New Developments in Screening for Inborn Errors of Metabolism*

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Because the mental retardation caused by some inborn errors of metabolism is to a large degree preventable by appropriate and early therapy, consideration is now being given to the possibility of mass screening for these disorders in the neonatal period. Experience with the prototype, the phenylketonuria (PKU) program, dictates that mass screening for inborn errors of metabolism is here to stay and will most certainly expand in the future—like it or not. Since I have recently gained some insight into the field as Metabolic Consultant to the State Health Department, I wish to review the PKU program in Virginia—the many pitfalls in the diagnosis and treatment of this disorder, and the feasibility of mass or selective screening for other inborn errors.

Mass Screening

In 1966, Virginia passed a law making it mandatory to test all newborns for the presence of PKU; indeed, today most states have such a law. The State Health Department responded to the legislature's charge to implement the program by: 1) establishing accurate Guthrie testing in three State laboratories, 2) establishing a quantitative serum phenylalanine (PA) and tyrosine procedure at its central laboratory in Richmond, and 3) providing nutritional and medical follow-up for diagnosed cases. Three full years of experience with the program have demonstrated its worth not only financially, but also in the prevention of mental retardation.

From the bookkeeping viewpoint, at $0.42 per test, 97% of the 76,000 births in the State of Virginia last year were screened for about $31,000. At an incidence of one in 13,000 it thus costs approximately $6,000 to uncover one case; it costs another $2,000 to provide therapy. In contrast, the cost to the State for the lifetime institutional care of the invariably severely retarded and untreated PKU patient is, very conservatively, $125,000. One state's estimate put it at twice that figure. A recent review of the rolls of the State's institutions discloses 45 such PKU patients. At present we are actively treating 30 children at home, most of them diagnosed since the inception of the testing program. These compelling statistics should make believers of any nonbelievers regarding the financial soundness of the program.

But we must soberly admit what this program hath wrought, especially in the way of need for a more precise diagnosis and long term follow-up of positively screened patients. In the classical phenylketonuric, with markedly deficient PA hydroxylase activity (Fig 1), the serum level will reach greater than 20 mg % by the second week and usually 30–40 mg % by the third week. 0-hydroxyphenylacetic acid usually appears in the urine when the serum phenylalanine level reaches 7 mg %, but phenylpyruvic acid is not excreted in the urine until the serum phenylalanine reaches 13 to 15 mg %. Because this latter substance is what makes the ferric chloride test positive, it can be seen that urine tested under age 2–3 weeks might be negative despite a high blood level. It is for this reason that urinary screening gave way to blood screening via the Guthrie test. Of those who have a *permanent* elevation of serum PA (ie, greater than 3–4 mg %), approximately ⅔ will follow this course where dietotherapy is necessary to allay mental retardation (Berman et al, 1969).

Because phenylalanine is an essential amino acid it cannot be completely eliminated from the diet; the aim therefore is to reach that amount of PA intake which will maintain the serum level between 3 and 7 mg % (Berry and Wright, 1967). This amounts to approximately ⅙ the normal intake, or 50–80 mg/kg per day in early infancy and 20–40 mg/kg per day in older infants and children. Why maintain the serum level at 3–7 mg when the normal is 1–1.5 mg %? If one aims for that normal level too much danger of undershooting exists, and PA deficiency is almost as deleterious to the developing infant as hyperphenylalaninemia. In fact, it has caused death in an occasional infant (Davens et al, 1965). Presently there is a co-

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operative study underway to determine if the 3–7 mg % range might be relaxed to 10–12 mg % without deleterious effect, as there is no knowledge of what level correlates with injury to the developing central nervous system. These ranges of intake are only rough guidelines, and frequent monitoring of serum PA levels by Guthrie testing is required to determine the more exact individual requirements. To make matters more difficult, these requirements often change with varying protein needs induced by infection, changes in the rate of growth, activity, etc.

Variability of Phenylalanine and Protein Needs

Of the 15 cases of phenylketonuria currently being followed in the MCV Metabolic Endocrine Clinic, I have selected two which demonstrate these extremely variable phenylalanine and protein needs. *The first case* (Fig 2) is that of a premature baby with a birth weight of 4 lb at 32 weeks gestation. The child was referred to MCV at age 3 weeks weighing 3 lb 4 oz, and having a serum PA level of 38 mg % with a normal serum tyrosine and without detectable phenylpyruvic acid in the urine. Extreme lethargy, persistent vomiting, and abdominal distension were present on admission and were not ascribable to other causes (ie, sepsis, organic bowel disease, etc), and might have been due to the high serum PA level. For this reason an extremely restricted PA intake of 16 mg/kg per day was instituted and consequent to the fall in the phenylalanine level, clinical symptoms disappeared and weight gain ensued. However, with a rapid weight gain the PA needs markedly increased and even intakes of 80–90 mg/kg per day did not suffice to maintain the serum PA level above 2 mg %. For this reason it was thought that this case might merely be one of transient hyperphenylalaninemia and a full phenylalanine intake of 120 mg/kg per day was offered in the form of regular milk. This resulted in a rise of serum phenylalanine to 22 mg %. Therefore, an intake of 80–90 mg/kg was again used with a consequent fall in the serum PA again to less than 2 mg %. Regular milk supplying 160 mg/kg PA per day raised the level to 15 mg %, and despite a drop in intake to 100–120 mg/kg per day, it continued to rise to 28 mg %. This time a decrease in intake to 80–90 mg/kg brought the infant to the correct range. By this time the rate of growth had also slowed somewhat. Since discharge this child's serum PA level has been maintained between 3–7 mg % on an even more reduced intake of 50 mg/kg per day. This infant, then, with the catch-up growth typical of the premature, initially required considerably more than the expected amount of dietary PA (90–100 mg/kg per day).

The second case is that of a 9 year old girl with an I.Q. of 85 who had findings in early infancy of classical PKU (Fig 3). She was maintained in fairly

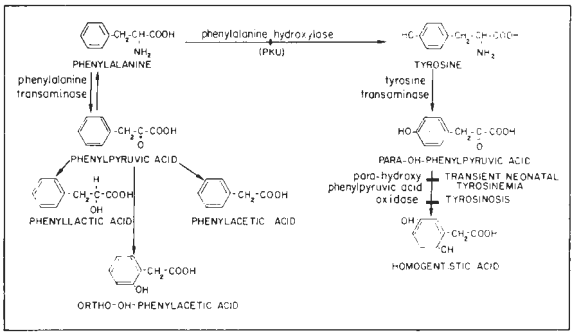

Fig 1—The classical phenylketonuric with markedly deficient PA hydroxylase activity.

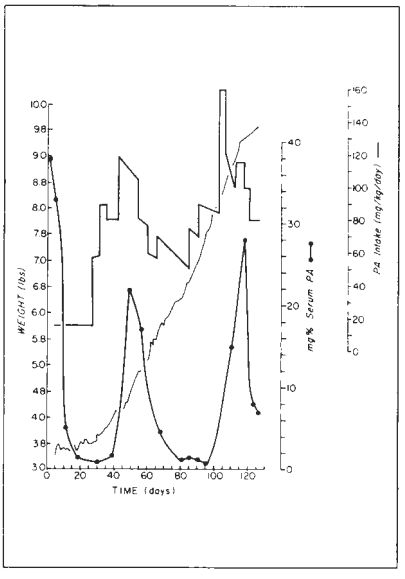

Fig 2—The course of case one.

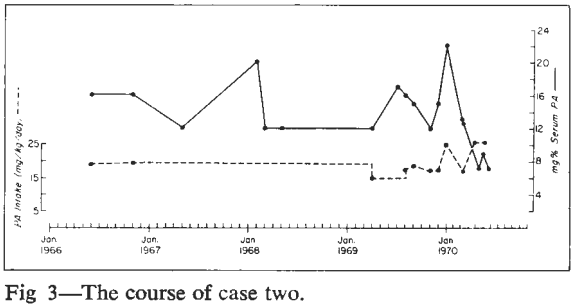

Fig 3—The course of case two.

good control through the first five to six years at the usual intake of 20–30 mg/kg of PA per day. Thereafter, because almost all brain growth has occurred by this age and because by intelligence testing and gross observations there is no deterioration in function when the diet is discontinued, the phenylketonuric child is usually gradually introduced to a normal PA intake. However, one very recent careful study suggests a measurable decrease of motor skills and learning processes may occur with a discontinuation of the diet at this age (Anderson et al, 1968). Because of this study, because the mother and child were most comfortable with the diet, and because the mother was fearful of any possible deterioration, PA restriction was continued. However, for the past three years, despite a low dietary PA intake of 16 mg/kg per day, the serum PA has been no lower than 12 mg %. An increase in dietary intake of PA (as natural food protein) to 24 mg/kg per day in December, 1969, merely caused an increase in the serum PA to 22 mg %. When the protein intake was increased in March, 1970, from a suboptimal level of 0.7 gm/kg per day to 1.3 gm/kg per day (by providing additional Lofenalac), a lowering of the serum PA to 7 mg % was effected, despite a small increase in total PA intake. This child therefore had high serum PA for several years because tissue protein breakdown was occurring in the face of inadequate protein intake. The changing dietary needs demonstrated by these two cases emphasize the need for constant monitoring with medical and nutritional supervision on an individual basis.

Other Causes of Hyperphenylalaninemia

Other than classical PKU there are at least five to six additional causes of hyperphenylalaninemia. Approximately 2/1000 normal newborns have a positive Guthrie test in the screening program, but the vast majority are due to *transient neonatal tyrosinemia.* This entity is believed to be due to a delay in the maturation in the liver of the enzyme parahydroxyphenylpyruvic acid oxidase, and large amounts of parahydroxyphenylpyruvic acid are usually found in the urine. Secondarily, serum PA rises and gives the positive Guthrie test, but serum tyrosine is characteristically much higher than phenylalanine. The values usually return to normal without therapy in one to two months, but occasionally not for six to eight months. No clinically apparent motor or intellectual deficits result from this transient defect and no treatment is indicated. However, until the tyrosine levels return to normal it is impossible to be certain that the enzyme defect is not of a permanent nature, then called *tyrosinosis* (or tyrosinemia). This disorder frequently causes severe liver and kidney damage and early death. Because it is ameliorated by a low tyrosine diet it becomes imperative that neonatally elevated tyrosine levels picked up by positive Guthrie tests be followed serially until they become normal.

Another cause of hyperphenylalaninemia of a transient nature is a *delay in the maturation of the enzyme PA hydroxylase* where the infant initially has classical findings of PKU but later in infancy tolerates a normal phenylalanine intake without hyperphenylalaninemia. Because of this possibility the dietary restriction of all infants with what initially appears to be classical PKU should be relaxed periodically to demonstrate persistence of the defect, as was done in my first example.

Of the approximate ⅓ of infants with permanent hyperphenylalaninemia who do not have the classical course of PKU, we encounter two generally described types. One is called *atypical PKU,* where levels of greater than 20 mg % are not associated with mental retardation despite there being no treatment. These children are usually uncovered as an older sibling of a newly diagnosed case from the screening program. The other term, simply *hyperphenylalaninemia,* is applied when the serum PA level never rises above 20 mg % without dietary restrictions and is usually not associated with mental retardation. The PA tolerance tests unfortunately do not distinguish these groups from the classical PKU patient, but there is some evidence to suggest that the absence in the urine of the breakdown products of PA, namely phenylpyruvic acid and orthohydroxyphenylpyruvic acid, favors a diagnosis of one of these milder forms of the disease. Indeed, some qualified investigators recommend not treating an infant unless there are PA metabolites in the urine, no matter how high the level of serum PA. It is therefore clear that we may be unnecessarily treating, with inherent dangers, as many as ⅓ of patients thought to have classical PKU. There is obviously a need for better means of diagnosing these subgroups.

Finally, there is the rare infant who lacks the enzyme phenylalanine transaminase but who only has hyperphenylalaninemia when on a higher than normal PA intake. In this instance, of course, no phenylpyruvic acid or orthohydroxyphenylpyruvic acid appears in the urine. Space does not permit a more detailed discussion of these variants, but I have mentioned them to emphasize that whereas just five years ago hyperphenylalaninemia was thought to be a simple disease state, mass screening has disclosed a wide spectrum of clinical and biochemical presentations with variable prognoses and treatment.

Indications for Screening

With some of the machinery already established, the next logical question deals with what other inborn errors should be screened. To answer this it would be best at this point to review the ten principles of early detection outlined by a World Health Organization team a few years ago (Wilson and Junger, 1968). It

will be seen that by these criteria the PKU program is a valid one:

1) "The condition should be an important problem." Although not terribly common, PKU is important as it is a treatable cause of mental retardation.
2) "There should be an accepted treatment for patients with recognized disease." There is for PKU. For other aminoacidopathies it is not so well worked out—for galactosemia, to be mentioned shortly, there *is* an accepted treatment.
3) "Facilities for diagnosis and treatment should be available." These do exist but are most marginally funded, so that the more detailed work-ups required with increasing awareness of the heterogeneity of the disease are not possible. This will be a drawback to establishing other mass or selective screening programs.
4) "There should be a recognizable latent or early symptomatic state." In the instance of PKU this is hyperphenylalaninemia, but at best this is likely an early symptomatic stage. Even dietary therapy instituted at less than 2 months of age results in only a mean I.Q. of 85 ± 12 vs. the unaffected sibling comparison of I.Q. of 109 ± 11. Whether the treatment is started too late or is inadequate is not known.
5) "There should be a suitable test or examination." The Guthrie test fits this bill well.
6) "The test should be acceptable to the population." The Guthrie test again fits this bill well.
7) "The natural history of the condition, including the development from latent to declared disease should be adequately understood." Classical PKU qualifies to a good degree, although what level of PA or its metabolites is actually injurious is not yet known—nor is it known why some patients with significant elevations of serum PA do not develop retardation, as in atypical phenylketonuria.
8) "There should be an agreed policy on whom to treat as patients." We are in the same trouble here with PKU because, as mentioned earlier, some physicians would delay treatment until metabolic products in the urine appear. Some use the level of 15 and others 20 mg % before starting treatment.
9) "The cost of case finding should be economically balanced in relation to possible expenditure on medical care as a whole." As pointed out, PKU scores high here.

TABLE

Urinary Screening Tests for Metabolic Defects

Disease	$FeCl_3$	DNPH	C-N	Benedict's	C-TAB
1. Phenylketonuria	Green	+++	−	−	−
2. Maple Syrup	Navy Blue	+++	−	−	−
3. Tyrosinosis	Trans. Green	+++	−	±	−
4. Histidinemia	Green	++	−	−	−
5. Hyperglycinemia	−	+++	−	−	−
6. Fructose Intol.	−	±(?)	−	++	−
7. Galactosemia	−	−	−	++	−
8. Cystinuria	−	−	++	−	−
9. Homocystinuria	−	−	++	−	−
10. Hurler's	−	−	−	−	++
11. Morquio-Ullrich's	−	−	−	−	±
12. Fanconi's	±	±	±	+	−
13. Alkaptonuria	Brown				
14. Lowe's		+			
15. Hyperlysinemia		+			

DNPH = dinitrophenylhydrazine
C-N = cyanide nitroprusside
C-TAB = cetyl trimethylammonium bromide
± = sometimes positive

10) "Case finding should be a continuing process and not a once and for all project." This is certainly true for PKU.

On the basis of these criteria, what other inborn errors would qualify for mass screening? Most aminoacidopathies probably do not qualify at this time mainly because of their rarity and of the expensive chromatographic techniques required, making case-finding too costly. Reduction in cost of detection might be achieved by the application of the bacterial "inhibition assay" technique as used in the Guthrie test. In this procedure the excessive leucine of maple syrup urine disease prevents the inhibition of B. subtilis by 2-methyl leucine, and excessive histidine of histidinemia prevents inhibition of B. subtilis by azaserine. With more refined resins and increased automation, quantification by ion-exchange chromatography might be a useful tool for mass screening of aminoacidopathies, but this development is several years away. In the meantime pilot studies in specialized centers are proceeding, using one or two dimensional paper or thin layer chromatography, or high voltage electrophoresis as screening tools. In this way more knowledge of these rare diseases and their treatment can be obtained and we can then better satisfy criteria 2, 7, and 8, listed above. One such recent study (Clow, Saiver and Davies, 1969) of the plasma of over 36,000 neonates revealed 316 with hyperaminoacidemia, but all except six were only transient. Five of the six permanent elevations were hyperphenylalaninemias and one was a case of hypermethionemia.

Considerations are somewhat different concerning the disorder of carbohydrate metabolism, *galactosemia.* In this disorder a buildup in the blood and tissues of galactose and galactose-1-phosphate causes mental retardation, cataracts, liver and kidney disease, and usually early death. Whereas previous estimates have put the incidence at 1:50,000, recent mass screening surveys put it at between 1:20,000 to 1:30,000 (Hansen, 1969). Various screening tests are presently being evaluated—measuring either blood galactose levels via a bacterial inhibition assay technique or the actual levels of the deficient enzyme, galactose-1-phosphate uridyl transferase, from spotted filter paper or with 50 microliters of heparinized blood. As for phenylketonuria and maybe for most inborn errors, it has already been established that there are several variants of galactosemia—four at present. It is not totally clear which types require therapy, but because this disease satisfies most of the established principles, with good treatment available (lactose elimination) it would appear that as soon as it is determined which test is most suitable, it will be the next metabolic disorder to be mass screened. Indeed one state has already made testing for galactosemia mandatory and several other states are seriously considering it.

There are a host of other inborn metabolic disorders for which diagnostic tests are available, but their consideration for inclusion in mass screening are not as pressing as the aforementioned diseases, either because of their rarity (Wilson's disease), the lack of adequate therapy, or the lack of a test acceptable to the population (cystic fibrosis).

Selective Screening

Though to this point we have been talking about mass screening, I would like to discuss one type of selective screening—that is, the screening of a selected group. Mental retardates, specifically, are more likely to have metabolic disorders than the general population. At first blush it would seem to be a waste of time, effort, and money to seek a diagnosis once mental retardation has already ensued, because in almost all instances therapy cannot reverse this damage. On the other hand, several other considerations point out the validity of testing mental retardates for metabolic disorders.

1) Establishing the cause of retardation enables better prognosticating and genetic counselling.
2) If therapy is started early with only mild retardation present, further deterioration might be prevented or slowed.
3) Identifying the inborn error allows a better determination of its incidence and therefore its possible worthiness for inclusion in mass screening programs.
4) Identifying the inborn error may give more insight into the various modes of clinical and/or biochemical presentation and therefore allow earlier recognition of future cases and determination of appropriate therapy.

There are almost as many screening tests as there are inborn errors of metabolism, but by selecting a few tests that will identify several different disorders, a workable screening panel can be established. The table depicts such a panel of tests established initially by R. B. Young (Department of Pediatrics, Medical College of Virginia) and continued presently by myself as a service to physicians in the State. In the present practice, fresh acidified urine of any patient with suspected mental retardation, or with a clinical complex to suggest an inborn error, is mailed to the Pediatric Metabolism Laboratory at the Medical College of Virginia. These tests are performed once weekly and the results mailed promptly to the referring physician. With more experience with these programs, it is expected that these tests will have to be modified (as has recently been found to be the case for the cyanide nitroprusside and C-TAB tests), and others added.

Summary

I have briefly outlined the PKU program in the State of Virginia and tried to demonstrate how this screening experience has disclosed the heterogeneity of the disease plus the need for an individualized approach to dietary control. I have applied the principles of mass screening to examine the feasibility of testing for other inborn errors and, on this basis, feel that galactosemia will soon next join with PKU. Mass screening for other aminoacidopathies will await more refined testing techniques and a definition of their incidence and mode of therapy. In the meantime, accumulated experience thus far mandates the establishment of specialized lab facilities and multidisciplinary teams; these will be necessary to effect optimal evaluation and treatment of patients found to be positive by these screening techniques.

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