

ED 028 559

Recommended Guidelines for PKU Programs.

Childrens Bureau (DHEW), Washington, D.C.

Pub Date 66

Note- 18p.

EDRS Price MF-\$0.25 HC-\$1.00

Descriptors-Clinical Diagnosis, Dietetics, \*Exceptional Child Services, Family Role, Followup Studies, Identification, Infancy, Medical Evaluation, Medical Services, Medical Treatment, \*Mentally Handicapped, Nutrition, Psychological Services, \*Screening Tests, Social Services, \*Special Health Problems

A discussion of screening tests for phenylketonuria recommends and provides some data on two tests, lists five disadvantages of urine tests, and discusses three new tests. Also considered are the role of the central laboratory facility and seven suggestions for screening different types of infants at different times. Treatment or followup programs are mentioned with the focus on confirmatory tests and eight references to articles on procedures. Services included as beneficial to a comprehensive multidisciplinary program for longterm followup and care are pediatric, nutritional, nursing, social, psychological, and biochemical laboratory and consultation services. Other considerations discussed are the family of the PKU child, the clinical management of the patients, and the frequent monitoring of blood levels. (LE)

recommended guidelines

for **PKU** programs



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RECOMMENDED GUIDELINES FOR PKU PROGRAMS

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE  
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The Children's Bureau recommends that all newborns be screened for phenylketonuria (PKU). The ultimate objective of screening programs is to find affected children at a time when intervention may prevent the ill-effects of the disease, especially mental retardation.

The success of screening programs must, however, be measured in terms of the final outcome of patients placed under treatment or follow-up programs. Follow-up services are an integral part of a total program. Because of this inter-relationship, the Children's Bureau has brought together in this publication the PKU Guidelines previously issued separately under these titles:

1. Recommended Guidelines for Development of PKU Screening Programs (February 1966)
2. Recommended Guidelines for the Development of Comprehensive Follow-up Services for Children With PKU and Hyperphenylalaninemia (June 1966)

A few changes have been made in the material as originally issued, both for editorial reasons and to incorporate more recent experience. As revised, the guidelines represent current Children's Bureau recommendations for a comprehensive PKU program.

# RECOMMENDED GUIDELINES FOR PKU PROGRAMS

## SCREENING

The following elements, which are considered basic to a meaningful and efficient newborn PKU screening or detection program, reflect the recommendations of the Committee on Fetus and Newborn of the American Academy of Pediatrics <sup>1/</sup> and of the Children's Bureau Technical Committee on Clinical Programs for Mentally Retarded Children.

1. A screening or detection test should be chosen which has proven efficiency as a screening tool. It should require simplicity in collection (of sample), storage and determination.
2. Screening test determinations should be performed by adequate laboratory facilities handling sufficiently large volumes to acquire skill in recognition of abnormal findings. A system of quality control developed on a statewide or regional basis would insure reliability of results.
3. The initial test should be applicable before the newborn is discharged from the nursery.
4. A follow-up test should be obtained at a few weeks of age.
5. All presumptive positives should be checked by a specific diagnostic test for phenylalanine.
6. Adequate referral procedures to centers of treatment should be incorporated.

These essential elements of a sound program are further elaborated below.

<sup>1/</sup> Recommendation of the Committee on Fetus and Newborn for the Screening of Newborn Infants for Metabolic Diseases. Pediatrics, 35, 499, 1965.

## SCREENING TESTS

The screening procedures used should have been tried on sufficiently large numbers of newborns to disclose their efficiency as well as their limitations. In the initial field trials, the Guthrie bacterial inhibition assay was tried on over 400,000 newborns with the detection of 39 PKU's. Of these infants, 38 were detected on the initial blood spot taken at an average of 3-5 days of age. One was detected on a repeat sample taken at 4 weeks of age. The third-day blood specimen was reported negative.

The Children's Bureau is recommending the Guthrie test or the McCaman-Robins method for screening newborns at this point since both are simple and proven.

It is well to keep in mind that different filter papers have different effects on the growth zone of *B. subtilis* by the Guthrie method and Schleicher and Schuell No. 903 paper is recommended. Proline, phenylpyruvic acid, phenyllactic acid and a "TA" factor reverse inhibition by beta-2-thienylalanine, like phenylalanine.

The automated McCaman-Robins test of Hill and Summer (see An Automated Procedure for Blood Phenylalanine; *Clinical Chemistry*, 11, 541-546, 1965) provides the advantages of collection, mailing and storage of the bacterial inhibition assay and in addition makes possible determinations at the rate of 60 samples an hour.

### Urine Screening Tests

Urine screening tests have heretofore been used widely for screening of older infants and children. A screening program for newborn infants should supplement and not replace these efforts. Shortcomings of urine tests lie in the following:

- a. Urinary metabolites found in phenylketonuria are not excreted in amounts detectable by  $\text{FeCl}_3$  or salt stick tests until several weeks of life.
- b.  $\text{FeCl}_3$  diaper tests are only 95 percent accurate in PKU detection.



c. There is loss of sensitivity of the  $\text{FeCl}_3$  and buffered ferric salt stick tests on exposure of urine to time. (From 99 percent to 76 percent in 24 hours by the  $\text{FeCl}_3$  test-tube and from 94 percent to 68 percent by the diaper test in 24 hours. Sensitivity of the stick test drops from 99 percent to 91 percent in 24 hours.)

d. Samples for the more sensitive dinitrophenylhydrazine test are difficult to collect and difficult to channel to a central laboratory.

e. Urine Guthrie tests have been essentially abandoned because of the high rate of false-positive tests which render this approach impractical.

Paper chromatography is perhaps a much more reliable means of screening newborns assuming significant excretion of orthohydroxyphenylacetic acid but depends on appropriate collection of glomerular filtrate, preservation and indication of some measure of glomerular filtration rate (creatinine, 24 hour urine volume or other "common denominator" of reference).

Phenylpyruvic acid may not be excreted if blood phenylalanine levels drop below 15 mg%. Also, metabolites of certain substances, physiological and nonphysiological (such as bile pigments, aspirin, or certain maternally ingested medications that are breast-milk transmitted) interfere with most urine iron salt methods.

### Other Tests for Screening

Although widescale field trials have not been tried on several of the new methods, the relative simplicity, economy and logic of their approach may eventually make them adaptable to screening programs especially as some would detect more than PKU. We refer to three methods:

1. Efron, M. L., Young, D., Moser, H. W., MacCready, R. A. A simple chromatographic screening test for the detection of disorders of amino acid metabolism: A technic using whole blood or urine collected on filter paper. *New England Journal of Medicine*, 270, 1378-1383, 1965.

This method offers the advantage of viewing the status of nine other amino acids in addition to phenylalanine and its ready application to urine samples collected on filter paper as well as to blood samples. However, experience to date, although limited, would suggest this method is insensitive to blood phenylalanine levels under 8-10 mg%, thereby greatly limiting its reliability in screening for PKU in the newborn period.



2. Scriver, C. R., Davies, E., Cullen, A. M. Application of a simple micromethod to the screening of plasma for a variety of amino acidopathies. Lancet, ii, 230-232, 1964.

This method requires collection of samples in microHinton tubes. It may be pointed out that samples in wet form are not as stable as dried samples, require application to paper (added procedure), and present more problems in storing and mailing when used in a widespread program.

3. Smith, I. Chromatographic and electrophoretic techniques. Ed. 2, London, William Heinemann, Ltd., 1960.

It might be well to keep all of the above methods in mind and develop such policies that when these methods are adapted to large-scale screening, programs can readily switch to these procedures.

#### CENTRAL LABORATORY FACILITY

A critical point in developing a screening program is the availability of a central, good laboratory facility. A screening program adopted for large-scale use should be carried out in conjunction with a facility large enough to handle a sufficient volume of samples to detect several positive cases per year to assure skill and experience in laboratory diagnosis. The efficiency of procedure is materially increased when a single central laboratory is utilized. The development of a system of quality control on a statewide basis if several laboratories are performing tests, or on a regional basis if State, hospital or private laboratories are performing tests should be considered to insure a high degree of reliability of results.

#### PROTOCOL FOR SCREENING OF NEWBORNS

1. Sample of blood at discharge of full term baby (see Item 7).
2. Sample of blood at 2 weeks or at discharge in prematures, whichever is earlier. If under 5½ pounds at 2 weeks and initial sample negative, repeat once more at discharge.
3. Prematures with levels 4-15 mg% should have a specific test for phenylalanine and be followed every 3-4 days until the zygoty of the infant is clarified.

4. Prematures, with levels 15 mg% or higher, after a specific test for phenylalanine without elevation of serum tyrosine levels, may be given a provisional diet (restricted phenylalanine intake but not so restrictive as to result in the severe macrocytic anemia reported in one case: Sherman, J. D., Greenfield, J. B., and Ingall, D. Reversible bone-marrow vacuolizations in phenylketonuria. *New England Journal of Medicine*, 270, 810, 1964). These patients should be rechallenged with a normal diet or whole milk when they are 5½ pounds in order to establish or discard the diagnosis.

5. Full-term infants with serum phenylalanine levels of 15 mg% or more may be placed on provisional dietary treatment with arrangements for rechallenging with normal diets or whole milk at reasonable intervals until the zygosity is suitably established.

6. From siblings of known PKU's, cord blood should be obtained and blood checked at 3 and 5 days if full-term. These newborn siblings of known PKU children may then be placed on a phenylalanine-low diet until laboratory results are obtained and the homozygosity of the patient can be ruled in or out. If the newborn is premature, cord blood should again be obtained as well as blood samples every 3 days until full term if zygosity is not clear by 1 week of life. Methods used for determination of phenylalanine should be specific. Cord bloods will be slightly higher than maternal levels. In pregnancy, the heterozygote has higher levels of phenylalanine than during her non-pregnant state. Comparison of the cord blood and the 3-day sample should indicate whether the levels are rising or dropping. Diagnoses should therefore not be based on the cord sample alone.

7. Age of infant and initial blood screening sample:

The current minimum time following the infants' ingestion of milk when blood phenylalanine findings can be said to be reliable has been variously stated to be 24, 48 and 72 hours. The range reflects the innumerable variables to be considered: the zygosity of the newborn, the maturity of his PA-hydroxylase enzyme system, the amount of protein ingested (dilution of formula, amount of formula taken) and the rate of protein hydrolyzed. The results of the Guthrie field trials suggest that "1 day after feeding" 3 PKU infants had levels between 6-10 mg%. In 7 infants, levels of 10-20 mg% were reported 2 days after their first milk feedings. However, 1 patient was not detected 2 days after his

first milk feeding but was picked up at the repeat test at 4 weeks (blood PA more than 20 mg%). Three days after initial feedings, 11 infants with PKU had levels over 20 mg%. However, it is worth noting that 3 infants had levels between 10-12 mg% the same number of days after their first milk feedings.

It would be wise to consider newborns within the first week of life with levels 6 mg% or greater by the Guthrie method as presumptive positives. Levels between 4-6 mg% should be weighed in the light of:

- a. The age of the newborn.
- b. The sensitivity of the laboratory performing the tests.
- c. The assurance of the follow-up test at several weeks of age.

#### FOLLOW-UP TESTS

Because of the early discharge practices currently in vogue in many maternity hospitals, it is possible that some newborns may be discharged before they have been challenged with sufficient phenylalanine to reflect the defect of the disease. Where feasible, a second screening test of the blood is recommended at 4-6 weeks for all "negative" newborns. It is well to remember that formulas are quite dilute in most nurseries, that first intakes are usually small and that in many instances feedings are not begun before 12-24 hours of life. These considerations in addition to the unknown incidence of false negative results with the Guthrie procedure make the second test vital to a meaningful program. The inclusion of a urine test at 4-6 weeks of life is to be preferred over no follow-up testing. However, a blood test at 4-6 weeks of life has distinct advantages over a urine test. Local health resources must be utilized in the formulation of an effective follow-up testing program as well as to insure adequate follow-up of presumptive positives as a result of initial tests and to develop channels of referral for confirmation and treatment purposes. Failure in the successful coordination of any of these crucial steps may be the result of failure of effective planning in local health programs. Effort directed to filling these gaps to improve the total health program will help insure the success of screening programs.

## FOLLOW-UP SERVICES

On the basis of knowledge currently available, the following guide is offered for the development of a comprehensive follow-up program for patients presumed positive for PKU by screening procedures:

1. All presumptive positive patients should be given appropriate tests to confirm or reject the diagnosis of PKU with a minimum of delay.
2. Confirmed cases should be offered multi-disciplinary services for comprehensive long-term laboratory, clinical and allied health services and follow-up.
3. A total health program should be available for patients.
4. Family needs must be considered.

### CONFIRMATORY TESTS

Confirmatory tests must be carried out on all presumptive positive cases detected by a screening procedure. The choice of the particular test used will be influenced by the accuracy that can be achieved with a particular procedure, the volume of samples and speed of determination, adaptation to blood as well as by the specific talents of the local resources to do one over another of these tests.

It is desirable also to determine tyrosine levels in addition to phenylalanine levels because of the recent reports of unusual biochemical patterns suggestive of PKU but accompanied by elevations of serum tyrosine. While the diagnosis of PKU may tentatively be entertained in the presence of persistent elevation of serum phenylalanine above 15-20 mg% and without a concomitant elevation of serum tyrosine, patients with serum phenylalanine ranges below 15-20 mg% should be followed on a periodic basis in order to insure against misdiagnosis.



Methods for measuring PA levels in body fluids are:

1. McCaman, M. W. and Robins, E. Fluorometric method for the determination of phenylalanine in serum. *Journal of Laboratory and Clinical Medicine*, 59, 885, 1962.

Adapted to automation:

Hill, J. B., Summer, G. K., Pender, M. W. and Roszel, N. O. An automated procedure for blood phenylalanine. *Clinical Chemistry* 11, 541, 1965.

2. LaDu, B. N. and Michael, P. J. An enzymatic spectrophotometric method for the determination of phenylalanine in blood. *Journal of Laboratory and Clinical Medicine*, 55, 491, 1960.

3. Wong, P. W. K., O'Flynn, M. E. and Inouye, T. Micromethods for measuring phenylalanine and tyrosine in serum. *Clinical Chemistry* 10, 1098, 1964. (Modification of McCaman and Robins method for phenylalanine determination and modification of Udenfriend method for tyrosine.)

4. Smith, I. Chromatographic and electrophoretic techniques. Edition No. 2. London, William Heinemann, Ltd. 1960.

5. Piez, K. A. and Morris, L. A modified procedure for the automatic analysis of amino acids. *Analytical Biochemistry* 1, 187, 1960.

Tyrosine and phenylalanine levels in blood can be determined simultaneously by the methods of LaDu, electrophoresis, column chromatography and paper chromatography. Other methods which may be used to determine tyrosine levels are a colorimetric and a fluorescent method:

Udenfriend, S. and Cooper, J. R. The enzymatic conversion of phenylalanine to tyrosine. *Journal of Biological Chemistry* 194, 503, 1952.

Waalkes, T. P. and Udenfriend, S. A fluorometric method for the estimation of tyrosine in plasma and tissues. *Journal of Laboratory and Clinical Medicine* 50, 733, 1957.

Wong, et al, opus cited above.

## COMPREHENSIVE MULTI-DISCIPLINARY SERVICES FOR LONG-TERM FOLLOW-UP AND CARE

There should be identification of a leadership role to assure that appropriate referrals are made from screening programs. This may rest with the director of crippled children's services, the State or local director of maternal and child health, a physician on the State or local health department staff specializing in genetics or metabolic diseases, the local health officer, or the private physician.

Clinical resources should include not only laboratory services but the medical and allied health resources considered essential in the long-term comprehensive follow-up of patients; in most instances such comprehensive services are located in medical centers or in special PKU clinics:

### Pediatric Services

-- preferably mediated through a pediatrician knowledgeable in this disorder and the nutritional needs of infants and children, with active interest in the clinical care of patients and sufficient laboratory training to permit continued sound interpretation of laboratory data. Situations may exist where a private pediatrician assumes primary responsibility for the management of the patient's metabolic disorder. Benefit of skills not present in the primary physician may be extended to the patient through consultation from cooperative experts on a continuing basis in the many health areas of concern. In a coordinated approach to providing comprehensive follow-up care, assignment of the leadership role is vital in order that the further dispensation of responsibility can logically follow. Primary responsibility may rest in the local public health agency, the private pediatrician or the specialized clinician. The closely coordinated interrelationships between them and their capacity to call on other resources necessary for a well-rounded program of follow-up services will greatly influence the quality of patient care.

### Nutrition Services

-- for interpreting to the family the dietary changes recommended by the pediatrician, helping them to implement recommendations according to their social, economic or cultural patterns and for making available to the pediatrician information and dietary data pertinent to the care of the patient.

### Nursing Services

-- in order that some continuity of follow-up can be provided during the interval between clinic appointments, to provide good nursing well-baby and well-child consultation to parents to insure the integrated development of all aspects of the child, and to reinforce the medical and other recommendations and suggestions.

### Social Services

-- for provision of casework services which invariably are required for the appropriate management of a chronic condition with genetic implications to parents and patients, to assist in the arrangements for utilization of other health and educational resources as indicated, to apprise the staff of particular stress or other features of family interaction which may affect the patient.

### Psychological Services

-- for objective performance of psychological examinations to determine developmental levels of infants, intellectual development and school readiness of pre-school children, and evidence of learning disabilities in school children. Also, for determining the presence and extent of cerebral dysfunction, patterns of emotional deviations and conduct disorders, disturbed interpersonal and inter-family relationships, and assessments relative to other facets of maturation, mental development, general adjustment and allied phenomena through the use of specialized techniques.

### Biochemical Laboratory and Consultation Services

-- to include means for satisfactory determination of at least two blood amino acids, phenylalanine and tyrosine, with sufficient speed as to render results useful in the dietary management of the patient and consultation services for the interpretation of newer findings.

### Other Services

-- to include means for obtaining an electroencephalogram and consultation in interpreting readings and such other special assessments as may be necessary from time to time in the long-term developmental follow-up of patients.

## TOTAL HEALTH PROGRAM

Other health needs of the patient (preventive, acute, anticipatory) require appropriate and well-timed attention. Neglect of other considerations in deference to dietary problems of management may result in further compromise of the general physical or emotional health of the patient which in turn may directly affect his ultimate developmental performance.

## FAMILY CONSIDERATIONS

When any treatment of a child involves restrictions in diet, it has broad implications for the total family. How the parents feel about food, how much they use food as a weapon in the parent-child relationship, and how the other children in the home react, can mean the success or failure of the dietary regime.

Consideration of the child as a member of a family with many other responsibilities, all of which should be met, require that medicinal and dietary care be provided without undue drain on family resources.

Genetic implications of this disorder need careful interpretation and appropriate testing of members of the family.

## CLINICAL MANAGEMENT OF PATIENTS

### Critical Levels

Critical phenylalanine levels for the diagnosis of PKU or definition of the therapeutically desirable blood ranges have not been established. Until further experience is gained heterozygote blood ranges may be considered safe. (No intellectual disadvantage has been demonstrated in the heterozygote. Heterozygotes have been demonstrated to have a fasting phenylalanine level about 1.5 to 2.5 times normal.)

Once treatment has been initiated, where phenylalanine tolerance appears inordinate without rise in serum levels, periodic challenging with a normal diet may be helpful in further clarifying the status of the patient.



## Monitoring

Frequent monitoring of blood levels and assessment of growth are essential to guard against too restrictive or too liberal allowance of phenylalanine.

Laboratory tests must be accurate and readily performed in order to be meaningfully useful in making adjustments of management.

Reliance on the demonstration of urinary metabolites of phenylalanine by  $\text{FeCl}_3$  or buffered iron salt stick tests as an indicator of dietary control is unsatisfactory if the therapeutic ranges desired fall under 15 mg%.

Requirements for phenylalanine vary considerably from patient to patient as well as for the individual patient from time to time. In general more phenylalanine is required during early infancy and during other periods of growth spurt.

Phenylalanine should not be too rigidly restricted. Phenylalanine is an essential amino acid required for building protein and cannot be synthesized by the body. A consistently low serum phenylalanine and poor weight gain are suggestive of too rigid restriction of dietary phenylalanine. In more severe states of phenylalanine restriction, catabolism of body protein occurs with a paradoxical rise in serum phenylalanine levels. This is a serious state of malnutrition and cannot be corrected unless more dietary protein and phenylalanine are provided.

Too liberal an allowance of phenylalanine may militate against the therapeutic objectives of maintaining "normal" levels of phenylalanine during the crucial period of early development. While there is no universal agreement on the desirable therapeutic range of phenylalanine levels, it may be reasonable to keep ranges at levels found in heterozygotes for PKU as suggested above.

Through screening programs, a number of unusual biochemical patterns of phenylalanine metabolism are being unfolded. Whether these are in fact normal variations and developmental patterns, heterozygote developmental patterns, or a spectrum of disorders of phenylalanine metabolism remains to be seen. Changes in clinical management may very well result as more knowledge is uncovered. These suggestions for the development of long-term comprehensive services for children with PKU are offered on the basis of currently available findings.



LABORATORIES FOR TYROSINE DETERMINATION

The following clinic and laboratory directors accept blood samples for tyrosine determination to help in the diagnosis of phenylketonuria. It is advisable to work out details for collection of samples by directly contacting the center most conveniently located to you.

Dr. Carl Ashley  
The Oregon State  
Public Health Laboratory  
1400 Southwest 5th Avenue  
Portland, Oregon 97201

Dr. Donough O'Brien  
University of Colorado  
Medical Center  
4200 9th Avenue  
Denver, Colorado 80220

Mrs. Helen Berry  
Children's Hospital  
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