.1</td>
</tr>
<tr>
<td>Throneine</td>
<td>79.3</td>
<td>0.9</td>
<td>206.4</td>
<td>2.5</td>
<td>87.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Serine</td>
<td>257.6</td>
<td>2.7</td>
<td>524.8</td>
<td>5.5</td>
<td>453.2</td>
<td>4.8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>154.1</td>
<td>2.3</td>
<td>124.8</td>
<td>1.8</td>
<td>206.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Proline</td>
<td>131.1</td>
<td>1.5</td>
<td>416.0</td>
<td>4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>115.0</td>
<td>0.9</td>
<td>174.4</td>
<td>1.3</td>
<td>157.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Alanine</td>
<td>195.5</td>
<td>1.7</td>
<td>491.2</td>
<td>4.4</td>
<td>210.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Cystine</td>
<td>trace</td>
<td></td>
<td>trace</td>
<td></td>
<td>trace</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>213.9</td>
<td>2.5</td>
<td>456.0</td>
<td>5.3</td>
<td>199.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>8.0</td>
<td>0.1</td>
<td>59.2</td>
<td>0.9</td>
<td>11.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>54.1</td>
<td>0.7</td>
<td>152.0</td>
<td>2.0</td>
<td>73.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>78.2</td>
<td>1.0</td>
<td>248.0</td>
<td>3.3</td>
<td>131.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>69.2</td>
<td>1.25</td>
<td>198.4</td>
<td>3.6</td>
<td>52.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1751.5</td>
<td>28.9</td>
<td>1281.6</td>
<td>21.2</td>
<td>1438.5</td>
<td>23.8</td>
</tr>
</tbody>
</table>
# Amino Acid Content of Selected Foods

<table>
<thead>
<tr>
<th></th>
<th>Lactovit Powder</th>
<th>Lactacid Powder</th>
<th>Powdered Milk-Osjeck</th>
<th>Pasteurized Milk</th>
<th>Fresh Carrots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>7.37</td>
<td>7.85</td>
<td>8.60</td>
<td>7.96</td>
<td>0.45</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.51</td>
<td>2.83</td>
<td>2.92</td>
<td>2.28</td>
<td>0.45</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.67</td>
<td>3.56</td>
<td>3.67</td>
<td>3.40</td>
<td>0.60</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>7.51</td>
<td>8.06</td>
<td>9.65</td>
<td>7.38</td>
<td>3.02</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.14</td>
<td>4.29</td>
<td>4.92</td>
<td>3.98</td>
<td>0.45</td>
</tr>
<tr>
<td>Serine</td>
<td>4.93</td>
<td>5.49</td>
<td>5.50</td>
<td>5.12</td>
<td>0.60</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>21.34</td>
<td>23.85</td>
<td>23.75</td>
<td>21.58</td>
<td>2.87</td>
</tr>
<tr>
<td>Proline</td>
<td>9.16</td>
<td>9.69</td>
<td>10.03</td>
<td>9.08</td>
<td>0.45</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.93</td>
<td>2.06</td>
<td>2.07</td>
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<td>Alanine</td>
<td>3.30</td>
<td>3.38</td>
<td>3.70</td>
<td>3.40</td>
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<td>Valine</td>
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<td>Leucine</td>
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<td>Tyrosine</td>
<td>4.92</td>
<td>5.23</td>
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<tr>
<td>Phenylalanine</td>
<td>4.93</td>
<td>4.93</td>
<td>4.82</td>
<td>4.98</td>
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## AMINO ACID CONTENT OF SELECTED FOODS

<table>
<thead>
<tr>
<th></th>
<th>Lactovit Powder</th>
<th>Lactacid Powder</th>
<th>Powdered Milk-Osiek</th>
<th>Pasteurized Milk</th>
<th>Fresh Carrots</th>
<th>Percentage of Fresh Weight</th>
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<tbody>
<tr>
<td><strong>Lysine</strong></td>
<td>1.58</td>
<td>1.83</td>
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<tr>
<td><strong>Histidine</strong></td>
<td>0.54</td>
<td>0.66</td>
<td>0.82</td>
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<tr>
<td><strong>Arginine</strong></td>
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<td>0.98</td>
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<tr>
<td><strong>Valine</strong></td>
<td>1.36</td>
<td>1.48</td>
<td>1.95</td>
<td>0.22</td>
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<tr>
<td><strong>Methionine</strong></td>
<td>0.38</td>
<td>0.58</td>
<td>0.13</td>
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<td>0.01</td>
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<tr>
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<td>1.23</td>
<td>1.58</td>
<td>0.18</td>
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<tr>
<td><strong>Leucine</strong></td>
<td>2.00</td>
<td>2.27</td>
<td>2.91</td>
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<tr>
<td><strong>Tyrosine</strong></td>
<td>1.04</td>
<td>1.22</td>
<td>1.59</td>
<td>0.16</td>
<td>0.01</td>
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<tr>
<td><strong>Phenylalanine</strong></td>
<td>1.05</td>
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<td>1.35</td>
<td>0.18</td>
<td>0.02</td>
<td></td>
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</table>

| **Protein percentage** | 21.5            | 23.3            | 28.0                | 35.18 g./l.      | 6.62          |
| **Moisture percentage**| 2.8             | 4.11            | 4.13                | -                | -             |
The infants' blood and urine phenylalanine levels were determined regularly (in the beginning each day and later three times each week) by inhibition assay or by one-dimensional paper chromatography. The effectiveness of the diet was tested also by the ferric chloride test. The aim of the dietary treatment was to keep the serum phenylalanine levels in the range of 3-7 mg./100 ml., the urine phenylalanine levels between 25-150 gamma/ml., and the ferric chloride tests negative. The hospital regularly examined the blood for hemoglobin and proteins. The babies had psychological examinations, EEG's, and X-rays of the wrist. Two mothers were tested and found to have normal blood serum levels of phenylalanine.

After the infants had been on diets for 3-6 days, the blood and urine phenylalanine reached the desired levels. The phenylalanine intake then was increased up to 73 mg./kg./day.

The length of stay in the hospital was determined by two factors: the environment of the infant's family and the availability of milk with low concentration of phenylalanine.

JERVIS (United States): The diet should be maintained as long as there is myelination, which is complete by the time the child is 5 or 6 years of age. Even though it is difficult, the diet should be confirmed after the child is 6 years of age.

GRÜTER (Germany): Myelination continues after 5 or 6 years of age. The diet should be maintained even after 20 years, as protein synthesis continues in the higher tracts.

PALMSTIERNA (Sweden): Attempts are made in Sweden to move families having children with phenylketonuria from the rural areas to locations nearer medical centers in order that proper treatment may be obtained for the children.

General Comments: After an infant with phenylketonuria has been on the diet for 3 months, he should then be placed on a normal diet for 1 to 2 days to determine whether the blood phenylalanine level again rises sharply and continued diet control is necessary.

In families having children with phenylketonuria, the IQ of siblings, parents, and first cousins could be used for comparative values. IQ alone is not a sufficiently broad test.

Vomiting is a complication of low blood phenylalanine levels. The addition of fatty acids to the diet helped to improve dermatitis. With low blood phenylalanine levels, there is an increase in the blood level of other amino acids, and there may be an overflow aminoaciduria.

Children treated at specialized centers respond better than those who are not.

Children do well at blood phenylalanine levels of 4-6 mg. percent. Because of the difficulty in diet control, lower blood levels are often not sought. There is also the possibility of complications from phenylalanine deficiency.
SUGGESTED FOLLOWUP

Many technical laboratories do not have the staff with the ability to interpret and give advice on the results of tests to detect metabolic disorders which produce brain damage. The participants were asked to submit a list of special laboratory tests they would be willing to carry out on specimens sent to them.

The participants of the conference unanimously suggested that the Children's Bureau, Welfare Administration, United States Department of Health, Education, and Welfare, coordinate efforts to collect data on the blood levels and development of all siblings of infants with phenylketonuria detected by screening newborns.

The participants thanked the Federal Institute of Public Health of Yugoslavia for its hospitality. A followup conference was recommended, and it was suggested that further discussion might take place in September 1967 at the First Congress of the International Association for the Scientific Study of Mental Deficiency in Montpellier, France.

The Children's Bureau agreed to send periodically to the conference participants information on screening programs and new developments.
## PARTICIPANTS

### BELGIUM

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<thead>
<tr>
<th>Dr. Michel J. Thiriar</th>
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<tbody>
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<table>
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<th>Priv. Doz. Dr. R. Grüttnner</th>
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<tr>
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<tr>
<td>Martinistrasse 52</td>
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<td>2 Hamburg 20, Germany</td>
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<tr>
<th>Dr. Vasso Deliyanni</th>
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<th>Dr. B. D. Punekar</th>
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<tr>
<td>Head of the Department of Biochemistry</td>
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<tr>
<td>All-India Institute of Mental Health</td>
</tr>
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<td>Bangalore 27, India</td>
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</table>
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Warsaw, Poland

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**SCOTLAND**

<table>
<thead>
<tr>
<th>Dr. James W. Farquhar</th>
<th>Dr. John Scott</th>
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<td>Department of Bacteriology</td>
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<td>Stobhill General Hospital</td>
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**SWEDEN**

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<th>Professor Rolf Zetterstrom</th>
<th>Dr. Hans Palmstierna</th>
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<td>Karolinska Institutet Pediatrika Kliniken</td>
<td>Associate Professor</td>
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<tr>
<td>Kronprinsessan Lovisas Barnsjukhus</td>
<td>PKU - Undersökningen</td>
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**UNITED STATES OF AMERICA**

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<tr>
<th>Dr. Robert Guthrie</th>
<th>Dr. Mitchell I. Rubin</th>
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<tr>
<td>NARC Research Associate</td>
<td>Professor of Pediatrics and Chairman of Department</td>
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<tr>
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**YUGOSLAVIA**

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<tr>
<th>Mrs. Gordana Andrejević</th>
<th>Dr. Ljubica Basta</th>
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</thead>
<tbody>
<tr>
<td>Chief of Laboratory Department</td>
<td>Pediatrician</td>
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<tr>
<td>The Mother and Child Health Institute of the Republic of Serbia</td>
<td>Maternal and Child Health Service</td>
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<tr>
<th>Dr. Marij Avčin</th>
<th>Professor Vukan Ćupić, M.D.</th>
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<tr>
<td>Professor in Pediatrics</td>
<td>Director</td>
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-65-
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conference on
inborn errors
of metabolism

May 30 - June 3, 1966
Dubrovnik, Yugoslavia
Chairmen:
Prof. Vukan Čupić
Dr. Mitchell I. Rubin
MONDAY
MAY 30, 1966

Morning

CLINICAL MANIFESTATIONS

Phenylketonuria
Galactosemia
Maple Syrup Urine Disease
Histidinemia, etc.

Importance of standard psychological evaluation (in relationship to nonaffected siblings)
Neurologic evaluation
Untreated and treated clinical course
Effect of transitory elevation of tyrosine and phenylalanine in premature infants

Presenter: Dr. George Jervis
Discussser: Dr. Donough O'Brien

Afternoon

DISCUSSION SESSION

Chairman: Dr. George Jervis

COCKTAILS
TUESDAY
May 31, 1966

Morning
LABORATORY SCREENING AND DIAGNOSIS

Screening Methods

Various methods
Reproducibility
Blood vs urine in diagnosis

Confirmation
Methods available

Presenter: Dr. Robert Guthrie
Discussers: Dr. Werner Grüter
Dr. A. Szeinberg

DISCUSSION SESSION
Chairman: Dr. Robert Guthrie

Afternoon
INTERPRETATION OF LABORATORY DATA

Significant Blood Levels
Influence of Maturity and Age
Other Conditions Producing Elevated Blood Phenylalanine
(Without Actual Phenylalanine Hydroxylase Deficiency of the Homozygous Type ("Classic PKU")

Heterozygosity
Prematurity
Elevated phenylalanine and elevated tyrosine
Others

Presenter: Dr. John Menkes
Discussers: Dr. George Jervis
Prof. Rolf Zitterstrom

DISCUSSION SESSION
Chairman: Dr. John Menkes
WEDNESDAY
June 1, 1966

Morning

SCREENING TESTS FOR OTHER CONGENITAL ABNORMALITIES

Microbiological Methods
Chromatographic Methods
Chemical Methods

Presenter: Dr. D. C. Cusworth
Discusser: Dr. Robert Guthrie

DISCUSSION SESSION
Chairman: Dr. D. C. Cusworth

Afternoon

LABORATORY ORGANIZATION

Qualifications of Personnel
Equipment
Specimen Collection
Relationship of Screening Laboratory to Community
Laboratory Reference Center

Presenter: Dr. Benjamin White
Discussers: Prof. Vukan Ćupić
Dr. John Scott

DISCUSSION SESSION
Chairman: Dr. Benjamin White

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THURSDAY
June 2, 1966

Morning

DIETARY MANAGEMENT

Types of Diets and Availability and Evaluation

Effect of Regular Diet Given for Short Intervals (for Final Confirmation of Original Diagnosis)

Adverse Effects of Low Phenylalanine Diet

How Long Is It Necessary to Use Diet?

Presenter: Dr. Werner Grüter

Discussser: Dr. Bernard E. Cohen

Afternoon

DISCUSSION SESSION

Chairman: Dr. Werner Grüter
FRIDAY
JUNE 3, 1966

SUMMARY SESSION

Each presenter will present a short summary.

Establishment of a Center to pool information gathered in different countries and to act as an information bureau to participants in the program and other interested parties (newsletter); data pooling and processing.

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Rudolf P. Hormuth

Provided by the Maternal and Child Health Library, Georgetown University
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