Dr. Robert Guthrie did not have newborn screening in mind when, in 1959, he developed the bacterial inhibition assay for phenylalanine. His intent was to provide Dr. Robert Warner of Buffalo Children's Hospital with a simple means to monitor the blood phenylalanine levels in children with phenylketonuria (PKU) who were on the phenylalanine restricted diet, developed by Dr. Horst Bickel. Bickel and his colleagues had shown that the diet lowered the markedly increased blood phenylalanine level that is the major metabolic feature of PKU and to improve the very hyperactive and agitated behavior of mentally retarded children with PKU who, at that time, were previously untreated.

Monitoring this level is critical in determining how much or how little to restrict the diet. Insufficient restriction does not lower the phenylalanine level enough to provide benefit, while excessive restriction can produce a lower than normal level that may result in growth retardation and even sudden death.

Warner was treating a number of these children but had difficulty monitoring their blood phenylalanine levels because the assays to measure phenylalanine were very laborious. Thus, necessity concentrated the minds of both Guthrie and Warner. The assay developed by Guthrie worked extremely well, enabling Warner to monitor his patients at least weekly.

By this time, published studies

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were appearing of genetically at-risk siblings detected with PKU soon after birth in whom mental retardation was prevented by early presymptomatic dietary treatment. All of these infants had increased blood phenylalanine levels within 1 to 2 days after birth. However, there is a family history of the disorder in only 20% of infants with PKU. Thus, testing only those with family history would mean 80% of children with PKU would not be identified in time to prevent mental retardation. The challenge was to detect all infants with PKU in time for preventive therapy.

Guthrie began to consider this problem. He had an intense interest in preventing mental retardation since his second son was mentally retarded, although not from PKU. Then one day, his wife learned that her niece who was institutionalized for the mental retardation was found to have PKU. This intensified his interest. As he learned more about the disorder, he thought of using the simple bacterial assay he developed for monitoring the diet to test newborn infants for PKU before nursery discharge. However, the test he developed used serum, and he knew that collecting such a specimen from all newborns would be problematic.

In seeking advice from pediatricians, he learned that the least intrusive method of blood collection in the neonate is by a simple heel stick. If this blood could then be blotted onto a filter paper and sent to a central laboratory for testing, Guthrie realized, all newborn infants could be screened for PKU. Consequently, he revised his bacterial assay so that blood dried on filter paper could be used. Thus began the Guthrie test and the possibility of universal newborn screening.

The challenge then became to bring universal screening into reality. Dr. Robert MacCready, director of the Diagnostic Laboratories at the Massachusetts Department of Public Health, also had an interest in preventing mental retardation, stimulated by one of his children who had Down syndrome. He and Guthrie met during an annual meeting of the National Association for Retarded Children (NARC) at which MacCready learned about Guthrie’s test and the promise of preventing mental retardation in PKU. Upon returning to Massachusetts, MacCready decided to establish the bacterial assay in the Diagnostic Laboratories, and to ask hospital pathologists to collect and mail the laboratory a blood specimen from each newborn infant.

The screening began in September 1962. Of the first 53,000 specimens tested in the Massachusetts laboratory, nine cases of PKU were detected. This incidence of one in 6,000 was astonishingly high, since at that time the frequency of PKU was considered to be no greater than one in 20,000. The publication of this information not only attracted national attention to newborn screening but also resulted in passage of a law in Massachusetts requiring that every newborn be tested for PKU.

During the next few years, the advocacy of parent groups, notably the NARC, resulted in the passage of mandatory newborn PKU screening laws in the majority of US states. By then, it was clearly established that newborn screening was indeed leading to the prevention of mental retardation in PKU.
for the consequences of genetic disease had begun.

THE EVOLUTION OF SCREENING: BEYOND PKU

The key to further developments in newborn screening was the newborn dried blood specimen, now known as the Guthrie specimen. The original Guthrie PKU test required only one or two small disks punched from the specimen, leaving much blood for additional testing, and the success of PKU screening stimulated consideration of newborn screening for other metabolic disorders. Guthrie soon discovered simple modifications of his bacterial assay for phenylalanine could result in identifying increases in other amino acids involved in metabolic disorders. These disorders were known to be treatable with diet, and, if detected in neonates, the consequences might be prevented, as in PKU. For instance, leucine is increased in maple syrup urine disease (MSUD) and methionine in homocystinuria. A bacterial assay of a different nature suggested by Dr. Kenneth Paigen could identify the increased galactose level in galactosemia. Guthrie succeeded in convincing several states to add these tests to the newborn blood specimens. This laid the foundation for presymptomatic diagnosis of biochemical genetic disorders.

These expansions of newborn screening beyond PKU were important, but none demonstrated the impact that screening for congenital hypothyroidism (CH) did. In 1975, Dussault and his colleagues in Quebec reported a radioimmunoassay for thyroxine (T4) that could detect CH in the newborn blood specimen. This was exciting because CH was known to result in cretinism, a prominent cause of mental retardation, and treatment with thyroxine replacement was much simpler than the diets for the metabolic disorders. Newborn screening for CH has revealed the relatively high frequency of one in 4,000 with this disorder.10 Treated newborns have had normal growth and development, including cognitive outcomes within normal limits.11

The success of newborn screening for PKU and CH stimulated further expansions. These have included hemoglobin electrophoresis to detect hemoglobinopathies,12 immunoassay to detect congenital adrenal hyperplasia,13 and enzyme assay to detect biotinidase deficiency.14 The Guthrie specimen has proven utility as well in identifying non-genetic disease states, including congenital infections such as toxoplasmosis and HIV.15

ENTERING A NEW ERA

In the future, newborn screening might have its greatest affect. We are entering an era of expanded screening made possible by the application of tandem mass spectrometry (MS/MS) to the Guthrie newborn specimen. This biotechnological advance has the capability of identifying at least 20 to 25 biochemical genetic disorders, only three of which (PKU, homocystinuria, and maple syrup urine disease) are covered by current traditional newborn screening.16 MS/MS might also be valuable in second-tier testing for the confirmation of disorders screened by other means. The results of employing MS/MS in newborn screening are reviewed by Fearing and Marsden in the article on page 509.

Perhaps the best way to illustrate the importance of expanded screening by MS/MS is to describe a specific case. An infant received traditional screening and subsequently presented with severe cardiomyopathy and cardiac failure due to a metabolic disorder not covered by that screening. The disorder was a very long chain acyl-CoA dehydrogenase deficiency (VLCADD), producing transient hypoglycemia interpreted as culture-negative sepsis, hypotonia, and poor feeding in the neonate. Cardiorespiratory failure due to the cardiomyopathy appeared at 3 months, and a plasma acylcarnitine profile revealed increased levels of C14:2, C14:1, C16, and C18:1, a pattern characteristic of VLCADD. Treatment with diet resulted in improvement over several weeks.

The original Guthrie newborn screening blood specimen was recovered from storage and was found to also have the acylcarnitine pattern of VLCADD when analyzed by MS/MS.
SUMMARY

It is safe to predict that we are still at an early stage of newborn screening. There is a high probability that in the future, MS/MS or a similar technology will be applied to screening for many additional disorders, both metabolic and non-metabolic. The ability to examine DNA in the Guthrie specimen, currently used in second-tier screening, has opened up opportunities for primary screening of a huge array of potential disorders that previously could not be identified in the newborn. Among the possibilities under current discussion are type I diabetes, severe combined immunodeficiency, fragile X syndrome, hereditary hemochromatosis, and lymphoblastic leukemia. The major problems with these considerations, however, are that preventive treatment is not yet possible for most of these disorders, and for many, the abnormal finding determines only susceptibility for and not certainty of disease. Our experiences in the past with such newborn screening as that for histidinemia, which was found not to produce disease, and α1-antitrypsin deficiency, which was not medically beneficial and had negative psychological effects, are lessons that must be taken seriously when considering new avenues of screening.

Beyond further application to the Guthrie blood specimen and testing in a centralized laboratory is the broader concept of newborn screening exemplified by universal screening for hearing impairment. This screening is conducted directly on the newborn in the newborn nursery. This type of in-hospital universal screening may have wider application in the future.

Much activity is underway to develop a consensus on appropriate newborn screening. This activity has been led by the Genetic Disease Branch of the federal Maternal and Child Health Bureau (MCHB), Health Resources and Services Administration (HRSA), in collaboration with the American Academy of Pediatrics and, currently, in collaboration with the American College of Medical Genetics through a committee to promulgate criteria for inclusion in newborn screening. The aphorism, “good judgment comes from experience, and experience comes from bad judgment,” may be applied to newborn screening. Our challenge now is to use the experience we have from the previous bad judgements to guarantee future good judgements.

REFERENCES