Blood Phenylalanine Levels of Newborn Infants

A Routine Screening Program for the Hospital Newborn Nursery

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■ *If phenylketonuria is diagnosed during the first few weeks of life, a special diet can be given to prevent the brain damage that otherwise will occur.*

A simple, cheap, accurate laboratory test has been developed to diagnose the condition in infants two to four days old before they leave the hospital.

At the Donald N. Sharp Memorial Community Hospital in San Diego routine testing of all newborn infants has been done with this technique since August 26, 1963. Mass screening programs in the newborn nursery are already being carried out by large numbers of hospitals across the United States.

PHENYLKETONURIA is caused by an inherited error in metabolism which is autosomal and recessive. Approximately one adult in 50 is a carrier of this trait. The incidence is approximately one in 11,000 live births.[4] A person so affected lacks a liver enzyme, phenylalanine hydroxylase. Phenylalanine, which constitutes approximately five per cent of all natural protein food, is absorbed into the blood of these persons but is not converted to tyrosine because of lack of this enzyme. The phenylalanine concentration, therefore, rises in the serum. In an untreated infant with this disease the serum phenylalanine level rises to 15 to 60 mg per 100 ml. It is believed by some experts in the field that the damage in the undeveloped brain is already occurring by the time the blood phenylalanine level has risen to 10 mg per 100 ml.[12] This happens two to six weeks after birth.

The brain damage is permanent and irreversible, and nine out of ten persons with phenylketonuria who are not treated have permanent mental deficiency with a median intelligence quotient of 10 to 30. Most of these mental defectives end up in state-supported institutions. Their life expectancy is considered to be only slightly less than normal. This disease, therefore, not only brings heartbreak and hard-

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TABLE 1.—*Serum Phenylalanine Levels of 2 to 3-Day-Old Infants (2,088 Tested)*

Mg per 100 ml	*No. of Infants*
Normal range—	
0 to 1.0	56
1.1 to 1.5	272
1.6 to 2.0	709
2.1 to 2.5	591
2.6 to 3.0	324
Warranting further examination—	
3.1 to 3.5	89
3.6 to 4.0	17
4.1 to 4.5	13
4.6 to 6.5	12
Over 6.5	5
Total	2,088

ship to a family but is of public health importance. At present there are 89 known phenylketonuric patients in state-supported institutions in California and, based on present costs, the taxpayers of California can expect to pay $100,000 to $125,000 for the care of each of them.[10]

The disease was first described by Fölling[3] in Norway in 1934, and his ferric chloride urine test has been used for the past 30 years to test inmates of mental institutions. In the past six to seven years pediatricians have used this test in their offices (the wet diaper test) when checking for the disease.

There are some serious drawbacks in using the urine ferric chloride test. In some phenylketonuric patients with elevated serum phenylalanine the result of the test is negative. Usually the reaction to this test is not positive until the serum phenylalanine is greater than 20 mg per 100 ml.

In 1958 a paper-strip method for testing the urine was reported[1] and the Ames Company produced the Phenistix®, which now is widely used for testing in a physician's office. The Phenistix is more sensitive than the ferric chloride test, and there are fewer false negative results. One problem, however, is that the Phenistix generally will not detect the presence of the phenylpyruvic acid in the urine until the blood phenylalanine level rises to 10 to 20 mg per 100 ml.

The office testing method has some serious drawbacks. If the physician is busy an inexperienced nurse or doctor's assistant might do the testing and the result might not be read properly if the Phenistix shows a color other than the blue-green or grey it is supposed to show if the urine is positive for phenylpyruvic acid. Also the result must be read immediately, for the "positive" color disappears. Another drawback is that the infant may not be taken to the physician's office until several months after birth, time enough for some of the brain damage of phenylketonuria to take place. On many occasions an infant arrives at a physician's office with a dry diaper and the test cannot be done.

Having the hospital give the mother of a newborn infant a do-it-yourself Phenistix testing kit when she takes the baby home has not been too successful, for she may not understand the importance of carrying out the instructions.

In 1960, LaDu[9] described an ultra-violet method for a spectrophotometric serum phenylalanine determination. This method is complicated, requires expensive equipment and would be difficult to adapt to inexpensive mass screening.

A paper chromatography method has been described[2] which we have used in our laboratory for approximately two years. We believe that this could be adapted for the mass screening of large numbers of serum specimens. It is semi-quantitative at best, however, and we do not believe it to be the preferred method.

The first practical method for the mass screening of blood levels, a bacterial inhibition assay test, was first described by Guthrie in the latter months of 1961.[5,6,7]

A fluorometric method that was described in May of 1962 by McCaman and Robins[11,8] is used for screening all infants born in the Donald N. Sharp Memorial Community Hospital. Our routine newborn nursery screening program was instituted on August 26, 1963. To date, approximately 2,500* infants have been screened. The first 2,088 were tested both by the Guthrie bacterial inhibition assay method and the fluorometric method. These tests were run in parallel to evaluate the relative accuracy, to compare the difficulties of running the tests routinely, to appraise the sensitivity of the results of the test and to determine costs by each method.

From these parallel tests we concluded that the fluorometric method was accurate and more sensitive; the problems of performing the test routinely, using a medical laboratory technologist, were not a contraindication; and when at least a hundred specimens were tested at a time, the cost of performing the test was approximately 85 cents. The cost can be reduced by automating certain parts of the procedure.

It costs us, because of infection control precautions, and the distance of the nursery from the laboratory, approximately 80 cents to obtain the blood specimen from the infant.

In our opinion normal phenylalanine levels for infants two or three days old must be considered to be less than 3 mg per 100 ml. The phenylalanine levels for the first 2,088 newborns screened are presented in Table 1. Eighty-five per cent of them had a serum phenylalanine level of 3 mg or less per 100 ml. The 15 per cent of infants with levels greater

*This number has now been brought up to approximately 5,000.

than that are probably normal, but they must be carefully observed by their physicians to make sure that their phenylalanine levels drop to normal.

In our program, we notify the attending physician if an infant's serum phenylalanine level is greater than 3 mg per 100 ml. We urge the physician to check the infant in the first two to four weeks of life, because this infant may be the one in 11,000 we are looking for.

Table 2, in which the results obtained by the fluorometric procedure are compared with those of the Guthrie method, supports our belief that the fluorometric procedure is the one of choice when testing two to three-day-old infants. In San Diego, the infants are routinely discharged from the hospital on the second to third day of life.

The Children's Bureau estimated that until wide publicity was given to Guthrie's reports, no more than 10 per cent of all newborns in the United States were screened for phenylketonuria. Dr. Guthrie, in conjunction with the U. S. Department of Health, Education and Welfare, began a mass screening program of 400,000 newborn infants in July, 1962.

A state law has been passed in Massachusetts requiring every newborn to be tested for phenylketonuria before leaving the hospital.† Many hospitals throughout the country, particularly on the East Coast and in the Midwest, have voluntarily instituted programs. Hospitals in Nevada are screening practically all the newborns in that state.

California is behind in phenylketonuria testing in hospitals. Since in this state approximately 99 per cent of all births occur in hospitals, there is opportunity to screen almost every newborn infant in the state. Based on the number of live births in this state in 1963, we can expect approximately 35 babies with phenylketonuria to be born in this state in 1964.

The PKU problem has been brought to the attention of the general public by magazines and newspapers. As early as November, 1959, an article on the subject appeared in the *Saturday Evening Post.* Articles by science writers have appeared in several large newspapers throughout the country.

The late President Kennedy, in his nationwide address on mental health in February, 1963, stated, "We must seek out the causes of mental illness and mental retardation, and eradicate them."

Phenylketonuria is unique because it is now easily detected, and because its successful dietary management represents one of medicine's first footholds in the prevention of mental deficiency.

We believe that routine hospital screening of newborn infants will virtually eliminate the elevated phenylalanine cause of mental retardation.

We now have practical, inexpensive, accurate methods for mass screening of all newborn infants for phenylketonuria.

TABLE 2.—*Comparison of Sensitivity of Fluorometric Test With That of Guthrie Test in 136 Cases of Newborns Shown by Fluorometric Test to Have Suspiciously High Serum Phenylalanine Levels**

Serum Phenylalanine mg per 100 ml	*Fluorometric*	*Guthrie Elevated*
3.1 to 3.5	89	2
3.6 to 4.0	17	2
4.1 to 4.5	13	5
4.6 to 5.0	6	3
5.1 to 5.5	2	0
5.6 to 6.0	2	2
6.1 to 6.5	2	2
Over 6.5	5	4
Total	136	20

*Most of infants were discharged from hospital by third day of life.

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†State laws have now been passed in New York, Rhode Island, and Louisiana. Three other states, Illinois, Oregon, and Minnesota, have adopted the test pattern through legislation or as a matter of public policy. All hospitals in the state of Maryland will be screening their newborns before the end of 1964.