PHENYLKETONURIA: SCREENING PROGRAMS AND TESTING METHODS

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WITHIN the last few years it has become apparent that it is possible to prevent phenylketonuric (PKU) infants from becoming mentally retarded—provided they are detected early (within the first few months) and are placed on low-phenylalanine diets.[1] An infant whose treatment is started at one month of age may be expected to develop his full potential. When treatment is started at two months, mentality probably will be normal, but ten points more or less may be shaved off the potential I.Q. In most cases, when treatment is delayed until the second half of the first year of life, the infant will remain somewhat retarded but probably will not need institutionalization.[2] Some young children of preschool age also can be benefited significantly by diet management[3-6]; however, PKU children detected at school age and older are apparently beyond significant I.Q. improvement from present treatment.[7] Yet the discovery of PKU in older mentally retarded children at home or in institutions has great importance. Such knowledge alerts the family and the family physician to the possibility of similar involvement in subsequent siblings or even cousins.[8]

Since we are dealing with a recessively inherited condition, the chance for the normal "carrier" parents to have another PKU infant is approximately one in four (25 per cent). In fact most of the PKU infants to date who have been discovered early enough to receive maximum benefit from dietary treatment have been younger siblings of a family with already diagnosed mentally retarded PKU children. The unfortunate aspect of this method of diagnosis is the sacrifice of the older child before the presence of the disorder in the family is recognized. In each case it would have been a much more satisfactory situation had the older child also been diagnosed in early infancy and received treatment.

Early diagnosis is of such importance that it seems worthwhile to repeat that, for maximum benefit the PKU infant must be diagnosed and treatment must be initiated in early infancy before irreversible brain damage has occurred. To wait for the usual first symptoms of mental retardation to suggest the diagnosis is to wait too long.

This brings us to the importance of the recently instituted routine screening tests for PKU on apparently well infants whereby even the first cases in involved families can be discovered early and mental retardation prevented.[9-13] The value of such a discovery to an involved child and his family is of course immeasurable. The monetary saving to the taxpayers when any such child is spared a lifetime admission to a state institution for the mentally retarded is in the neighborhood of $100,000 for that one child. It is estimated that about 200 PKU infants are born in the United States each year. If each such infant were to be discovered early and thus prevented from becoming mentally retarded and

institutionalized, the theoretical saving to the nation's taxpayers for each year's successful detection would be in the neighborhood of $20,000,000 spread over the lifetime of these children. Thus, it goes without saying that an effective nation wide routine screening program for PKU would be very desirable—especially if it were also both simple and economical.

Screening Programs for PKU

Mentally Retarded Populations

The screening of mentally retarded populations for PKU has been carried on for many years in many institutions for the retarded. In many other institutions, however, such detection efforts are just starting. Although it is unlikely that children picked up by this method can benefit by dietary control, diagnosing older children is such an important way of protecting subsequent siblings that routine screening is being urged in all institutions and schools for the retarded, both public and private.[8] The success of such programs will depend on many cooperating organizations. Case findings will have to be followed systematically into the homes of the families in a way somewhat similar to the screening of families in which an active case of tuberculosis has been found.

A recent home survey of the siblings of 20 PKU patients at an institution of 3,000 population uncovered three previously undiagnosed cases.[8] Two of these were still young enough to receive benefit from treatment. On the basis of this experience several recommendations have been made for follow-up work with the families of PKU patients:

1. The families should be oriented as soon as possible; the orientation should include an explanatory letter or literature that describes the problem. The information should also be forwarded to the family physician. At least one information pamphlet for parents is now available.[14]
2. All siblings should be screened as soon as possible, even if the parents do not think they are retarded. Sometimes retardation in young children is unsuspected by the parents.
3. All newborn siblings should have serum phenylalanine determinations just prior to discharge from the newborn nurseries.* If this is not possible, they should have urine tests at two, four, six, eight and 12 weeks of age.
4. The families should be encouraged to notify other relatives so that young cousins, second cousins, nieces and nephews can be tested for PKU during early infancy.

Well-Baby Populations

In most cases where PKU has been discovered in early infancy, the diagnosis had been made previously in a defective older sibling,[1] as described above. If this were the only means by which young infants with PKU could be identified, it would mean the sacrifice of one child in each involved family.

A more recently instituted type of screening program involves a routine screening test on all infants. This is the only way to find PKU children in previously uninvolved families early enough for the infants to receive maximum benefit from treatment. It is suggested that the test be done at the first three well-baby checkups. Phenylpyruvic acid usually does not appear in the urine until the second or third week of life[15] and in some cases not until six weeks.[1] For this reason there is no value in testing babies in the newborn nursery.

At the present time, many California health departments have joined in a routine screening program for PKU.[9-11]

* We are not aware of any PKU infants who, when tested at 3 days of age, did not already have significantly elevated serum phenylalanine levels. However, until enough cases can be collected to prove that this is invariably true, it is suggested that the urines of infants with normal 3 day serum phenylalanine levels have periodic urine tests during the first several months of life.

The population area serviced by the participating health departments is in excess of three-fourths of the State of California. The program would not be possible except for the full cooperation and enthusiasm of all these health departments. The test being used, called the diaper test, will be described later in this article. As the program is long-range, no final results can be reported at this time. However, it has already been proved that routine testing by this method is easily incorporated into the regular well-baby program, and the simplicity of the test has helped promote the enthusiastic support of the workers in the clinics. Also, it has been proved that the test does uncover cases of PKU.[10,11] Already several PKU infants have been detected by routine screening programs both in the well-baby clinics and by private practitioners. The health departments of several other states have incorporated this type of screening program into their well-child conference programs.

Shortly after the diaper test detection program was initiated in California, a routine screening program was started independently in England. There it is advised that all babies be tested at about one month of age during the first postnatal visit to the clinic or hospital. The screening test now in use is the Phenistix test as a urine or diaper test[13] (described herein).

In Cincinnati, Ohio, a screening program has been started with the so-called filter-paper test[12] (also described herein). In this program each mother with a newborn infant receives instructions along with a piece of filter paper and a preaddressed, stamped envelope. She is instructed that, when the infant is about one month old, the filter paper is to be placed in the baby's diaper until soaked with urine. It is then removed, dried by open-air contact, and subsequently sent via the mails to a medical center.

An Evaluation of Testing Methods Used for PKU

Several tests and modifications of tests are now available for the identification of PKU. In 1958 a study was undertaken at the College of Medical Evangelists School of Medicine to determine, if possible, whether one test had any advantages or disadvantages over another for screening purposes. In this study several thousand evaluations were made on 132 consecutive urine samples taken from 20 untreated institutionalized cases of PKU. The effects of many factors on the urine tests were measured, for example, pH, specific gravity, turbidity, a.m. and p.m. voiding, age and sex of patient, and intervals of time between voiding and testing. Factors such as cost of the test and ease of administration were also compared. More recent evaluations by others are essentially in agreement with our findings.[16]

A more detailed report of this study is in preparation for publication, but the result can be quite simply stated at this time. All the tests studied were comparable in accuracy, and each test had enough specific merits to make it very valuable under certain circumstances (Tables 1 and 2). It was also found that the value of each of these tests is dependent on some simple but specific precautions which if not understood and carried out can easily cut the efficiency of that test considerably. We will attempt to include most of these vital pointers in the following discussion. Because PKU patients on rare occasions will fail to excrete phenylpyruvic acid, it is not wise to exclude the possibility of this disease on the basis of one negative urine test. Also, it should be remembered that the diagnosis is of great concern to the family and involves an expensive, long-term treatment. Therefore, all "positive" urine tests should be confirmed with a phenylalanine blood level before the diagnosis

Table 1—Percentage of 104 PKU Urine Samples Giving Definitely Positive Reactions After Increasing Intervals of Exposure at Room Temperature*

Exposure Time (Hours)	$FeCl_3$ (Test Tube)	$FeCl_3$ (Diaper Test)	Dinitrophenyl-hydrazine (DNPH)	Phenistix
2	99.1	94.2	100	99.1
4	95.2	93.3	100	98.1
8	95.2	91.3	100	98.1
12	92.3	89.4	100	97.1
18	90.4	84.6	100	96.2
24	76.0	68.3	100	91.3

* Adapted from a report to the Technical Committee on Clinic Programs for Mentally Retarded Children, Washington, D. C., Feb. 26-27, 1959.

of PKU is definitely established. Treatment can be begun on the presumptive diagnosis of two confirmatory urine tests if a phenylalanine blood level cannot be obtained immediately. In such a case, however, a sample of pretreatment serum should be frozen for later phenylalanine determination when this is possible.

The Test-Tube Test with Ferric Chloride

The test-tube test with ferric chloride is the oldest, the best known, and the most widely used of any of the diagnostic urine tests for PKU.[17] The color reaction of ferric chloride with phenylpyruvic acid is practically pathognomonic. Immediately there is a medium-dark blue-green to grey-green color response which begins to fade in a matter of seconds, or minutes, depending upon the concentration of the phenylpyruvic acid in the urine and the strength of the ferric chloride solution being used. This fading of the color response back to a neutral or negative urine color is most useful in differentiating the true phenylpyruvic acid reaction from most so-called false-positive reactions. Ingested salicylates, for example, will give the urine an immediate blue-purple color response upon application of ferric chloride. Unlike the phenylpyruvic acid reaction, however, this color response does not fade away for days. Some of the color responses which can be obtained when a ferric chloride solution is added to urine samples are green from bile, homogentisic acid (alcaptonuria), the catecholamines (pheochromocytoma), and the urine of maple syrup urine disease[18]; red-brown from diacetic acid (acidosis); grey from melanin (malignant melanoma); light violet from chlorpromazine (Thorazine®) ingestion; and purple from proclorperazine (Compazine®) ingestion. Color reactions are fairly stable except with homogentisic acid. In this case the green color is extremely fleeting, disappearing within a second or two. The ferric chloride solution has certain remarkable advantages as a test reagent. It is a simple and inexpensive solution to make. One penny's worth of this solution is adequate to make more than 100 test-tube tests (5 drops to 1 cc of urine each). The green color response is obvious even to the untrained eye. Extensive testing has demonstrated that this is an adequately sensitive and accurate test, yielding only five per cent false-negative tests on PKU urine samples standing four hours at room temperature (Tables 1 and 2).

There are some important points to

remember about the preparation, care and use of ferric chloride solutions:

1. Distilled water is preferable as the diluent for an aqueous solution.
2. A 10 per cent solution is preferred over a 5 per cent solution. The stronger solution gives a quicker, more intense, shorter-lasting color reaction.
3. Stock solutions are best stored in large plastic (polyethylene) bottles, or in large stained-glass bottles, and kept in a refrigerator.
4. Smaller bottles can be filled and refilled as needed from the stock supply. A half-ounce or one-ounce size polyethylene squeeze bottle with dropper nozzle is preferred. Ferric chloride solution will keep nicely in such bottles for many months without refrigeration even in the hottest climates. However, it is probably best, when possible, to keep such solutions away from excessive heat.
5. Ferric chloride solutions will bleach alkali out of some glass bottles and rubber droppers. The result is a cloudy yellow precipitate of ferric oxide, with a correspondingly diminished potency of the solution. Whenever a solution of ferric chloride becomes a markedly cloudy yellow upon being shaken, the solution should be discarded and replaced with a new supply. Since heat hastens deterioration, it is advisable to keep the solution refrigerated when it is not in use, particularly if glass bottles with rubber dropper bulbs are used.
6. The earliest medical literature on the subject, and even the modern-day textbooks, stress that the urine should be acidified before ferric chloride is added. This is not at all necessary if 3 to 5 drops of the 10 per cent solution are added to 1 cc of urine. The marked acidity of the ferric chloride solution (pH 1.8) instantaneously counteracts any excess basicity of the urine. One might run into trouble if one slowly added a 5 per cent solution of ferric chloride to a 5 cc sample of quite basic urine. In such a case, ferric hydroxide might form and could nullify expected results with the phenylpyruvic acid.
7. A definitely positive, immediate color reaction with ferric chloride can disappear on occasion in less than half a minute—thus necessitating observation and evaluation immediately following the addition of ferric chloride. The blue-green to gray-green transient color response is characteristic enough to differentiate the PKU urine from most other so-called "false-positive" reactions. The fact that the ferric chloride reagent will demonstrate the presence of various drugs and unusual metabolites other than phenylpyruvic acid does not impair the value of this test but rather makes it more interesting and more valuable.

The Diaper Test

The diaper test is a modification of the ferric chloride test-tube test. A drop of 10 per cent ferric chloride is placed on a baby's wet (or dried) diaper, and a blue-green to gray-green color appears immediately. The concomitant use of a drop of acid is neither necessary nor desirable. As with the test-tube test, the color is transient. Many

Table 2—Some General Comparisons of Five Current Testing Methods*

	Filter Paper	$FeCl_3$ (Test Tube)	$FeCl_3$ (Diaper Test)	Dinitrophenylhydrazine (DNPH)	Phenistix
Ease of testing	+++	++	++++	++	+++
Ease of interpretation	+++	++++	+++	+++	++++
Low cost	++	+++	++++	+++	++
Sensitivity	++	+++	+++	++++	++++
Other pathology detectable	+++	++	++	++	+

* Adapted from a report to the Technical Committee on Clinic Programs for Mentally Retarded Children, Washington, D. C., Feb. 26-27, 1959.

times a definitely positive test will fade in less than half a minute. Such fading starts in the center of the spot, and the last to fade is the green-rimmed periphery. Whether positive or negative, the spot on the diaper leaves a permanent, tan-colored stain. This is perhaps worth mentioning to the mother. Although thousands of infants have been tested, only a few mothers have mentioned this on a subsequent visit, and these mothers were not disturbed about the stain. When diapers get older, the spot where the ferric chloride is placed is more likely to tear than is adjacent cloth. Again, in practice this has been no problem.

The pointers made earlier concerning the preparation, care and use of ferric chloride solution apply here also. It is possible for the same false-positive reaction to be obtained with the diaper test. The test has proved exceptionally simple to perform and very inexpensive —one penny's worth of solution can test more than 500 infants. The test is also very reliable. Of 104 PKU urine specimens allowed to stand at room temperature for four hours, 97 (or 93 per cent) were still definitely positive by this test (Tables 1 and 2). Trace and one-plus reactions were considered negative; only two-three- and four-plus reactions were considered definitely positive. This was done to eliminate a reasonable objection, that the average relatively inexperienced tester (i.e., nurse or nurse's aid in an office or clinic) might not recognize the most weakly positive tests.

In practice it was found that in a considerable number of cases the infant did not have a wet diaper and the mother had no wet diaper with her. We are now advocating that mothers be instructed to bring along with them on the initial and subsequent clinic visits the most recently wet diaper from home. In this way approximately 30 per cent more infants will be tested who would otherwise miss being tested.

The Phenistix* Test

Another modification of the ferric chloride test is the Phenistix dip stick, a commercially prepared paper strip which is impregnated with a buffered ferric salt.[19,20] This reagent, according to our studies, is second only in sensitivity to the dinitrophenylhydrazine (DNPH) test which will be discussed later. Of 104 PKU urines, 102 (or 98 per cent) were still definitely positive with Phenistix after standing for four hours at room temperature (Tables 1 and 2). This test is excellent for routine screening of apparently well infants because the dip stick can be pressed against the wet diaper or dipped into a urine solution. These sticks are stable almost indefinitely if kept in their special container until used. False-positive reactions do occur with Phenistix but less frequently than with ferric chloride. The color reaction of Phenistix with phenylpyruvic acid is the same as with the test-tube test. The color may also fade away within a minute or so; thus it would be impossible to place a Phenistix in a baby's diaper and expect to come back later and get an accurate test result.

At present the Phenistix costs approximately eight cents a test; this is certainly inexpensive and should be no barrier to those physicians who wish to utilize it as a screening test in their practices. Phenistix is slightly more cumbersome than the "diaper test" for routine screening; on the other hand, it leaves no stain on the diaper.

The Filter-Paper Test[12]

The filter-paper test is performed on ordinary white filter paper which has been wetted with urine, has been dried, and then sent through the mail to the testing laboratory. The test is the same as the diaper test. The difference be-

* The Phenistix used in this study was supplied through the courtesy of the Manufacturer, Ames Company, Inc., Elkhart, Ind.

tween the filter-paper test and the diaper test is that the wet diaper which is tested is probably no more than several hours old, whereas the filter paper urine may be several days old before it is tested.

The question arises, how long does the dried, urine-soaked paper continue to give an accurate test? To evaluate this problem, 30 urines from known cases of PKU were soaked on separate sheets of filter paper, were dried and tested with a drop of ferric chloride. The remaining portion of each filter paper was cut into six smaller pieces and each piece placed in a separate envelope to simulate conditions of the filter-paper screening program. Every day a new set of 30 envelopes was opened and the contents tested. Inasmuch as the program as set up in Cincinnati involves experienced laboratory personnel, even the faintest trace of positive reaction was considered as positive. By the use of such criteria, the 30 specimens initially were all positive, but two of them were only very faintly positive. After three days, the minimum time one can expect between voiding and testing under such a program, three (10 per cent) of the test specimens were negative. At five days, five (16.7 per cent) were negative. After one week, six (20 per cent) were negative. Considering these results, it would be a mistake to take one negative test as assurance that no PKU is present.

The cost per test is approximately 7.5 cents (not including laboratory salaries). This cost could be cut in half if parents would supply their own stamps on the return envelopes. The advantages and indications of filter-paper test program are (1) that the screening program can be initiated in practically all young infants (at least those who are born in hospitals); (2) that this test can be administered to patients in out-of-the-way and distant places and perhaps to infants for whom no wet diaper is available for testing at the office or clinic visit (Table 2).

Finally, this test has special merit as performed by the Cincinnati group because the filter papers are tested also for the presence of protein and galactose. This requires more complex laboratory facilities and equipment and increases the cost, but in turn the discovery of another even rarer mental deficiency syndrome, galactosemia, is made possible. The mental deficiency of galactosemia, like that of PKU, is preventable if detected in early infancy.

The Dinitrophenylhydrazine Test-Tube Test[21]

Properly prepared and used, the dinitrophenylhydrazine (DNPH) reagent is the most sensitive and reliable of the various urine tests for PKU. False-negative test reactions using DNPH on known untreated cases of PKU are very rare (less than one per cent). In our experience with over 100 consecutive urine samples, there were no false-negatives even after 18 hours of standing at room temperature (Tables 1 and 2). Yet because of this very rare possibility, it is advised that urines of mental defectives be so tested on at least two separate occasions, especially in institutions and homes for the mentally retarded, where patients are available and urine specimens more easily obtained. Because other substances rarely found in urine will react to give a positive test with the DNPH, any positive test should be cross-checked with a ferric chloride test. Of the aforementioned substances which can give a color response with ferric chloride, only diacetic acid and the urine of maple syrup urine disease may give positive reactions with DNPH.[18]

To prepare the DNPH reagent, about 4 gm of 2-4 dinitrophenylhydrazine (an orange powder) is added to a liter of one normal hydrochloric acid. This mixture is heated in a hot water bath overnight to make a supersaturated ap-

proximately 0.3 per cent solution of DNPH. The supernatant clear-yellow solution is filtered off and stored in a dark glass bottle. From the larger stock bottle, smaller stained glass dropper bottles can be repeatedly filled for clinical use. Although it is perhaps not necessary, we have kept our stock solutions under refrigeration and have found them to be stable almost indefinitely. A dark granular precipitate sometimes settles on the bottom of a bottle, but this usually goes back into solution if the bottle is shaken.

The test is performed in the following manner: One-half to one cubic centimeter of urine is placed in a test tube. To this is added an equal amount of DNPH. Immediately after addition of the DNPH, the mixture is a clear, pale yellow-orange solution, and it will remain so if the test is negative. The test is positive when in the course of one to five minutes the solution gradually becomes an opaque, bright yellow color. This is a permanent reaction and will be essentially unchanged many hours later. There is a possibility of some confusion in interpreting test results if the urine sample is initially cloudy. When the tester is in doubt, he should duplicate the test and compare the opacities of the older and fresher mixtures, and he should cross-check with a ferric chloride or Phenistix test. It is worth testing a freshly made solution of DNPH against the urine of a known case of PKU. I know of one large state hospital which concluded that the DNPH test was not very sensitive. A check of the solution being used there revealed that it had not been prepared properly and was far from a saturated solution. It is worth mentioning that, like the ferric chloride solution, the cost of chemicals for the DNPH solution is extremely small. By the use of 1 cc of solution for each test, it is possible to make over 200 tests for one penny's worth of solution.

Serum Phenylalanine Determination

The main value and indication for the serum phenylalanine determination is for final confirmation of the diagnosis of PKU in cases seemingly positive by urine tests. No infant or child should be started on the long-term program of a low-phenylalanine diet without a blood sample taken first. This serum specimen can be safely stored in a freezer and tested later if an immediate determination of the serum phenylalanine level is inconvenient or impossible. In this way there need be no long delay in initiating dietary treatment because of a local lack of laboratory facilities.

Relatively few hospitals are set up to make such determinations, not because this is so difficult, but because demands for such tests are so few as to make training of personnel and acquisition of necessary equipment for such procedures impractical for most laboratories. In most large cities commercial laboratories are available where serum phenylalanine levels can be determined at a cost varying from $15 to $30 per determination. Many medical school laboratories also are equipped to perform this test. Anywhere from ½ cc to 6 cc of blood (0.1 to 2.0 cc of serum) will be necessary for this determination, depending on the methods employed by the particular laboratory[22-25] If one is not already familiar with the requirements of the particular laboratory to which one is sending the serum specimen, it is wisest to play safe and obtain a 6 cc blood specimen. This will permit drawing off at least 2 cc of serum, which should be the maximum desired for this determination by any laboratory. When the blood clot has retracted, the serum should be removed and sent to the laboratory for determination. It can be sent by mail in a standard laboratory mailing container. If going any distance, it should be sent by airmail during the early part

of the week so that the sample will not arrive at the week-end and sit in the post office for an extra day or two before delivery.

Sterile technic in handling the serum is indicated. Serum should be sent—not whole blood. A little hemolysis or slight pinkness of the serum is permissible, but the amount of hemolysis which takes place in a whole blood specimen sent through the mails makes test results invalid. A serum sample should be kept in the deep freeze until it is ready to be sent to a laboratory for evaluation. If anticipating delay in transit, send the specimen packed in dry ice. A serum specimen can be safely stored for many months if kept frozen.

Several of the medical centers in the United States doing research in this field have been approached and have expressed willingness to perform serum phenylalanine determinations on specimens sent to them by persons who have no local facilities. The following names and addresses may be used for such purposes:

1. Marvin D. Armstrong, Ph.D.
   The Fels Research Institute
   Yellow Springs, Ohio
2. Helen K. Berry, M.A.
   George M. Guest, M.D.
   The Children's Hospital
   Elland Avenue and Bethesda
   Cincinnati 29, Ohio
3. Joseph A. Garrisi, M.D.
   Department of Pediatrics, School of Medicine
   College of Medical Evangelists
   1720 Brooklyn Avenue
   Los Angeles 33, Calif.
4. Bert N. La Du, M.D.
   Department of Health, Education, and Welfare
   National Institutes of Health
   Bethesda 14, Md.
5. Donough O'Brien, M.D.
   Department of Pediatrics
   University of Colorado Medical Center
   4200 East 9th Avenue
   Denver 20, Colo.

Since none of these centers can guarantee to provide these services indefinitely, it would be wise to verify by a letter of inquiry before sending serum specimens. The desired test and the return address should be clearly stated and postage to cover return mail should be enclosed.

There are two other indications for the use of the serum phenylalanine level other than the final confirmation of PKU cases detected by urine tests. Subsequent siblings born into a family where PKU is present in an older child have a 25 per cent chance of also having PKU. Newborn infants with this disorder have normal cord blood phenylalanine levels; that is, between 1 and 5 mg per 100 ml of serum. By two or three days of age, however, the serum phenylalanine level has risen to abnormally high levels (10 to 15 mg), and a blood specimen at this time is adequate to make the diagnosis.[15] However, it takes a week or longer (rarely up to six weeks) before the serum levels are sufficiently high that phenylpyruvic acid can be detected in the urine. Thus, it is suggested that blood specimens be taken on the newborn siblings of known cases of PKU just before their discharge from the newborn nursery. Babies in a newborn nursery are too young to be urine-tested for PKU. Lastly, it might be advisable to determine the serum phenylalanine level on a child who clinically seems to suggest strongly the diagnosis of PKU but whose urine tests are negative. There are cases of PKU on record whose serum phenylalanine levels, although definitely elevated above normal, are just at the renal threshold level, so that spillage of metabolites into the urine is inconsistent. The clinical findings other than mental retardation which might make one especially suspicious of PKU are blondness, which in 80 per cent of cases exceeds that of the parents and normal brothers and sisters, and an irritable personality pattern. About 25 per cent of PKU children have a history of con-

vulsions and from 20 to 40 per cent have a history of eczema.[26] Also the urine and sweat have a characteristic musty, mousy or barnlike odor which becomes more obvious a few hours after being passed.

Summary

1. A brief background sketch of phenylketonuria (PKU) is made, including the relatively recent knowledge that the mental deficiency of this disease is preventable, provided the diagnosis is made in early infancy and proper dietary management instituted at that time.

2. All mental-deficiency populations should be screened for phenylketonuria, at home, in special schools and in institutions.

3. The families and private physicians of any diagnosed cases of phenylketonuria should be alerted to this condition so that subsequent siblings and cousins will be diagnosed early enough to permit maximum benefit from treatment and so that all siblings of all ages can be tested for this condition. Such orientation is best accomplished when verbal explanation is followed up with appropriate reading material on the subject.

4. Routine screening of all well babies for phenylketonuria is essential if the maximum number of PKU infants are to be diagnosed early enough for optimum benefit from treatment.

5. Three organized screening programs for the detection of infant phenylketonurics within "normal, well-baby" populations are briefly described. Each of these programs, from widely separated geographical areas, utilizes a different method of testing. The hospital newborn nursery is not a suitable place to screen for phenylketonuria because PKU infants rarely give positive urine tests until after the first week of life.

6. Six tests for PKU are outlined and the advantages and disadvantages of each test are discussed. The tests described are (1) the test-tube test with ferric chloride, (2) the diaper test with ferric chloride, (3) the Phenistix (dipstick) test, (4) the filter paper test with ferric chloride, (5) the dinitrophenylhydrazine test-tube test, and (6) the serum phenylalanine determination. The first five of these are simple urine tests. The second, third and fourth tests are particularly suitable for mass well-baby screening programs.

7. All the tests studied are comparable in accuracy and each test has enough specific merits to make it particularly valuable under certain circumstances.

8. The maximum value of each type of test is dependent on some simple but specific precautions and knowledge essential to the reliability of the test. Most of these vital factors are included in the discussions of the respective tests.

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REFERENCES

1. Horner, F. A., and Streamer, C. W. Effect of a Phenylalanine-Restricted Diet on Patients with Phenylketonuria: Clinical Observations in Three Cases. J.A.M.A. 161:1628–1630, 1956.
2. Centerwall, W. R. Unpublished data.
3. Bickel, H. Aminoaciduria in Childhood. Ph.D. Thesis, University of Birmingham, England, 1952.
4. Bickel, H., Gerrard, J. W., and Hickmans, E. M. Influence of Phenylalanine Intake on Phenylketonuria. Lancet 2:812–813, 1953.
5. Woolf, L. I., Griffiths, R., and Moncrieff, A. Treatment of Phenylketonuria with a Diet Low in Phenylalanine. Brit. M. J. 1:57–64, 1955.

6. Armstrong, M. D., and Tyler, F. H. Studies on Phenylketonuria. I. Restricted Phenylalanine Intake in Phenylketonuria. J. Clin. Invest. 34:565–580, 1955.
7. Hsia, D. Y., Knox, W. E., Quinn, K. V., and Paine, R. S. A One-Year Controlled Study of the Effect of Low-Phenylalanine Diet on Phenylketonuria. Pediatrics 21:178–202, 1958.
8. Centerwall, W. R., and Centerwall, S. A. Phenylketonuria: Report of a Survey to Discover Treatable Siblings of Institutionalized Patients. M. Arts & Sc. 13:173–175, 1959.
9. Centerwall, W. R. L. Phenylketonuria. J.A.M.A. 165:392, 1957.
10. ————. Phenylketonuria. J.A.M.A. 165:2219, 1957.
11. ————, and Centerwall, S. A. Early Diagnosis and Management of Phenylketonuria. Scientific Exhibit, AMA Annual Meeting, San Francisco, 1958.
12. Berry, H. K., Sutherland, B. S., Guest, G. M., and Warkany, J. Simple Method for Detection of Phenylketonuria. J.A.M.A. 167:2189–2190, 1958.
13. Gibbs, N. K., and Woolf, L. I. Tests for Phenylketonuria: Results of a One-Year Programme for Its Detection in Infancy and Among Mental Defectives. Brit. M. J. 2:532–535, 1959.
14. Centerwall, S. A., and Centerwall, W. R. An Introduction To Your Child Who Has Phenylketonuria. Loma Linda, Calif.: College of Medical Evangelists Press (7 pp.), 1958.
15. Armstrong, M. D.; Centerwall, W. R.; Horner, F. A.; Low, N. L.; and Weil, W. B. The Development of Biochemical Abnormalities in Phenylketonuric Infants. In Chemical Pathology of the Nervous System. New York: Pergamon Press, 1960 (in press).
16. Nelson, T. L. Personal correspondence, 1959.
17. Fölling, A. Uber Ausscheidung von Phenylbrenztraubensäure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillität. Ztschr. physiol. Chem. 227:169–176, 1934.
18. Menkes, J. H., Hurst, P. L., and Craig, J. M. A New Syndrome: Progressive Familial Infantile Cerebral Dysfunction Associated with an Unusual Urinary Substance. Pediatrics 14:462–467, 1954.
19. Baird, H. W. III. A Reliable Paper-Strip Method for the Detection of Phenylketonuria. J. Pediat. 52:715–717, 1958.
20. Wortis, J., and Giancotti, A. M. A New Simple Test Paper for Mass Detection of Phenylketonuria. A.J.P.H. 49:463–464, 1959.
21. Penrose, L. S., and Quastel, J. H. Metabolic Studies on Phenylketonuria, Biochem. J. 31:266–274, 1937.
22. Udenfriend, S., and Cooper, J. R. Assay of L-Phenylalanine as Phenylethylamine after Enzymatic Decarboxylation; Application to Isotopic Studies. J. Biol. Chem. 203:953–960, 1953.
23. Henry, R. J., Sobel, C., and Chiamori, N. Method for Determination of Serum Phenylalanine with Use of the Kapeller-Adler Reaction. Am. J. Dis. Child. 94:604–608, 1957.
24. Berry, H. K. Paper Chromatographic Method for Estimation of Phenylalanine, Proc. Soc. Exper. Biol. & Med. 95:71–73, 1957.
25. La Du, B. N., and Michael, P. J. An Enzymatic Spectrophotometric Method for the Determination of Phenylalanine in Blood. J. Lab. & Clin. Med. 55:491, 1960.
26. Paine, R. S. The Variability in Manifestations of Untreated Patients with Phenylketonuria (Phenylpyruric Aciduria). Pediatrics 20:290–302, 1957.

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