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What is Newborn Screening?

Newborn screening is a form of preventive health care in which babies are tested within the first days of their life to discover evidence of diseases for which the principal symptoms may not yet be apparent.

In order for screening to be successful a simple and reliable test must exist. Also, there must be a treatment that makes a difference when the disease is detected early. Screened diseases are varied; they may be genetic, endocrinologic, metabolic or hematologic. What they all have in common is that without timely treatment they will cause severe harm to the child.

Unlike treatment-based health care processes, newborn screening is population-based. This means that tests are not applied just to babies that are sick, but to all babies, including the vast majority, who will appear to be completely healthy. A screening test is intended to reveal whether a baby is more likely than other babies to have a disorder. It does not provide the information that a baby definitely has a disorder. If a screening test shows an abnormal result, then diagnostic testing is needed in order to confirm the presence of a disease.

It is important to recognize that the newborn screening process involves far more than just the screening test. The institution of a program requires that systems are in place for the efficient collection of samples from all newborns, for the reporting of results and possible recall of a child for diagnostic testing. Most important, a system must be in place to ensure that babies with confirmed disorders receive the timely treatment that they require.

What are the Reasons for Starting a Newborn Screening Program?

In the words of Dr Harry Hannon, former Chief of the Newborn Screening Branch, with Centers for Disease Control and Prevention (CDC),

*Newborn screening is critical to ensuring that we don’t have unnecessary suffering*
by parents and newborns – and that we prevent as many adverse outcomes of the disorders as possible and give ... (the affected children) ... as close to the natural life that they would have expected without the disorder as possible.

While reducing suffering is a natural objective for all health professionals, investment in preventive care yields significant savings when the total cost of a screening program is compared to the cost of providing lifetime care and support for people whose diseases could have been effectively treated had they been detected sufficiently early.

**Figure 1.** In materials produced to explain the benefits of newborn screening, the University of the Philippines-National Institutes of Health (UP-NIH) together with PhilHealth, the Philippine Health Insurance Corporation has presented the cases of two children both positive for congenital hypothyroidism. The 7-year old girl on the left of the picture was successfully treated after newborn screening highlighted the disorder. The 14-year old boy was not screened, and the lack of timely treatment resulted in developmental retardation.

**Early History of Newborn Screening**

The history of newborn screening began in the early 20th century when the British physician and pioneer in medical genetics, Sir Archibald Garrod used the term, inborn error of metabolism as the title for his 1908 Croonian lecture before the Royal College of Physicians in London. The four inborn errors of metabolism that he considered were albinism, alkaptonuria, pentosuria, and cystinuria. Working only a few years after the rediscovery of Mendel’s work, Garrod had established that a problem in a specific biochemical pathway was connected with a gene mutation.

A real movement towards newborn screening began with the work of Dr. Robert Guthrie, and his work with children affected by the disease, phenylketonuria (PKU). Following the birth in his own family of a child with a development disorder (though not PKU), he became interested in such children and particularly in the potential that early identification of PKU allowed. It was shown that infants identified as having the disease could be treated with a low-phenylalanine diet, and that with early identification and
treatment, infants had normal cognitive development compared to untreated infants who developed severe mental retardation.

Dr Guthrie was a cancer researcher, familiar with development of bacterial inhibition assays (BIA). In such assays a bacterial strain and growth medium are developed such that growth of the bacteria cannot take place unless the test substance is added to the medium. When unknown samples are added to the medium and the bacteria inoculated, subsequent growth of the bacteria is proportional to the amount of the substance in the unknown samples.

In 1962, he developed a BIA that was the first simple, sensitive, and inexpensive screening test for PKU. This test came to be known as the Guthrie test. When Guthrie also introduced a system for collection and transportation of blood samples on filter paper, cost-effective, wide scale genetic screening became possible.

Following the implementation by the State of Massachusetts in the mid 1960’s of the first universal newborn screening program, screening initially for just the one disorder, hyperphenylalaninemia (HPA), other states were quick to follow suit. The disease still forms the backbone of screening programs worldwide.

Soon after the advent of the Guthrie test, bacterial tests were also developed to allow screening for additional disorders, such as maple syrup urine disease (MSUD) and homocystinuria. The arrival of the radioimmunoassay technology in the 1970’s made it possible to develop a suitable inexpensive and simple test for thyroxine (T4). Low levels of this hormone are associated with congenital hypothyroidism (CH), which has a higher incidence than even PKU. Another important disease for which screening was implemented was galactosemia.

Over the following decade radioimmunoassays or immunoradiometric assays (or non-radioactive counterparts to the two aforementioned techniques) were introduced for analytes such as thyroid stimulating hormone (hTSH, providing an additional facility in CH screening), for 17α-OH-progesterone (17-OHP, for congenital hyperplasia screening) and for immunoreactive trypsin (IRT, helping in early identification of cystic fibrosis).
Collecting the Dried Blood Spot Sample

The process for newborn screening may be thought to start with the collection of blood samples, which will take place typically between day 2 and day 5 (when the actual day of birth is numbered as 0). The use of dried blood spots in newborn screening is the generally preferred method, since it is easy to obtain the samples and only a small amount of blood is needed.

Depending on the timing and other local factors the blood collection may be at the site of the baby’s delivery, at a postnatal clinic, or at home. The blood collection will usually be performed by a midwife or nurse, and a blood specimen card should be filled in, providing all necessary information to accompany the sample.

Blood is taken from the baby’s heel, which should be gently warmed. The region that is to be punctured, within the shaded area in the illustration, should be cleaned with a suitable solution, e.g. 70% alcohol solution, and allowed to air-dry. A sterile lancet is then used to make the puncture, to a depth not greater than 2 mm. The first drop of blood that forms may be wiped away with a dry sterile gauze. The foot should not be squeezed.

![Figure 2. A spot of blood is obtained from one side of the bottom of the baby’s heel.](image)

A drop of blood large enough to fill the circle printed on the collection card should be allowed to form and this is then applied to the rear side of the card only. The blood has to fill the circle completely and saturate the paper right through.
Only one drop of blood should be applied to each circle, and each circle should be filled. The wound can then be treated according to the local practice, and the card is allowed to dry in air at ambient temperature (15° C - 22° C). Cards must not be stacked on top of one another while drying.

The dried specimens are sent directly to the laboratory for measurement.

**Timing of Sample Taking and Need for Retesting**

Although there are obvious practical benefits in being able to perform primary screening while the child is still at the maternity hospital, in most cases sample collection could reasonably take place after the 5 days suggested above. However, it is important to remember that for many of the disorders screened, babies confirmed to be positive need to be on treatment at least within a few weeks of birth. Since retesting will be required for all babies initially found to be screen positive, it is important that strict control should be retained over the timing of the primary screening. For this reason some traditionally used markers that are better suited for measurement after 5 days have recently come under the spotlight. For example, measurement of tyrosine, a marker for tyrosinemia type 1, at the time while a child is still at the maternity hospital may lead to an incorrect screening result. A preferred marker for tyrosinemia type 1 may be succinylacetone, which is clearly detectable much sooner after birth.

Babies born preterm may require a repeat test for CH at the equivalent of 36 weeks gestation. Repeat testing for several disorders may also be necessary in children that have received a blood transfusion shortly after birth.

Babies who screen positive are referred to a specialist team directly from the laboratory and local physicians are informed of this. These steps are intended to ensure that treatment is started in a timely fashion for the disease in question.
Carnitine Uptake Defect (CUD)

Background
Carnitine uptake defect (CUD) is a condition that prevents the body from using fats for energy, particularly during periods without food (fasting). Carnitine, a natural substance acquired mostly through diet, is used by cells to process fats and produce energy. In people with CUD, proteins called carnitine transporters do not work properly. These proteins normally bring carnitine into cells and prevent the escape of carnitine from the body in urine. CUD is estimated to occur in less than 1 of 100,000 live births. However, it has been reported to have an incidence rate of 1:40,000 live births in Japan.

Clinical
Typically, initial signs and symptoms of this disorder occur during infancy or early childhood and often include changes in brain tissue (encephalopathy) resulting in functional abnormalities; an enlarged, poorly pumping heart (cardiomyopathy); confusion; vomiting; muscle weakness; and low blood sugar (hypoglycemia). Serious complications such as heart failure, liver problems, coma, and sudden unexpected death are also a risk. Severe illness due to CUD can be triggered by periods of fasting or illnesses such as viral infections, particularly when eating is reduced.

This condition is sometimes mistaken for Reye syndrome, a severe disorder that develops in children while they appear to be recovering from viral infections such as chicken pox or flu.

Testing
Newborn screening using tandem mass spectrometry of a dried blood spot identifies low level of free carnitine (C0). \((C0+C2+C3+C16+C18:1+C18)/Cit\) is also found as informative ratio. Plasma and urine carnitine analysis will reveal decreased free and total carnitine (C0) in plasma and overexcretion of carnitine in urine. The newborn’s mother should be investigated, as well, because several cases of maternal CUD have been identified following an abnormal newborn screening result in their offspring. Transporter assays and OCTN2 gene sequencing establish the diagnosis.
Treatment
Treating CUD patients with oral L-carnitine supplementation is followed by a slow increase of plasma carnitine levels. If the infants’ levels reflect maternal primary carnitine deficiency, the rise in plasma levels is fast and this should prompt the work-up towards the diagnosis of maternal primary carnitine deficiency. CUD patients should also avoid fasting and sometimes low-fat, high carbohydrate diet is used in addition to L-carnitine. Guidelines for the management of carnitine deficiency and other fatty acid mitochondrial disorders have been established.

Because the diagnosis and therapy of CUD is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

Inheritance
This disorder follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

Long Chain L-3 hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)

Background
Long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is a disorder of mitochondrial fatty acid β-oxidation. LCHAD is one of two enzymes that carry out the third step (of 4) in the β-oxidation of fatty acids – the other enzyme being short-chain hydroxyacyl-CoA dehydrogenase (SCHAD), which acts on shorter-chain substrates. LCHAD activity resides on the mitochondrial trifunctional protein, which acts to catalyze 3 sequential steps in β-oxidation. LCHAD deficiency occurs as an isolated defect (described here) or together with deficiency of the other 2 enzymes in mitochondrial trifunctional protein deficiency. LCHAD deficiency impairs oxida-
tion of dietary and endogenous fatty acids of long-chain length (16 carbons and longer). LCHAD is estimated to occur in at least 1 in 75,000 live births.

Clinical
LCHAD deficiency can present clinically from day one to 3 years of age. Two clinical scenarios have been described. One group of LCHAD deficiency patients presents with symptoms of cardiomyopathy, which may lead to death. Several cardiac problems have been described, including cardiomegaly, left ventricular hypertrophy, and poor contractility. Onset may be acute or chronic. A second group of patients presents, usually following fasting, with non-ketotic hypoglycemia, vomiting, hypotonia, and hepatomegaly. Rhabdomyolysis may occur. Both presentations are highly variable and may have overlapping features. Symptoms may be initiated by a seemingly innocuous illness (a cold or otitis media), leading to prolonged fasting. Symptoms often precede onset of hypoglycemia. Hypoglycemia occurs from an inability to meet gluconeogenic requirements during fasting despite activation of an alternate pathway of substrate production – proteolysis. Physical examination of the acutely ill child may find mild to moderate hepatomegaly and muscle weakness. Laboratory examination of blood may reveal hypoglycemia, elevated CK and abnormal transaminases. Unique among the fatty acid oxidation disorders, LCHAD patients may develop a sensorimotor peripheral neuropathy and pigmentary retinopathy over time. Fatty liver is noted at autopsy, often leading to a misdiagnosis of Reye’s syndrome or Sudden Infant Death Syndrome (SIDS) in an infant.

A complication of pregnancy, HELLP Syndrome (hemolysis, elevated liver enzymes, and low platelets), has been described in women carrying a fetus affected with LCHAD deficiency.

Testing
Newborn screening using tandem mass spectrometry of a dried blood spot identifies elevated levels of several long chain hydroxyacylcarnitines (C16-OH, C16:1-OH, C18-OH, C18:1-OH and C16-OH/C16 is also found to be an informative ratio). Biochemical testing of blood and urine for carnitine, acylcarnitines, acylglycines, and organic acids is diagnostic for this disorder. Dicarboxylic and hydroxydicarboxylic acids are usually found with urine organic acid analysis, but may be “normal” when the patient is not acutely ill. Analysis of LCHAD activity in fibroblasts can reveal affected
individuals compared to heterozygous carrier and normal fibroblast lines. LCHAD activity should be assayed after antibody precipitation of SCHAD activity, due to the overlap in substrate recognition.

LCHAD patients have a common mutation (1528G>C) in the -subunit of mitochondrial trifunctional protein. Detection of mutations in the DNA of affected individuals allows for confirmation of biochemical test results and accurate detection of asymptomatic carriers among other family members. Prenatal diagnosis is possible by enzyme assay of cultured amniocytes or by in vitro probe of the β-oxidation pathway. DNA analysis can also be used for prenatal diagnosis of affected fetuses in at-risk pregnancies when both parents carry a known mutation.

**Treatment**

Fundamental to the medical management of LCHAD is the avoidance of fasting, particularly during periods of high metabolic stress, such as illness. Overnight fasts should last no longer that twelve hours and infants should receive late evening feedings to reduce this period. The addition of food-grade uncooked cornstarch mixed in liquid at bedtime has helped some infants decrease the frequency of morning hypoglycemia. A diet high in natural fat should be avoided. Medium-chain triglyceride supplementation bypasses the metabolic block and provides safe calories. Supplementation with oral L-Carnitine has not been shown to be beneficial in avoiding or ameliorating clinical symptoms.

High carbohydrate intake should be encouraged during illness, with initiation of intravenous glucose supplementation if the child is unsuccessful in keeping down fluids, or unable to take adequate oral feedings. For individuals with LCHAD deficiency, it is imperative that the lethargic patient receive parenteral dextrose to avoid hypoglycemia during evaluation.

Because the diagnosis and therapy of LCHAD deficiency is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.
Inheritance
This disorder follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)

Background
Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is a disorder of fatty acid β-oxidation, occurring in at least 1 in 25,000 live births. The enzyme deficiency is medium-chain acyl-CoA dehydrogenase, one of four mitochondrial acyl-CoA dehydrogenases that carry out the initial dehydrogenation step in the β-oxidation of fatty acids. MCAD deficiency results in an impaired ability to oxidize dietary and endogenous fatty acids of medium-chain length (6-12 carbons).

Clinical
MCAD deficiency generally presents between the second month and the second year of life, although onset as early as two days and as late as adulthood has been reported. Clinical presentation is often triggered by a seemingly innocuous illness like otitis media or a viral syndrome. The initiating event is probably prolonged fasting, which increases lipolysis and the need for fatty acid oxidation. Symptoms include vomiting, lethargy, apnea, coma, cardiopulmonary arrest, or sudden unexplained death. Initial symptoms often precede the onset of profound hypoglycemia, and are probably related to high free fatty acid levels. Hypoglycemia occurs from an inability to meet gluconeogenic requirements during fasting despite activation of an alternate pathway of substrate production (protein catabolism). Physical examination of the acutely ill child is remarkable for mild to moderate hepatomegaly, and some patients may also have demonstrable muscle weakness. Without prior indication of metabolic disease, 20–25 percent of patients with this disease will die with their first episode of illness. Cerebral edema, and fatty liver,
heart, and kidneys are noted at autopsy, often leading to a misdiagnosis of Reye’s syndrome or Sudden Infant Death Syndrome (SIDS). This disorder accounts for about one percent of SIDS deaths.

**Testing**

Newborn screening by tandem mass spectrometry of the heel stick dried blood spot identifies elevated levels of octanoylcarnitine (C8 acylcarnitine), usually accompanied by decanoyl (C10), hexanoyl (C6) and decenoyl (C10:1) carnitine esters. Also C8/C2 and C8/C10 ratios have been found informative for MCAD. When symptomatic, laboratory examination of blood may reveal hypoglycemia, metabolic acidosis, mild lactic acidosis, hyperammonemia, elevated BUN, and high uric acid levels. Serum transaminases are usually elevated. The urine often shows inappropriately low or absent ketones due to impaired fatty acid oxidation. Low serum and urine carnitines are typically found in the untreated patient. Biochemical testing of blood and urine for carnitine, acylcarnitines, acylglycines, and organic acids is diagnostic for this disorder. A generalized dicarboxylic aciduria is noted, characterized by elevations of suberylglycine and hexanoylglycine. In fibroblasts, the activity of medium chain acyl-CoA dehydrogenase is severely deficient in affected individuals, while heterozygous carriers for the disease usually have intermediate levels of activity, but are otherwise clinically and metabolically unaffected.

Detection of mutations in the MCAD gene on chromosome 1 in affected individuals confirms the biochemical results and accurately detects asymptomatic carriers among other family members. A common 985A>G mutation is responsible for up to 85% of cases. DNA analysis of postmortem tissue is possible when plasma and urine samples are not available. Prenatal diagnosis is possible by enzyme assay of amniocyte cultures. DNA analysis in amniocytes or chorionic villi can also be helpful in the diagnosis of an affected fetus in at-risk pregnancies.

**Treatment**

Fundamental to the medical management of MCAD is the need to avoid fasting, particularly during periods of high metabolic stress, such as illness. Overnight fasts should be managed with nighttime or late evening feedings where appropriate. The addition of food-grade cornstarch mixed in liquid at bedtime has also helped to decrease the frequency of morning hypoglycemia in some
patients. High carbohydrate intake should be encouraged during illnesses, with initiation of intravenous glucose supplementation if the child is unsuccessful in keeping down fluids or unable to take adequate oral feedings.

The preventive efficacy of a low fat diet versus a normal fat diet is unclear, but high intake of long and medium-chain fatty acids should be avoided. Supplementation with oral L-carnitine has been associated with a reduction in the frequency and severity of episodes. The continued need for carnitine supplementation post-puberty is uncertain, and has not been adequately studied.

Because the diagnosis and therapy of MCAD deficiency is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**
This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

**Trifunctional Protein Deficiency (TFP)**

**Background**
Mitochondrial trifunctional protein (TFP) deficiency is a defect in mitochondrial fatty acid β-oxidation. Three enzyme activities that act sequentially in the oxidation of fatty acids reside together on the TFP enzyme complex located on the inner mitochondrial membrane. The enzymes are long-chain-2-enoyl-CoA hydratase, long-chain hydroxyacyl-CoA dehydrogenase (LCHAD), and β-ketoacyl-CoA thiolase. The TFP complex consists of two different protein subunits (α and β) coded for by two nuclear genes. The TFP complex has specificity toward fatty acids of ten carbons (C10) or longer. TFP is estimated to occur in less than 1 in 100,000 live births.
Clinical
Diverse clinical presentations have been reported in patients having TFP Deficiency. The usual presentation is in infancy and follows a period of fasting associated with a minor illness. Patients develop non-ketotic hypoglycemia, hypotonia, and lactic acidemia. Areflexia and cardiomyopathy is often found on physical exam, and sudden death can occur. Patients may have elevated CK levels and even rhabdomyolysis, and a few have had hyperammonemia. Low carnitine levels have been measured in serum and muscle. Hepatic steatosis is found at biopsy. Many of these patients succumb to severe muscular hypotonia with respiratory distress.

Testing
Newborn screening of a dried blood spot using tandem mass spectrometry detects elevations of several long-chain and hydroxy acylcarnitines (i.e. C16-OH, C16:1-OH, C18-OH and C18:1-OH also the C16-OH/C16 ratio has been found informative for TFP). These findings are characteristic but not definitive of TFP Deficiency, because isolated LCHAD deficiency shows similar findings. Quantitative urine organic acid determination is usually not helpful, as elevation of C6 to C14 dicarboxylic and 3-hydroxy-dicarboxylic acids may or may not be present. Plasma acylcarnitine profile can demonstrate elevations of the above acylcarnitines noted in a dried blood spot. Definitive testing is performed by direct enzyme testing using leukocytes or fibroblasts or by probing cultured fibroblasts for the TFP activities using labeled fatty acid substrate.

TFP deficiency can be caused by mutations in either the α-subunit or β-subunit genes for TFP. No common mutation in TFP deficiency has been reported, but prenatal diagnosis is theoretically possible if both mutations are known.

Treatment
Supportive care for the acutely ill child involves treating hypoglycemia, lactic acidosis, and hyperammonemia with IV fluids containing glucose and bicarbonate. Administration of L-carnitine should be considered. Avoidance of fasting is important to prevent symptomatic episodes.

Because the diagnosis and therapy of TFP Deficiency is complex, the pediatrician is advised to manage the patient in close collaboration with a consult-
ing pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**
This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

**Very Long Chain Acyl-CoA Dehydrogenase Deficiency (VLCAD)**

**Background**
Very long chain acyl-CoA dehydrogenase deficiency (VLCAD) is a disorder of β-oxidation of fatty acids. The enzymatic deficiency is one of four mitochondrial acyl-CoA dehydrogenases that carries out the initial dehydrogenation step in the β-oxidation of fatty acids. VLCAD deficiency impairs oxidation of dietary and endogenous fatty acids of long chain length (16 carbons and longer). The buildup of the long chain fatty acid acyl-CoA intermediates results in toxic effects to normal metabolism. The gene is on chromosome 17 and encodes a protein that functions on the inner mitochondrial membrane. VLCAD is estimated to occur in at least 1 in 75,000 live births.

**Clinical**
Two general presentations have been reported with VLCAD deficiency, although both can vary considerably. Infants can present with severe, sepsis-like symptoms resembling a Reye-like syndrome, which is often lethal. The patient may be hypoglycemic with fasting and have metabolic acidosis, elevated liver enzymes with hepatomegaly (due to steatosis), cholestasis, hypertrophic cardiomyopathy, proteinuria, and hematuria. A second presentation has later onset and exhibits lethargy and coma with fasting. These patients have hypoketotic hypoglycemia, hepatomegaly, recurrent “infections”, and
easy fatigue resulting in recurrent sore muscles. Some present with exercise-induced rhabdomyolysis.

**Testing**
Newborn screening using tandem mass spectrometry detects increased levels of C14:1, C14:2 and C14 acylcarnitines indicating a probable case of VLCAD deficiency (the C14:1/C16 ratio has also been found informative for VLCAD). Clinical testing may reveal hypoglycemia with elevations of lactate, pyruvate, ammonia, and CK. Elevated dicarboxylic acids, both saturated and unsaturated, are often seen on urine organic acid analysis when the patient is ill. Enzyme studies performed on cultured fibroblasts can also be used to indirectly detect VLCAD activity using a labeled probe for β-oxidation.

**Treatment**
VLCAD deficiency patients are treated with carnitine supplementation and strict avoidance of fasting. Maintaining glucose homeostasis is accomplished with frequent feedings, restricting dietary fat and increasing carbohydrates, using medium-chain triglycerides (MCT) oil supplementation and possibly cornstarch if necessary to prevent hypoglycemia. Workup of a suspected VLCAD deficient patient should rule out medium chain acyl-CoA dehydrogenase deficiency (MCAD) or glutaric aciduria type II (GA-II), because MCT oil supplementation is contra-indicated for these disorders. For individuals with VLCAD, it is imperative that the lethargic patient receives parenteral glucose to avoid hypoglycemia.

Because the diagnosis and therapy of VLCAD deficiency is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**
This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition, but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is
a 25% chance with each pregnancy that they will have a child affected with the disorder.
ORGANIC ACID DISORDERS

3-Hydroxy-3-Methylglutaric Aciduria (HMG)

Background
3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) lyase has a dual function in the breakdown of leucine and in regulating production of ketone bodies. It is located predominantly in mitochondria, but is also found in peroxisomes. In the last step in leucine metabolism, it cleaves 3-hydroxy-3-methylglutaric acid, producing acetyl-CoA and acetoacetate, one of the ketone bodies. HMG was first described in 1971 and more than 60 patients have subsequently been diagnosed. HMG is estimated to occur in less than 1 in 100,000 live births.

Clinical
HMG typically presents within the first week of life, though some patients have onset later in the first two years of life. The onset of symptoms is initiated by fasting, infection, dietary protein load, or simply the stress of birth. Symptoms progress from vomiting, lethargy, tachypnea and dehydration to coma and possibly death. Hepatomegaly and neurologic abnormalities are seen on physical exam. Laboratory studies reveal non-ketotic hypoglycemia, metabolic acidosis, hyperammonemia and elevated liver transaminases. Abnormal urine organic acids are present as well as the distinctive elevated plasma acylcarnitine species.

Testing
Newborns can be screened for HMG using tandem mass spectrometry analysis of a dried blood spot. The finding of elevated six-carbon dicarboxylic acylcarnitine (C6-DC) and C5-hydroxy acylcarnitine (C5-OH), suggests the metabolic defect. Also C5-OH/C8 ratio has been found informative for HGM. To make a diagnosis, further testing is required. Urine organic acid analysis of a patient with HMG will reveal elevation of 3-hydroxy-3-methylglutaric, 3-methylglutaconic and 3-hydroxyisovaleric acids. A diagnosis should be confirmed by measurement of HMG-CoA lyase enzyme activity in fibroblasts or leukocytes. Prenatal diagnosis is possible by measuring 3-hydroxy-3-methylglutaric acid in amniotic fluid and by assaying HMG-CoA lyase enzyme activity in cultured amniocytes and chorionic villi cells.
Mutations in the HMG-CoA lyase gene on chromosome 1 have been identified in a number of patients and prenatal diagnosis can be accomplished using DNA analysis.

**Treatment**
Acute symptoms of HMG-CoA lyase deficiency should be treated with IV glucose, bicarbonate for the metabolic acidosis and restriction of protein (leucine). During an acute episode, patients may require assisted ventilation. For the long-term treatment, affected patients should avoid fasting and restrict protein intake.

Because the diagnosis and therapy of HMG-CoA lyase deficiency is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**
This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

**Glutaric Acidemia Type I (GA I)**

**Background**
Glutaric acidemia, type I (GA I), was first described in 1975. The disease is caused by a genetic deficiency of the enzyme, glutaryl-CoA dehydrogenase (GCD), which leads to the buildup of glutaric acid in the tissues and its excretion in the urine of affected patients. GCD is involved in the catabolism of the amino acids, lysine, hydroxylysine, and tryptophan.

Over 200 cases of GA I have been reported in the medical literature. GA I is one of the most common organic acidemias and has an estimated incidence
of about 1 in 50,000 live births. Because of the initial slow progression of clinical symptoms, GA I is frequently undiagnosed until an acute metabolic crisis occurs.

**Clinical**
Newborns with GA I may appear normal at birth or have macrocephaly. Development is typically normal during the first year of life until the infant experiences an acute encephalopathic crisis brought on by an intercurrent illness. Symptoms are characterized by metabolic acidosis, dystonia, athetosis, and seizures. The patient is often left with permanent dystonia and long-term loss of motor function. Neurologic recovery is rare and incomplete. As an alternate presentation, an affected infant may be delayed in achieving early motor milestones and appear irritable, jittery, hypotonic, and have impaired voluntary movements. This may progress as a gradual neurological disorder with preservation of mental abilities. Both presentations involve destruction of the caudate and putamen resulting in the movement disorder typical of GA I. Affected patients have a very high risk for neurologic problems before age five.

**Testing**
Newborn screening using tandem mass spectrometry of the heel stick dried blood spot identifies patients with GA I by the presence of glutaric acid covalently bound to carnitine (C5-dicarboxylic acylcarnitine, C5-DC). Also the C5DC/C5-OH, C5DC/C8 and C5DC/C16 ratios have been found informative for GA I. This permits the earliest possible diagnosis and initiation of treatment for presymptomatic patients. In acutely ill patients, large amounts of glutaric acid can be detected in blood and urine by organic acid analysis. Analysis of the urine for abnormal organic acids in a suspected patient may reveal glutaric acid, 3-hydroxyglutaric acid (which is pathognomonic for GA I), and possibly glutaconic acid. These organic acids may be missing, however, in patients who are not acutely ill, in which case acylcarnitine analysis or enzymatic testing is preferred. GCD enzyme activity can be assayed in cultured fibroblasts, cultured amniocytes and chorionic villus (direct or cultured). Prenatal diagnosis has also been accomplished by finding elevated glutaric acid in amniotic fluid. DNA mutation analysis for prenatal diagnosis requires knowing the mutation(s) in the parents prior to testing. Free carnitine levels are often low and acylated carnitine levels are high at diagnosis. Plasma amino acids are usually normal and not helpful in diagnosis.
Several different gene mutations have been found to cause GA I. There has been no correlation of the DNA mutation with the clinical severity of the disorder for a given patient.

**Treatment**

Early, aggressive treatment prior to onset of clinical symptoms may prevent development of neurological damage. At the onset of any sickness or metabolic decompensation, prompt, vigorous initiation of IV fluids, including glucose and carnitine, with monitored administration of insulin, is recommended. Restriction of protein, i.e. Lysine and Tryptophan restriction, has not produced clear clinical benefits.

Because the diagnosis and therapy of GA I is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is suggested that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**

This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

**Isovaleric Acidemia (IVA)**

**Background**

Isovaleric acidemia results from a defect in the metabolism of the amino acid, leucine. The first patient with isovaleric acidemia was described in 1966 and the deficiency of isovaleryl-CoA dehydrogenase activity was found a few years later. Isovaleryl-CoA dehydrogenase functions in the inner mitochondrial matrix. The gene is located on chromosome 15. IVA is estimated to occur in less than 1 in 100,000 live births.
Clinical
Isovaleryl-CoA dehydrogenase deficiency has two general presentations. The first occurs within days or weeks of life as an acute, overwhelming illness with vomiting and ketoacidosis progressing to lethargy, coma and death in greater than 50% of the patients. Other laboratory findings include variable hyperammonemia, hypocalcemia, neutropenia, thrombocytopenia, and pancytopenia. A second cohort has onset later in the first year of life or after. These patients develop chronic, intermittent illnesses brought on by infection or a large protein intake. Laboratory findings will be as noted above, but perhaps not so severe. Both groups are susceptible to infection. The patient commonly has a distinctive odor of “sweaty feet” during an illness because of the volatile isovaleric acid that accumulates.

Testing
Newborns can be screened for IVA using tandem mass spectrometry analysis of a heel-stick dried blood spot specimen. The finding of elevated five-carbon acylcarnitine (C5) indicates either isovaleryl-CoA dehydrogenase deficiency or 2-methylbutyryl-CoA dehydrogenase deficiency. To differentiate the two diseases, further testing is required. Also the C5/C0, C5/C2 and C5/C3 ratios have been found informative for IVA. Urine organic acid analysis of a patient with IVA will reveal an elevation of isovalerylglycine with lesser elevation of 3-hydroxyisovaleric acid. The odiferous isovalerate is found in a urine specimen only during acute illness when its levels are significant. Due to its volatility (thus producing the odor), it is lost prior to and during specimen preparation for urine organic acid determination. In contrast, patients with 2-methylbutyryl-CoA dehydrogenase deficiency have 2-methylbutyrate and 2-methylbutyrylglycine in their urine. Prenatal diagnosis is possible by measuring isovalerylglycine in amniotic fluid and by measuring isovaleryl-CoA dehydrogenase enzyme activity in chorionic villus specimens or cultured amniocytes. The activity can also be measured in fibroblasts and leukocytes.

Treatment
Treatment of patients with isovaleric acidemia involves reducing protein intake, particularly the branched-chain amino acid leucine. During an acute episode, aggressive use of glucose and electrolytes is necessary. Glycine supplementation has proven beneficial because this amino acid is conjugated to isovalerate, forming the less harmful isovalerylglycine. Carnitine treatment
is similarly effective. Strict dietary control and aggressive treatment have resulted in normal development in some patients. However, many patients with isovaleric acidemia show neurologic abnormalities from acute illness.

Because the diagnosis and therapy of isovaleric acidemia is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist and dietician. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**

This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

**3-Methylcrotonyl-CoA Carboxylase Deficiency (3-MCC)**

**Background**

3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency has been recognized since 1984. It is a defect in the degradation of the amino acid leucine. As a carboxylase enzyme, 3-MCC requires biotin for activity. There are four carboxylases in man that utilize biotin and each can be deficient singly or together. If biotin metabolism is defective, activities of all four carboxylases will be low, resulting in multiple carboxylase deficiency. Some of the biochemical findings in 3-MCC deficiency overlap with those seen in multiple carboxylase deficiency, necessitating careful testing to distinguish the two disorders. 3-MCC is estimated to occur in at least 1 in 75,000 live births.

**Clinical**

The clinical presentations of 3-MCC deficiency range from severe to benign. The age of onset of symptoms is usually during the first several years of life,
but later onsets and even asymptomatic adults have been reported. Symptoms often have onset with an infection, illness, or prolonged fasting. Patients with 3-MCC deficiency can lapse into catabolic stress leading to vomiting, lethargy, apnea, hypotonia, or hyperreflexia and seizures. Patients may have profound hypoglycemia, mild metabolic acidosis, hyperammonemia, elevated liver transaminases, and ketonuria. Plasma free carnitine levels may be very low. Other patients may present with failure to thrive beginning in the neonatal period or developmental delay. Some individuals with 3-MCC deficiency have no apparent symptoms. Asymptomatic women with 3-MCC deficiency may pass along the 3-MCC metabolite transplacentally to their infants, who are then found to have elevated 3-MCC by newborn screening with tandem mass spectrometry, but who themselves do not have the disease.

Testing
Newborn Screening using tandem mass spectrometry reveals an elevation of C5-hydroxy acylcarnitine (C5-OH) from the dried blood spot of an affected patient. Also the C5-OH/C8 and C5-OH/C0 ratios have been found informative for 3-MCC. Diagnosis of 3-MCC deficiency then requires further testing. Urine organic acid analysis finds elevation of 3-hydroxyisovaleric acid and usually 3-methylcrotonylglycine. Following carnitine supplementation, 3-hydroxyisovalerylcaritnine is usually elevated in an acylcarnitine profile using tandem mass spectrometry. If C3 acyl carnitine is elevated, the disorder is multiple carboxylase deficiency. To further confirm isolated 3-MCC deficiency, the enzyme activity should be assayed in fibroblasts or leukocytes, along with at least one other carboxylase having normal enzyme activity. 3-MCC activity can also be measured in chorionic villus specimens. Mothers of all infants found to have elevated 3-MCC with newborn screening should be tested with a blood acyl carnitine profile to determine whether they have 3-MCC deficiency rather than their infant. The testing should also extend to other family members.

Treatment
Treatment of 3-MCC deficiency involves reducing dietary leucine intake using a special leucine-depleted formula or instituting a general protein restricted diet. With onset of illness, IV glucose is needed and the acidosis must be corrected. Both carnitine and glycine supplementation have proven beneficial. Patients should undergo an early trial of biotin supplementation
on the possibility that the defect is with biotin metabolism rather than isolated 3-MCC; biotin may be discontinued if there is no response.

Because the diagnosis and therapy of 3-MCC deficiency is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**

This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

**Methylmalonic Acidemias (MUT)**

**Background**

Methylmalonic acidemia (MUT) can result from several different genetic disorders, including methylmalonic-CoA mutase deficiency and defects of enzymes in cobalamin (vitamin B12) metabolism. Methylmalonic acidemia is one of the most studied metabolic defects, having been first reported in 1967. The incidence is estimated to be 1 in 48,000 births, but is probably higher due to lack of recognition and diagnosis. Multiple DNA mutations for MUT have been identified.

**Clinical**

Because of the dependence of methylmalonyl-CoA mutase activity upon cobalamin metabolism and function, the different defects producing MUT have a similar clinical presentation. The picture of methylmalonic acidemia as recurrent vomiting, dehydration, respiratory distress, muscle hypotonia, and lethargy that can lead to coma and death is often seen in the first week of life. Metabolic acidosis is pronounced. Ketoacidosis, hyperglycinemia, hypoglycemia, and hyperammonemia are often found, along with leukopenia, thrombocytopenia, and anemia. This same scenario can present later in the first
month of life, manifesting as failure-to-thrive and intellectual disability. All patients are reportedly susceptible to infection. A long-term complication of MUT is renal failure.

**Testing**

Newborns can be screened for MUT using tandem mass spectrometry analysis of a heelstick dried blood spot specimen. The finding of elevated three-carbon acylcarnitine (C3) indicates a possible metabolic defect, either MUT or propionic acidemia. Also the C3/C2 and C3/C16 ratios have been found informative for MUT. To make a diagnosis, further testing is required. Urine organic acid analysis of a patient with MUT will reveal massive elevation of methylmalonic acid, together with precursor metabolites β-hydroxypropionate and methylcitrate. These metabolites and others inhibit mitochondrial function. Methylmalonyl-CoA mutase activity and cobalamin metabolism can be studied in several tissues. A trial of vitamin B12 therapy has diagnostic importance in identifying those patients who have defects in cobalamin metabolism. Prenatal diagnosis is possible by measuring methylmalonic acid in amniotic fluid or maternal urine, and by enzyme activity studies in cultured amniocytes.

**Treatment**

Treatment of patients with MUT involves reducing protein intake, particularly the branched-chain amino acids valine and isoleucine, along with methionine and threonine. Special formulas are commercially available for this purpose. All patients should be given a trial of cobalamin supplementation to evaluate a response, since the management of B12-responsive patients is considerably easier and the prognosis is better. Carnitine supplementation has proven beneficial. Oral antibiotics help control infections and hypothetically reduce intestinal bacteria, which produce Propionic acid that can be absorbed through the gut and contribute to methylmalonic acid production. Strict control is most crucial throughout childhood. Several older patients with mild metabolic defects are reported to function untreated.

Because the diagnosis and therapy of MUT is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist and dietician. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.
Inheritance
This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

β-Ketothiolase Deficiency (BKT)

Background
β-ketothiolase (mitochondrial acetoacetyl-CoA thiolase) is an enzyme with a dual function in metabolism. It acts in the breakdown of acetoacetyl-CoA generated from fatty acid oxidation and regulates production of ketone bodies. It also catalyzes a late step in the breakdown of the amino acid isoleucine. β-ketothiolase deficiency was first described in 1971 and more than 40 cases have been reported. BKT is estimated to occur in less than 1 in 100,000 live births.

Clinical
β-ketothiolase deficiency has a variable presentation. Most affected patients present between 5 and 24 months of age with symptoms of severe ketoacidosis. Symptoms can be initiated by a dietary protein load, infection or fever. Symptoms progress from vomiting to dehydration and ketoacidosis. Neutropenia and thrombocytopenia may be present, as can moderate hyperammonemia. Blood glucose is typically normal, but can be low or high in acute episodes. Developmental delay may occur, even before the first acute episode, and bilateral striatal necrosis of the basal ganglia has been seen on brain MRI. Some patients may develop cardiomyopathy. An exaggerated ketogenic response to fasting or illness should raise suspicion of this disease.

Testing
Newborns can be screened for BKT by analysis of a dried blood spot using tandem mass spectrometry. The finding of elevated C5:1 and C5-OH suggests the metabolic defect. Also the C5-OH/C8 ratio has been found informative for BKT. To make a diagnosis, further testing is required. Urine
organic acid analysis of a patient with BKT will find elevations of 2-methyl-3-hydroxybutyric acid, tiglic acid, and tyglylglycine. A diagnosis should be confirmed by measuring enzyme activity in fibroblasts or leukocytes. Prenatal diagnosis is possible by measuring enzyme activity in cultured amniocytes or chorionic villus cells.

A variety of mutations have been identified in patients with BKT. There are no common mutations, however, that would permit rapid screening. The potential for prenatal diagnosis exists if the mutations are known in a family.

**Treatment**

The acute acidosis of BKT should be treated aggressively with sodium bicarbonate, keeping in mind the possibility of iatrogenic hypernatremia. Plasma levels of glucose, electrolytes, and ammonia should be normalized. Carnitine supplementation may be helpful.

For the long-term, affected patients should avoid fasting, eat frequently, and restrict protein intake. Intravenous glucose can be used when the patient is febrile or vomiting. Carnitine supplementation is reasonable. With appropriate monitoring and therapy, there is a good prognosis for normal development.

Because the diagnosis and therapy of BKT is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**

This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.
Propionic Acidemia (PROP)

Background
Propionic acidemia (PROP) is characterized by the accumulation of propionic acid due to a deficiency in propionyl CoA carboxylase, a biotin dependent enzyme involved in amino acid catabolism. Propionic acid may also accumulate in multiple carboxylase deficiency and methylmalonic acidemia. Multiple mutations for PROP have been identified. PROP is estimated to occur in at least 1 of 75,000 live births.

Clinical
Patients with PROP typically present in the first days of life with dehydration, lethargy, hypotonia, vomiting, ketoacidosis, and hyperammonemia. Seizures, neutropenia, thrombocytopenia, and hepatomegaly may be present. Untreated patients can progress to coma and die. Most patients who survive the neonatal period have episodes of metabolic acidosis precipitated by infection, fasting, or a high protein diet. In some cases, episodic hyperammonenemia seems to predominate over the metabolic acidosis. Psychomotor retardation is a life-long complication. Some patients have first presented later in infancy with encephalopathy and associated ketoacidosis, or developmental delay.

Testing
Newborns can be screened for PROP using tandem mass spectrometry analysis of a heelstick dried blood spot. The finding of elevated three-carbon acylcarnitine (C3) indicates a possible metabolic defect, either PROP, methylmalonic acidemia, or less likely, a defect in biotin metabolism. Also the C3/C2 and C3/C16 ratios have been found informative for PROP. To make a diagnosis, further testing is required. Urine organic acid analysis of a patient with PROP will demonstrate massive elevations of propionic acid and related compounds such as methylcitrate, propionylglycine, β-hydroxypropionate, and tiglic acid. In PROP, carnitine deficiency due to increased renal excretion of propionyl carnitine is often seen.

Treatment
Treatment of PROP involves reducing protein intake, particularly the amino acids Valine, Isoleucine, Methionine, and Threonine that feed into the defective pathway. This requires placing the infant on a special metabolic formula
depleted in these amino acids. Until the diagnosis of PROP is clearly established, all patients should be given a trial of cobalamin and biotin to evaluate a response. Carnitine supplementation has proven beneficial. Oral antibiotics help control infections and hypothetically reduce intestinal bacteria, which produce propionic acid that can be absorbed through the gut and contribute to metabolic stress. Prevention of constipation is important. Strict control is most crucial throughout childhood. Rarely, older patients with mild forms of PROP are reported to function untreated.

Because the diagnosis and therapy of metabolic disorders like PROP is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**
This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

**Multiple Carboxylase Deficiency (MCD)**

**Background**
There are four carboxylase enzymes in man that require biotin for activity. These enzymes are propionyl-CoA carboxylase, 3-methylcrotonoyl-CoA carboxylase, pyruvate carboxylase, and acetyl-CoA carboxylase. If biotin metabolism is defective, all four carboxylases will be deficient. Biotin is covalently linked to a key lysine residue in each carboxylase by action of holocarboxylase synthetase. When the carboxylase proteins are degraded, biotinoyl-lysine is subsequently cleaved by biotinidase releasing free biotin that can be reutilized. The two defects in biotin metabolism associated with Multiple Carboxylase Deficiency (MCD) are caused by deficient activity of holocarboxylase synthetase and biotinidase. The disorders tend to present clinically
at different ages, with holocarboxylase synthetase deficiency being known as early-onset (neonatal) multiple carboxylase deficiency and biotinidase deficiency referred to as late-onset multiple carboxylase deficiency. Both respond to biotin supplementation. MCD is estimated to occur in less than 1 in 100,000 live births.

Clinical
Patients affected with deficient holocarboxylase synthetase usually present in the first days or weeks of life with poor feeding, lethargy, hypotonia, and seizures, sometimes progressing to coma. Generalized rash and alopecia may be present. Affected patients exhibit metabolic acidosis and mild to moderate hyperammonemia. In contrast, biotinidase deficiency, which constitutes the vast majority of patients with multiple carboxylase deficiency, typically presents after several months of life with neurocutaneous symptoms including developmental delay, hypotonia, seizures, ataxia, hearing loss, alopecia, and skin rash. In some patients, the disease can be life-threatening.

Testing
Biotinidase deficiency is readily detected by measuring the activity of the enzyme on a heel stick dried blood spot. Newborn screening using tandem mass spectrometry may reveal an elevation of C5-hydroxy acylcarnitine (C5-OH) from the dried blood spot of a patient affected with holocarboxylase synthase deficiency. Also the C5-OH/C8 ratio has been found informative for MCD. Diagnosis of holocarboxylase synthetase deficiency requires further testing. Urine organic acid analysis reveals elevations of \( \beta \)-hydroxyisovaleric acid, \( \beta \)-methylcrotonylglycine, and tyglylglycine. Urine may also contain metabolites seen in propionyl CoA carboxylase deficiency and \( \beta \)-methylcrotonyl CoA carboxylase deficiency. Discriminating these disorders is important to ensure proper therapy is initiated.

Treatment
Treatment of patients with MCD involves administration of high doses of biotin. An excellent and rapid clinical response to biotin is characteristic of both enzyme defects associated with MCD. This highlights the importance of accurate and timely diagnostic evaluation of affected infants.

Because the diagnosis and therapy of MCD is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pedi-
atriac metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**
This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.
AMINO ACID DISORDERS

Argininosuccinic Aciduria / Citrullinemia

Background
The finding of elevated citrulline in a newborn screen dried blood spot suggests one of two metabolic defects: argininosuccinic acid synthetase deficiency or argininosuccinate lyase deficiency. Both are disorders of the urea cycle and are associated with severe, episodic hyperammonemia. Argininosuccinic acid synthetase deficiency (commonly called citrullinemia) occurs in 1:57,000 births and causes a dramatic elevation of plasma citrulline. Argininosuccinate lyase deficiency causes a less dramatic increase of plasma citrulline, but is no less clinically devastating. It is found in 1:70,000 births.

Clinical
Both forms of citrullinemia have a similar clinical presentation. With an early onset presentation, the newborn appears normal for the first 24 hours. Symptoms develop in association with worsening hyperammonemia. By 72 hours, lethargy, feeding difficulties and vomiting usually appear. The patient develops hypothermia, respiratory alkalosis and often requires ventilation. Seizures progressing to coma and death are typical in untreated patients. Physical examination reveals encephalopathy, which is due to brain edema and swollen astrocytes from glutamine accumulation and the resulting water retention. Patients with argininosuccinate lyase deficiency may exhibit hepatomegaly. These patients are frequently mistaken for a case of sepsis. A key laboratory abnormality suggesting a urea cycle defect is low blood urea nitrogen, which should dictate measurement of ammonia. Patients who survive the newborn period may have a neurologic impairment. These neonatal onset patients have recurrent episodes of hyperammonemia associated with viral infections or increased dietary protein intake. Some patients with either disorder have a later onset with a less severe course making diagnosis difficult.

Testing
Newborn screening by tandem mass spectrometry using a dried blood spot can detect elevated levels of citrulline with either disorder. Also the Cit/Arg ratio has been found informative for ASA. Argininosuccinate lyase deficiency
patients have measurable levels of argininosuccinic acid in plasma, which is not normally detected. The activity of either enzyme can be measured from a liver biopsy. Both genes have been isolated and mutations identified. DNA studies can be performed for prenatal diagnosis when the mutation is known from both parents. Biochemical studies of cultured amniocytes and chorionic villus tissue are also informative. The presence of argininosuccinic acid in the amniotic fluid of argininosuccinate lyase deficiency patients has been used for prenatal diagnosis.

**Treatment**
The symptoms of citrullinemia seem to originate from the hyperammonemia rather than citrulline accumulation. Acute hyperammonemia may necessitate hemodialysis, which is more effective for lowering ammonia than peritoneal dialysis or arteriovenous hemofiltration. Sodium benzoate is given to conjugate glycine, a major amino acid that contributes ammonia to the urea cycle, forming hippurate, which is subsequently excreted in the urine. Intravenous arginine results in ammonia clearance by enhancing formation of citrulline in argininosuccinic acid synthetase deficiency or argininosuccinate in argininosuccinate lyase deficiency. Both of these metabolites are excreted in the urine and draw off excess nitrogen from ammonia. Patients who survive the initial presentation are placed on protein restriction. Patients with either defect having onset in the newborn period face a poor outcome and significant risk of neurological damage or demise.

Because the diagnosis and therapy of these metabolic disorders is complex, the pediatrician is strongly advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**
These disorders most often follow an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder,
there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

**Homocystinuria (HCY)**

**Background**
The finding of elevated methionine in a dried blood spot upon newborn screening suggests one of two metabolic defects: 1) Homocystinuria due to cystathionine β-synthase (CBS) deficiency or 2) hepatic methionine adenosyltransferase deficiency. The most likely defect is a deficiency of CBS, which causes a connective tissue disease with several manifestations. Many patients with homocystinuria have been described since the deficiency was first reported in 1962. Methionine accumulates at the beginning of a metabolic pathway that sequentially converts this amino acid to homocysteine, cystathionine and cysteine. The step in the pathway that converts homocysteine to cystathionine is catalyzed by CBS. Although it is highly elevated, homocysteine is not detected with newborn screening because of its reactive nature with many components in blood, including itself with the formation of the dimer cystine. The elevation of methionine, therefore, is used to detect this disorder in newborn screening. The incidence of CBS deficiency is about 1 in 60,000, although several investigators believe this disease is more common.

**Clinical**
While the metabolic defect is present at birth, initial symptoms of homocystinuria usually have onset later in infancy and childhood. Developmental delay may be the first sign and is a harbinger of intellectual disability, but is not obligate. An early and distinctive finding is dislocation of the lens of the eye (ectopia lentis). Patients are at high risk for developing thromboembolism that may occur at any age. These may lead to stroke, seizures, permanent neurologic sequelae and death. Increased clotting ability makes surgery a risk. Osteoporosis is a long-term complication of homocystinuria.

**Testing**
Newborn screening of a dried blood spot using tandem mass spectrometry reveals elevated levels of methionine, which should prompt testing plasma for amino acids, including homocysteine. Elevated methionine and homocysteine in plasma indicate CBS deficiency, while an isolated increase in
methionine suggests hepatic methionine adenosyltransferase deficiency. Also the Met/Phe ratio has been found informative for HCY. In affected patients, the presence of homocystine in the urine is a consistent finding, especially after early infancy. CBS enzyme activity can be measured in many tissues, including fibroblasts, lymphocytes, liver, amniocytes, and chorionic villi (biopsy or cultured cells). Deficient enzyme activity may be followed with DNA mutation analysis for the several known mutations in the CBS gene.

**Treatment**

Treatment of CBS deficiency usually begins with a trial of oral vitamin B6 (pyridoxine) supplementation, with daily measurement of plasma amino acids. CBS requires pyridoxine as a coenzyme for enzymatic activity. Overall, about 25% of patients respond to large doses of pyridoxine, although the percentage may be lower for patients identified through newborn screening. This pyridoxine response usually coincides with the presence of some residual enzyme activity. Dietary restriction of methionine in conjunction with cystine supplementation reverses the biochemical abnormalities to some extent and appears to reduce the clinical symptoms. Special formulas are available commercially, but the diet is difficult to maintain long term. In an attempt to decrease homocysteine levels, folic acid, and betaine can be supplemented to induce recycling of this amino acid to methionine for alternate metabolism. Vitamin B12 (cobalamin) may also be helpful.

Because the diagnosis and therapy of homocystinuria is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**

This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.
Maple Syrup Urine Disease (MSUD)

Background
Maple syrup urine disease (MSUD) was first described in 1954 in a family with four successive affected newborns. Each died with a progressive neurologic disease in the first weeks of life. MSUD is caused by a deficiency in the ability to decarboxylate branched-chain amino acids. This enzyme activity resides in the branched-chain a-ketoacid dehydrogenase complex in the mitochondrial membrane. MSUD is estimated to occur in less than 1 in 100,000 live births but is as common as 1 to 176 in Old Order Mennonites.

Clinical
The most common form of MSUD presents with overwhelming symptoms in the first days of life. Patients appear normal at birth, but begin to have feeding difficulties with vomiting, progressing to lethargy and coma. The infant may have a high-pitched cry and the odor of maple syrup may emanate from the diaper. Metabolic acidosis with increased anion-gap is typically present, and plasma branch-chain amino acids (leucine, isoleucine, and valine) are seen. Hypoglycemia may occur. Neurologic deterioration is progressive and rapid. Cerebral edema results in encephalopathy exhibited as alternating hyper- and hypotonia, scissoring of the legs, opisthotonos, abnormal respirations, coma, and death. If the patient survives this period, any infection or metabolic stress is life threatening. Other less acute presentations have been reported, including an intermittent form associated with episodic ataxia and acidosis, and a milder, more chronic intermediate form with less severe acidosis. With all forms of MSUD, neurologic symptoms are typically evident by two years of age. Variable phenotypes arise from different mutations in the branched-chain a-ketoacid dehydrogenase complex and the residual metabolic capacity of a given patient.

Testing
Newborn screening of a dried blood spot specimen using tandem mass spectrometry measures valine and the sum of leucine, isoleucine, and alloisoleucine, the branchedchain amino acids. Also the Val/Phe, (Leu+Ile)/Phe and (Leu+Ile)/Ala ratios have been found informative for MSUD. Leucine, isoleucine, and valine are nutritionally required amino acids. In MSUD, these amino acids are not metabolized (decarboxylated) and accumulate to very high levels, the highest being leucine. Oxidative decarboxylation is the sec-
ond step in the degradative metabolic pathway, which is blocked in MSUD and results in the buildup of three organic acids - 2-oxoisocaproic acid from leucine, 2-oxo-3-methylvaleric acid from isoleucine, and 2-oxoisovaleric acid from valine - which are detected in high levels in the urine of affected patients. During a crisis, patients are acidic from elevated organic acids and lactate, and are typically ketotic. The diagnosis of MSUD is based on measuring elevated plasma branched-chain amino acids along with alloisoleucine, and the abnormal urine organic acids. Deficiency of the branched-chain a-ketoacid dehydrogenase enzyme complex activity can be measured in cultured fibroblasts. Prenatal diagnosis can be accomplished by measuring enzyme activity in chorionic villi cells or cultured amniocytes.

**Treatment**

Treatment often begins with aggressive intervention in an acute metabolic crisis. Hemodialysis, if available, can lower the levels of the branched-chain amino acids and organic acids in the plasma. Generous administration of IV fluids helps eliminate organic acids through renal loss. IV glucose provides an alternate energy source that reduces protein catabolism, which is a major source of branch-chain amino acids in acutely ill infants. Restriction of protein intake is usually a life-long requirement and commercial formulas that are depleted in branched-chain amino acids are available. Carnitine supplementation is useful in removing organic acids and repleting carnitine stores. Some patients respond to high-dose thiamine supplementation, a cofactor of the enzyme complex, and all newly diagnosed critically ill infants should be treated with this vitamin. Close monitoring of patients who survive the newborn period or who have a later presentation is required to reduce morbidity and mortality.

Because the diagnosis and therapy of MSUD is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**

This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symp-
Symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

**Phenylketonuria (PKU)**

**Background**
Phenylketonuria (PKU) is a disorder of amino acid metabolism that was recognized as a genetic defect as early as 1930. Phenylalanine is an essential amino acid that is converted to tyrosine by action of the enzyme phenylalanine hydroxylase. A block in this reaction due to deficient activity of phenylalanine hydroxylase causes PKU and results in severe central nervous system symptoms. Phenylalanine hydroxylase requires tetrahydrobiopterin as a cofactor and its deficiency, caused by an enzyme defect in the synthesis or recycling of tetrahydrobiopterin, can also result in PKU. The incidence of PKU is approximately 1 in 12,000 Caucasians. Historically, newborn screening originated with Dr. Robert Guthrie who developed a test for elevated phenylalanine (PKU) in dried blood spots.

**Clinical**
PKU babies typically appear normal at birth and in the neonatal period. Infants may later exhibit irritability, posturing, increased deep tendon reflexes, a peculiar “mousy” odor, and vomiting. Pale pigmentation develops in hair and skin, and seizures are sometimes present. Phenylalanine accumulates within the first days of life and tyrosine levels tend to be low. Although various phenylalanine metabolites are present, phenylalanine itself appears to be the toxic molecule in PKU. High phenylalanine levels prevent transport of other amino acids across the blood-brain barrier, inhibiting synthesis of key neurotransmitters and disrupting protein synthesis in the brain. This produces severe intellectual disability and white matter disease.

Women with untreated PKU who become pregnant are at high risk for having newborns with neurological damage. This is caused by the high phenylalanine levels in the untreated mother that cross the placenta during pregnancy and are toxic to the developing fetus. Mothers affected with PKU need to be on dietary control prior to conception to avoid the toxic effects of phenylalanine on their baby.
Testing
Newborn screening for PKU began with the use of a bacterial bioassay. Subsequently greater sensitivity has been achieved by the implementation of fluorometric methods and tandem mass spectrometry. The latter technology allows for measurement of both phenylalanine and tyrosine, showing elevated phenylalanine levels in conjunction with an increased phenylalanine to tyrosine ratio that is indicative of PKU. This methodology makes PKU screening effective, reliable, and efficient. Several hundred DNA mutations in the phenylalanine hydroxylase gene can cause PKU (98% of cases), as well as mutations in other genes necessary for tetrahydrobiopterin production (2% of cases). All newborns with PKU should be tested for tetrahydrobiopterin defects. Hyperalimentation with parenteral amino acid supplementation produces elevated phenylalanine levels in non-PKU infants, but the phenylalanine to tyrosine ratio is not elevated. Some infants have mutations in phenylalanine hydroxylase that result in mild hyperphenylalaninemia and no serious neurological disease.

Treatment
Patients with PKU need to maintain normal, physiological levels of phenylalanine and tyrosine for life. Studies have shown that periods of elevated Phenylalanine affect brain development and function. Newborns diagnosed with PKU should begin dietary treatment as soon as possible. Several commercial PKU formulas and various phenylalanine-restricted foods are available. Phenylalanine (and tyrosine) should be measured on a regular basis to follow dietary control.

Because the diagnosis and therapy of PKU is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist and dietician. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

Inheritance
This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is
a 25% chance with each pregnancy that they will have a child affected with
the disorder.

**Tyrosinemia Type-1 (TYR I)**

**Background**
Elevated blood tyrosine levels are seen in three inherited disorders of tyro-
sine metabolism. Tyrosinemia Type I was described in 1957 and is caused by
deficiency of fumarylaceto-acetate hydrolase (FAH). While a predominance
of patients are of French Canadian or Scandinavian decent, people from
other ethnic groups have also been diagnosed. Overall, TYR I is estimated to
occur in less than 1 in 100,000 live births but is as common as 1 in 12,500 in
French Canadians.

**Clinical**
TYR I usually presents in the first few months of life with progressive hepato-
renal symptoms. Infants exhibit failure-to-thrive, hepatomegaly, liver dys-
function, together with metabolic acidosis and electrolyte disturbances due
to renal tubular dysfunction (renal Fanconi syndrome). Diminished bio-
synthetic function of liver, which results in decreased clotting factors and a
bleeding diathesis, often precedes large elevations in serum transaminases.
Liver disease progresses to cirrhosis, hepatic failure, and death in undiag-
nosed patients. At any time, patients may develop acute hepatic crises with
ascites, jaundice, and gastrointestinal bleeding. Neurologic episodes of pain-
ful paresthesias, weakness, paralysis, and respiratory insufficiency occur.
There is a high risk for development of hepatic nodules and hepatocellu-
lar carcinoma. Most untreated patients die in infancy or early childhood.
Patients with Type I disease do not have intellectual disability.

**Testing**
Tyrosine and succinylacetone are readily measured in a newborn screening
dried blood spot using tandem mass spectrometry. Also the Tyr/Cit ratio has
been found informative for TYR I. Mild to moderate elevations of tyrosine
that decrease and become normal with follow-up testing is consistent with
transient tyrosinemia of the newborn. This transient elevation is a pattern
associated with liver immaturity or dysfunction.
High tyrosine levels or the presence of succinylacetone may point to an inherited metabolic defect. Workup of such patients includes measuring plasma amino acids and looking for succinylacetone on urine organic acid analysis. Elevations of plasma tyrosine, often with methionine and perhaps a generalized aminoacidemia, are seen in TYR I. The finding of succinylacetone in urine is pathognomonic for Type I disease. FAH activity is deficient in lymphocytes, erythrocytes, and liver tissue of TYR I patients. Prenatal diagnosis for TYR I can be accomplished by detecting succinylacetone in amniotic fluid and finding deficient FAH activity in chorionic villus cells or cultured amniocytes.

**Treatment**

Normalization of the tyrosine level is hastened by dietary supplementation with vitamin C. Patients with Type I disease must be treated aggressively with dietary restriction of tyrosine and phenylalanine, and administration of 2(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). This drug inhibits 4HPPD and lowers tyrosine metabolites that are responsible for much of the Type I morbidity. Liver transplantation is a cure for patients with Type I disease, providing normal FAH activity.

Because the diagnosis and therapy of tyrosinemia is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**

This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.
Biotinidase Deficiency

Background
Biotin is part of the vitamin B complex that functions as a cofactor for four carboxylase enzymes in man. When biotin is covalently linked to a key lysine residue in the carboxylases, the enzymes are activated. When the carboxylase enzymes are degraded, biotinyl-lysine is produced. Biotinidase subsequently hydrolyzes biotinyl-lysine to release free biotin, allowing it to be recycled and made available for activating newly synthesized carboxylase enzymes. In the absence of normal biotinidase activity, the patient develops functional biotin deficiency and its clinical symptoms. Biotinidase deficiency is also known as late-onset multiple carboxylase deficiency, which distinguishes it from an earlier onset form caused by holocarboxylase synthetase deficiency.

Clinical
Newborns with biotinidase deficiency appear normal at birth. Biotin deficiency develops over time with clinical symptoms beginning at a few weeks to several years of age. If untreated, patients will develop metabolic ketoacidosis and organic aciduria. Symptoms include ataxia, hypotonia, developmental delay, conjunctivitis, skin rash and alopecia, seizures, hearing loss, breathing problems and optic atrophy. There is variable expression of these symptoms probably related to dietary biotin intake and the degree of residual biotinidase enzyme activity. Partial biotinidase deficiency appears as a milder disease with most patients exhibiting chiefly the cutaneous symptoms, particularly when the patient is under metabolic stress.

Testing
Newborn screening of biotinidase activity from dried blood spots can identify affected patients shortly after birth. Both complete and partial deficiencies can be detected. The diagnosis is confirmed by measuring biotinidase activity in serum or analyzing DNA to detect the most common genotypes. In clinically symptomatic cases, urine analysis by gas chromatograph/mass spectrometry will identify elevation of β-hydroxyisovalerate, lactate, β-methylcrotonylglycine, β-hydroxypropionate and methyl citrate. These build up due to the inactivity of the four biotin requiring enzymes.
Treatment
Biotinidase deficiency is treated with oral biotin supplementation, which prevents development of the clinical symptoms.

Because the diagnosis and therapy of metabolic disorders is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

Inheritance
This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

Cystic Fibrosis (CF)
Background
Cystic fibrosis (CF) was first recognized as a clinical entity in 1938. Its genetic nature and autosomal recessive inheritance pattern were described in 1946. In 1948, patients with CF were observed to lose excess salt in their sweat which led to development of the chloride sweat test (a diagnostic test still in use). Documentation of clinical manifestations (pancreatic insufficiency and bacterial endobronchial infections) over the next 3 decades resulted in earlier diagnosis. In the 1980s, problems in epithelial chloride transport were linked to CF. In the late 1980s, elevations in pancreatic immunoreactive trypsinogen (IRT) in newborn blood were associated with CF. Finally, in 1989, DNA mutations associated with CF were identified on chromosome 7. The gene product was called the cystic fibrosis transmembrane conductance regulator (CFTR). During the 1990s, major insights were gained into the function of CFTR and the pathophysiology of CF. Dramatic improvements in early diagnosis and treatment have followed close behind.
Clinical
More than 1,600 mutations within the CFTR gene have been identified, and while some mutations are often associated with severe sequelae, even siblings with identical CF mutations may have dramatically different clinical courses. CF may affect the lung and upper respiratory tract, GI tract, pancreas, liver, sweat glands, and genitourinary tract. Nutritional abnormalities secondary to pancreatic insufficiency also have predictable consequences for growth and development. Organ dysfunction can occur at widely different ages and progression of the disease is highly variable. Although CF is a multi-system disease, lung involvement is ultimately the major cause of morbidity and mortality.

Testing
Initial screening of newborn bloodspots measures IRT. This pancreatic exocrine product is significantly elevated in over 90% of affected newborns. An elevated IRT should prompt additional genetic evaluation or sweat testing to confirm the diagnosis. If the patient being screened had meconium ileus or other bowel obstruction, IRT screening is not reliable and additional screening or diagnostic tests should be considered as indicated.

Treatment
Early diagnosis by newborn screening has allowed earlier combined anti-inflammatory and antibiotic therapies to combat upper respiratory infections and nutritional supplementation to avoid nutritional deficits. Dramatic progress has been made in improving quality of life for these newborns.

Because the diagnosis and therapy of cystic fibrosis is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric pulmonologist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

Inheritance
This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is
Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD)

**Background**
Glucose-6-phosphate dehydrogenase (G6PD) functions throughout the body, but its deficiency is seen predominantly in its effects on the red blood cells. G6PD anchors the production of NADPH and glutathione to protect the body from oxidative insults. Erythrocytes are especially sensitive to oxidative damage. G6PD deficiency can result in neonatal jaundice and in life threatening reactions to several medications, foods and infections. G6PD deficiency affects 400 million people around the world and is the most common genetic enzyme deficiency in man. Population and epidemiology information point to G6PD deficiency as providing some resistance to malaria.

**Clinical**
Babies with G6PD deficiency appear normal at birth. They may experience neonatal jaundice and hemolysis that can be so serious as to cause neurologic damage or even death. Barring such severe complications in the newborn period, infants with G6PD deficiency generally experience normal growth and development. Exposure to certain antimalarial drugs and sulfonamides, infection stress (such as upper respiratory or GI infections), environmental agents (e.g. moth balls), and eating certain foods (e.g. fava beans), each of which impact the patient’s ability to handle oxidative reactions, can cause acute hemolytic anemia. Conversely, uniform testing for several years by the United States military found no significant adverse effects in G6PD deficient males with their health or military performance under proper care and avoidance.

**Testing**
Newborn screening for G6PD deficiency can be done by enzyme analysis or primary DNA screening. Confirmatory testing using a quantitative assay should be performed for diagnosis of G6PD deficiency.
Treatment
Infants with G6PD deficiency may be at increased risk for pathological newborn jaundice and may warrant close monitoring for associated complications during the newborn period. Otherwise, treatment of G6PD deficiency is avoidance. For the infant, this means avoidance of several medications routinely prescribed for infections and illness. Strict attention to the ingredients of prepared foods and restaurant meals is required as fava beans are a frequent addition to prepared foodstuffs. Patients should not be exposed to moth balls containing naphthalene. The adverse affects of infection on patients with G6PD Deficiency can be acute and life threatening. Over exertion from exercise and work leading to dehydration and hypoglycemia can precipitate clinical symptoms. As mentioned above, patients mindful of these limitations can lead a normal life of exercise and choice of vocation.

Because the diagnosis and therapy of this disorder is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric hematology specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

Inheritance
G6PD deficiency is inherited as an X-linked defect. Males with a G6PD deficiency mutation on their X chromosome are affected. Females with one G6PD deficiency mutation are carriers at a 50% risk to pass their G6PD deficiency X chromosome to a male child. As an X-linked disorder, G6PD deficiency would generally be thought to affect only males. However, females having a G6PD deficiency mutation on both of their X chromosomes also have clinical symptoms. Some carrier females have been reported to have symptoms. Therefore, all members of an identified family should have G6PD testing and genetic counseling. The risk for having an affected male pregnancy is one chance in two for a carrier female. G6PD deficiency is found in populations from areas of the world where malaria is prevalent.

Congenital Adrenal Hyperplasia (CAH)

Background
The deficiency in one of the five enzymes required in the steroidogenic pathway for the biosynthesis of cortisol (hydrocortisone) results in a group of diseases known collectively as congenital adrenal hyperplasia (CAH).
The diseases are inherited as autosomal recessive disorders. As a result of impaired cortisol synthesis by the adrenal cortex, there is excessive secretion from the pituitary of adrenocorticotropic hormone (ACTH), or corticotrophin, which stimulates the adrenal cortex to synthesize and secrete more cortisol. ACTH stimulation causes diffuse hyperplasia of the adrenal gland, and usually the disease is recognized in infancy. Greater than 90% of cases of CAH are caused by reduced or absent activity of the steroid 21-hydroxylase enzyme, known as CYP21, or Classic CAH. This form of CAH presents in early infancy, early childhood or adolescence, depending upon the magnitude of the deficient enzyme activity. In severe cases, very low CYP21 activity causes low aldosterone secretion, salt loss and hypovolemia. Combined with hypotension and hypoglycemia from cortisol deficiency, this results in neonatal death during the first month of life if not recognized and adequately treated. Because the androgen synthetic pathway does not require CYP21 activity, there is excess androgen secretion, and virilization in the female fetus causing varying degrees of sexual ambiguity at birth.

**Clinical**

Male infants with CAH are normal at birth. In severe cases, salt wasting becomes evident within 7-10 days. By 2-3 weeks, failure to thrive, unexplained vomiting, poor feeding, hypovolemia and shock develop. The same sequence of symptoms develops in untreated female infants with CAH, but virilization with sexual ambiguity at birth leads to an early diagnosis of CAH and adequate treatment in many patients. However, complete female virilization presents at birth with the clinical phenotype of a male infant with bilateral cryptorchidism. In this presentation, the diagnosis of CAH may be missed and the incorrect sex assigned. Approximately 75% of children with classic CAH have the saltlosing CAH. Milder forms of CAH, the so-called Simple Virilizing CAH, have normal aldosterone secretion and present with virilization in infant girls, but the diagnosis in boys may not be evident until childhood when androgen excess causes sexual precocity without testicular enlargement. Late diagnosis is associated with markedly advanced skeletal maturation and accelerated linear growth initially, but early natural puberty and ultimately short stature. In the mildest form of CAH (attenuated, or late onset 21-hydroxylase deficiency), both cortisol and aldosterone secretion are normal, but at the expense of chronic mild-to-moderate excess production of androgenic hormones. These children present in childhood or adoles-
ence with early onset of sexual hair (premature pubarche) and/or hirsuit-
ism, oligomenorrhea and acne in females, or infertility in both sexes.

**Testing**
The immediate steroid precursor in classic CAH and the substrate for CYP21 is 17- hydroxyprogesterone (17-OHP). The measurement of 17-OHP in the newborn blood spot can discriminate infants with salt-wasting or Simple Virilizing CAH from non-affected infants. The newborn screening test usually does not detect attenuated or late onset nonclassical CAH patients. When values exceed the normal range, 17-OHP analysis is repeated using organic extraction to remove interfering substances. The normal values for 17-OHP vary with birth weight and gestational age, and cutoffs should be adjusted accordingly.

Serum confirmation tests include a repeat 17-OHP value, other steroid pre-
cursors to be certain that a mildly elevated 17-OHP is not caused by another form of CAH (e.g., 11-hydroxylase deficiency), and tests related to salt loss, such as serum Na and K, and renin activity. Confirmatory DNA testing is also available.

**Treatment**
Oral hydrocortisone in a physiologic replacement dose is the treatment of choice for CAH. The more potent glucocorticoids are contraindicated in the growing child and adolescent because of the difficulty in determining a physiologic versus pharmacologic dose. In children with salt-losing CAH, 9·-fluorohydrocortisone should maintain normal electrolyte balance without excessive natriuretic or glucocorticoid effects. Monitoring plasma 17-OHP and androstenedione levels, growth velocity, and an occasional bone age offer the basic tools for adequate, effective therapy.

Because the tests to select and interpret at the time of initial diagnosis and during therapy are often complex, the pediatrician is advised to manage the patient with CAH in close collaboration with a consulting pediatric endo-
crine specialist. It is recommended that parents travel with a letter of treat-
ment guidelines from the patient’s physician, and the child should wear a bracelet or necklace for emergency identification that they are cortisol deficient.
Inheritance
The forms of CAH are each inherited as autosomal recessive diseases. DNA carrier testing of families and prenatal diagnostic testing is available. Early identification of affected fetuses is important to avoid virilization of female infants. For families at risk for an affected child, oral dexamethasone is started as early in pregnancy as possible after pregnancy is diagnosed. If started before 6 weeks of fetal life, virilization is prevented or considerably limited. Once the sex of the fetus is determined, maternal therapy can be discontinued for a male fetus; once DNA tests of the female infant are known, maternal therapy can be discontinued for an unaffected female fetus.

Congenital Hypothyroidism (CH)

Background
The deficiency of thyroid hormones in the neonate has been known since antiquity. Most cases prior to the 20th century were caused by iodine deficiency. Though still a prevalent nutritional disease worldwide, iodine deficiency rarely causes congenital hypothyroidism (CH) in western countries. Permanent neurodevelopmental deficits were known to occur when CH was not recognized and adequately treated by 2 to 3 months of postnatal age. Since the advent of newborn screening for CH in 1973, intellectual disability as a consequence of CH has been virtually eradicated among affected infants detected by screening within the first 2-3 weeks of age. The incidence is approximately 1:4,000 in iodine sufficient populations. The etiology in 70 to 80% of the non-familial cases is unknown. Maternal hypothyroidism may adversely affect the fetus to rarely cause findings of CH, or to be associated with mildly decreased IQ outcomes when maternal hypothyroidism occurs during the first half of pregnancy despite normal fetal and neonatal thyroid function.

Clinical
In greater than 95% of newborn infants with CH, there are no symptoms or signs of CH when the diagnosis is suspected by newborn screening. During the first 2 to 6 months of life, an affected, untreated infant with moderate to severe hypothyroidism may have persistent hyperbilirubinemia, edema, an umbilical hernia, enlarged fontanelles, and an absent, hypoplastic, normal or enlarged thyroid gland; then gradually develops lethargy, poor feeding, mac-
roglossia, hypothermia, constipation, dry and sallow skin, hoarse cry, circumoral pallor, and mottling of the skin.

**Testing**

There are two newborn screening tests performed in blood to detect hypothyroidism: thyroid stimulating hormone (TSH) and thyroxine (T4). When the thyroid gland is defective, known as Primary CH, TSH values are elevated and T4 values usually are low, although T4 values may be within the normal range in mild CH. Primary CH accounts for > 95% of cases. When there is defective hypothalamic or pituitary regulation of the thyroid gland, known as Central CH, T4 values are low; the TSH values may be low, normal, or mildly elevated.

Serum confirmatory tests usually are limited to 2 tests: TSH and free thyroxine, or FT4 (the small fraction of T4 that is not bound to serum proteins and represents the more biologically relevant measurement). An elevated serum TSH test is diagnostic of primary CH, the most common form of CH. A low serum FT4 with a normal or low TSH is diagnostic of central CH. Infants with central CH should have other tests to evaluate hypothalamic-pituitary function since ACTH/cortisol, growth hormone and/or gonadotropin deficiencies are associated with hypoglycemia (typically within hours of birth), and in male neonates, hypogonadism (micropenis, small testicles, cryptorchidism).

An image of the thyroid gland is often obtained by thyroid ultrasound and/or a radionuclide thyroid scan. These tests determine whether it is enlarged (goiter), absent (athyreosis), small (hypoplasia), or malformed and not in the normal location in the neck (ectopia). In these situations, the infant most often has a permanent form of primary CH. The thyroid gland may be normal in size and in the normal location in the neck (eutopic) on ultrasound, especially in familial causes of CH and transient CH.

**Treatment**

Treatment of hypothyroidism is relatively uncomplicated. As soon as tests to confirm the diagnosis of CH are obtained, levothyroxine (L-thyroxine) should be started promptly. Central CH may require additional management based on associated findings. Evaluation and monitoring should be performed in conjunction with a pediatric endocrine specialist.
Inheritance
Approximately 15 to 20% of affected infants with CH have one of several inherited forms of CH collectively known as Familial Thyroid Dyshormogenesis. These diseases are caused by mutations in the enzymes that are required for thyroid hormone synthesis, metabolism and end organ responsiveness, and are inherited as autosomal recessive traits. With similar inheritance patterns the mutations in genes for the synthesis of hypothalamic-pituitary hormones and their receptors infrequently cause CH. There are very rare mutations in genes that regulate thyroid and pituitary gland embryogenesis. The mode of inheritance is unknown. Though not a cause of CH, thyroxine binding globulin (TBG) deficiency is caused by mutations in the gene that is required for the synthesis of this major plasma binding protein for T4. The mode of inheritance usually is X-linked, though autosomal recessive forms of TBG deficiency have been reported.

Sickle Cell and Other Hemoglobinopathies

Background
Sickle cell disease was the first hemoglobinopathy to be linked to an inherited structural defect in the beta globin gene, and the first in which the point mutation resulting in the defect was identified and characterized. The scope of newborn screening for sickle cell disease, which began over 30 years ago, has evolved to include other hemoglobin diseases.

Today, evaluation of newborns for hemoglobinopathies encompasses detection of point mutations which lead to structural defects in the alpha or beta globin chains (hemoglobinopathies) such as sickle cell disease, and detection of defects in rate of production of either alpha or beta globin chains (thalassemias). Taken together, the inherited disorders of hemoglobin are some of the most common genetic disorders in the world. Because the different hemoglobin disorders coexist at a high frequency in many populations and because individuals may inherit more than one type, hemoglobin disorders present a complex series of clinical phenotypes.

Clinical
In the newborn period, a transition occurs from primarily fetal hemoglobin (HbF) production to adult hemoglobin (HbA). This transition temporarily masks symptoms of disease. As a result, diseases associated with red blood
cell sickling (Hb S, C, S/C, S/O, and S/D diseases) usually present during the first or second year of life, although milder cases may present much later. Usual presenting features are failure to thrive, repeated infection in infancy, painful dactylitis, and pallor. At this stage typical hematologic findings are established. Heterozygotes (e.g., Sickle cell trait) are carriers with no clinical symptoms.

Thalassemias present with a wide clinical diversity depending upon the degree to which the alpha or beta chains are being synthesized. In beta thalassemia major, or complete absence of beta chain production, newborns are asymptomatic. But as HbF declines, affected infants present with severe anemia (usually within the first two years). Beta thalassemia minor (partial synthesis) has a variable clinical course. In alpha thalassemia, absence of alpha chains is uniformly fatal to the newborn within days of birth, whereas partial production, as in hemoglobin H disease, may produce variable clinical symptoms.

**Testing**
Primary screening for hemoglobinopathies is by isoelectric focusing (IEF) of blood eluted from a dried blood spot. IEF separates the hemoglobins and identifies most common variants by band mobility. DNA probes which specifically identify HbS, HbC, HbE, HbO Arab and HbD are used to confirm abnormal findings by IEF. Reduced HbA and second tier DNA testing for common beta thalassemia mutations help to identify beta thalassemias. The presence of Hb Barts and/or Hb H in the IEF pattern detects alpha thalassemia.

**Treatment**
Apart from bone marrow transplantation, there is no curative treatment for hemoglobin disorders. Management for newborns affected with sickling diseases typically involves oral penicillin given daily and maintained throughout childhood, and vaccination against *Pneumococcus, Meningococcus*, and *H. influenzae*.

Because the diagnosis and therapy of this disorder is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric hematology specialist.
**Inheritance**

Structurally abnormal hemoglobins follow autosomal recessive inheritance. Most abnormal hemoglobins cause little or no clinical manifestations in heterozygotes (one copy of the mutation), including Hb S, C, D, E, and O Arab. Because the production of alpha chains is controlled by four gene loci (two on each pair of chromosomes), each gene controls only about 25% of total alpha chain production, and hemoglobinopathies due to alpha chain abnormalities are uncommon compared to beta chain abnormalities.

**Galactosemia**

**Background**

Individuals intolerant to ingested galactose were described as early as 1908, but it wasn’t until 1935 that elimination of galactose from the diet was shown to reverse the acute toxicity syndrome associated with galactosemia. The Leloir pathway, the main pathway of galactose metabolism, was elucidated in the early 1950s. In 1955, the accumulation of galactose-1-phosphate in red blood cells of infants with impaired galactose metabolism was demonstrated. Methods were subsequently developed to measure galactose-1-phosphate uridyl transferase (GALT) and galactose-1-phosphate in newborn blood spots.

GALT deficiency accounts for about 95% of galactosemias. Although many mutations in the GALT gene have been documented, most cases of GALT deficiency are accounted for by a few high frequency mutations. In about 5% of cases of galactosemia, the metabolic defect is in galactokinase (GALK), and very rarely, the defect is found to be in uridyl diphosphate galactose epimerase (GALE).

**Clinical**

GALT deficiency most often presents as a life threatening illness within the first two weeks after birth. Poor feeding, poor weight gain, vomiting and diarrhea, lethargy, and hypotonia are initial symptoms. On physical examination, infants are jaundiced with hepatomegaly, may have a full fontanelle and show prolonged bleeding after venous or arterial sampling. Cataracts are often present. Lab tests revealing liver disease, renal tubular dysfunction, and hematologic abnormalities are common. Hemolytic anemia and septicemia may occur. Long-term complications include impaired neuropsychological
development and late neurologic complications. Ovarian failure due to prenatal toxic effects of galactosemia is common in females and not reversible.

In GALK deficiency, the only consistent clinical finding is cataracts. In GALE deficiency, patients are, with very rare exceptions, asymptomatic, with normal growth and development.

**Testing**

Screening for galactosemia includes testing for elevated total galactose (galactose plus galactose-1-phosphate), and measuring GALT enzyme activity. Elevated total galactose and/or reduced GALT activity should trigger additional evaluation of the patient. The galactose level can be fractionated into free galactose and galactose-1-phosphate, and DNA analysis for the common mutations associated with GALT deficiency can be performed on the initial blood spot specimen. This combined approach quickly reveals from a single blood spot whether the galactosemia is a GALT deficiency, or is due to GALK or GALE deficiency. Confirmatory testing usually includes serum studies of galactose and the associated enzyme activity to characterize the patient’s phenotype.

**Treatment**

Immediate exclusion of dietary galactose (breast feeding, cow’s milk) should be instituted at the first suspicion of galactosemia. Infant soy formulas are recommended unless there is significant liver disease. For the newborn who is seriously ill at the time of diagnosis, supportive care may include treatment with vitamin K and fresh-frozen plasma to correct clotting abnormalities. Gram-negative sepsis should be assumed and appropriate IV antibiotics given.

Because the diagnosis and therapy of this disorder is complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist. It is recommended that parents travel with a letter of treatment guidelines from the patient’s physician.

**Inheritance**

This disorder most often follows an autosomal recessive inheritance pattern. With recessive disorders affected patients usually have two copies of a disease gene (or mutation) in order to show symptoms. People with only one copy
of the disease gene (called carriers) generally do not show signs or symptoms of the condition but can pass the disease gene to their children. When both parents are carriers of the disease gene for a particular disorder, there is a 25% chance with each pregnancy that they will have a child affected with the disorder.

Severe Combined Immunodeficiency (SCID)

Background
Severe combined immunodeficiency (SCID) is a group of disorders characterized by the absence of both humoral and cellular immunity. Infants with SCID die of infections by age 2 years unless immunity is reconstituted by treatment. The defining characteristic for SCID is always a severe defect in T cell production and function, with defects in B-lymphocytes as a primary or secondary problem and, in some genetic types, in NK cell production as well. SCID is also commonly known as the “bubble boy” disease.

Clinical
Infants generally have a normal physical examination result before the onset of infection. The pathophysiology and molecular biology vary among different forms of SCID, however, the lack of T-cell and B-cell function is the common endpoint in all forms of SCID. Lymphopenia usually occurs from the absence of T cell, and sometimes from the absence of natural killer cells. Functional antibodies are decreased or absent. Infections are usually serious, and may include pneumonia, meningitis or bloodstream infections with average age at the onset of symptoms at 2 months.

Testing
T-cell Receptor Excision circles (TRECs) are circular DNA fragments generated during T-cell receptor rearrangement. In healthy neonates, TRECs are made in large numbers, while in infants with SCID they are barely detectable. Following PCR amplification TREC copy number in blood can be used to distinguish T-cell lymphopenic SCID infants from healthy babies. However, low TRECs copy numbers can also be the results of other immunodeficiency, such as DiGeorge Syndrome, and sometimes as a result of the use of immunosuppression drugs. Confirmatory tests are needed for the diagnosis of SCID and for the determination of the form of SCID.
Figure 3. TREC\textsuperscript{s} are circular DNA fragments. They are generated when a section of chromosome 14 (14q11-2), which contains genes responsible for coding T-cell receptors, rearranges. The TREC\textsuperscript{s} are amplified by PCR.

**Treatment**

Infections are treated with specific antibiotic, antifungal, and antiviral agents and administration of intravenous immunoglobulin. Restoration of a functional immune system is essential. The preferred treatment is bone marrow/stem cell transplantation. Early detection and treatment can result in markedly improved survival rates. Enzyme replacement therapy is available for adenosine deaminase deficiency (a form of SCID). Gene therapy is still in the experimental phase.

**Inheritance**

SCID occurs in approximately 1 in 50,000-100,000 live births. Over 10 different genetic defects have been identified that account for SCID in humans. The most common type is linked to the X chromosome, making this form affect only males. This X-linked form accounts for approximately 50% of SCID cases. Other forms of SCID usually follow an autosomal recessive inheritance pattern or are the result of spontaneous mutations. Approximately 25% of the patients with an autosomal recessive SCID are JAK3 deficient, and 40% are adenosine deaminase deficient.
The technologies used in today’s screening laboratories, depend on the scope of the screening program and on the particular analytes that are being measured. Immunoassays (page 63) and enzymatic assays (page 66) have normally represented the backbone methods, especially for start-up programs, for screening from one to several diseases. At a relatively early stage in the development of immunoassay technologies, possibilities to run multi-label assays were explored. Multi-analyte testing is associated with benefits such as

- Savings in time, labor, reagents
- Increased test throughput
- Reduced overall costs
- Reduced sample volumes

Although a technique like time-resolved fluorometry allows measurement of several labels simultaneously from a single assay well, the need for expanded screening has actually come to be met by an alternative technology, tandem mass spectrometry (page 68). This allows the assessment of large numbers of analytes from a single dried blood spot sample.

When it became available in the early 1990’s tandem mass spectrometry screening led to a large expansion of potentially detectable congenital metabolic diseases. Consequently, additional tests have been added to many screening programs.

For some disorders screened, the need is to distinguish between a number of similar substances, or different forms of the same substance. For detecting hemoglobinopathies, for example, the isoelectric focusing (IEF) technique (page 69) allows clear separation of different forms of hemoglobin. Although immunoassay and chromatography options also exist for hemoglobinopathy screening, IEF remains the technology of choice for many programs.

Genetic testing has become a standard part of newborn screening recently and there are numerous methods available that can be applied for nucleic acid analyses. However, most of the methods do not meet the needs of new-
born screening for high-throughput and fluent, efficient processes. Here (page 70) we mention an end-point PCR application that does provide these missing features, and is consequently suitable for use in routine screening of newborns.

**Immunoassay**

Immunoassays are specific protein binding assays that rely on the high specificity and affinity of the recognition reaction between an antibody and the determinant in an antigen, i.e. the chemical structure against which the antibody is formed.

The introduction of reporter groups to one of the components of the immunological reaction facilitates monitoring of the binding. During the 1960’s and 70’s the evolution of immunoassay technologies took place through the use of radioisotopic labels. In the late 1970’s and 80’s the advent of non-radioactive labels led to new assay improvements, to allow assay automation and even more sensitive assays.

The first quantitative immunoassays were competitive radioimmunoassays (RIA) introduced in the early 1960’s. Since then, competitive radioimmunoassays have been used both for small molecular haptenic antigens as well as for larger antigens.

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*Figure 4. Example of a competitive immunoassay employing PerkinElmer DELFIA® technology with time-resolved detection of fluorescence.*
Due to its limitations (restricted assay dynamics and sensitivity more dependent on antibody affinity than label detection) and after the development of solid-phase separation systems and sandwich assay design, the competitive assay has lost its place as a method of choice for larger antigens. Still, the competitive assay has to be used when measuring analytes, which because of their small size, can only bind one antibody at a time, i.e. antigens unable to form a sandwich.

The invention of non-competitive immunoassay technology has generally been credited to Miles and Hales, who in 1968 labeled anti-insulin antibodies with $^{125}\text{I}$ and used them in a non-competitive two-step immunoradiometric assay (IRMA) of insulin. They described their assay as an immunoradiometric assay because they used labeled antibodies instead of labeled antigens. Later on, this term and corresponding terms relating to other detection technologies, for example, immunofluorometric assay (IFMA) have become more generally used to describe reagent excess, non-competitive assay designs - performed most often as a sandwich assay. Subsequently, two-site sandwich type assays have proved to be the most suitable assays for most antigens - or antibodies - having at least two epitopic sites.

In their day, Miles and Hales calculated that IRMA techniques should provide improvements in analytical sensitivity and specificity, and that further advantages could be gained by replacing the radioactive iodine with a high activity non-radioactive alternative. Since the sensitivity of competitive assays is defined by the association constant of antibodies, while the sensitivity of non-competitive assays is defined by the total error, non-specific binding and the affinity of antibodies, it has been possible to create non-competitive assays that are several orders of magnitude more sensitive than competitive assays.
Figure 5. Example of a sandwich-type, immunofluorometric assay employing PerkinElmer DELFIA® technology with time-resolved detection of fluorescence.

Time-Resolved Fluorometry

The examples of immunoassay designs shown in figures 4 and 5 involve the use of a lanthanide chelate fluorophore and its detection by means of time-resolved fluorometry. The time-resolved detection relies on two important properties of the fluorophore, which contribute to the sensitivity of the assay. The fluorophore has a long decay time (figure 6) and it has a wide Stokes’ shift (figure 7). Measurement can then be made at a time and wavelength at which background is minimal.

Measurement takes place by repeatedly exciting the fluorophore, and after each excitation, measuring emitted fluorescence for a period that starts after an interval. For a measurement cycle of 1 millisecond, measurement might take place between 400 and 800 microseconds, as in the example shown in figure 6.

The lanthanide most commonly used in time-resolved fluorometry in both FIA and IFMA assay designs is europium. However there are several other lanthanides with similar properties, but each with its own unique fluorescence profile. Multi-analyte time-resolved fluorometry assays are available.

Time-resolved fluorometry has wide application because the labels employed are small, easy to apply and non-toxic.
Enzymatic Assay

Enzymes are proteins that catalyze chemical reactions. The molecules at the beginning of the reaction are called substrates, and the enzyme converts them into different molecules, named as the products. Enzymes are not consumed by the reactions they catalyze. The enzymatic assays are based on the very high specificity as to which reactions they catalyze and the substrates that are involved in these reactions.

Enzyme activity can be affected by other molecules. Inhibitors are molecules that decrease enzyme activity; activators are molecules that increase activity. Many drugs and poisons are enzyme inhibitors. Activity is also affected by temperature, chemical environment (e.g., pH), and the concentration of substrate.
Since the tight control of enzyme activity is essential for homeostasis, a malfunction (mutation, overproduction, underproduction or deletion) of a single critical enzyme can lead to a genetic disease. The importance of enzymes is shown by the fact that a lethal illness can be caused by the malfunction of just one type of enzyme out of the thousands of types present in our bodies. One example is classical galactosemia. A mutation in single amino acid in the enzyme galactose-1-phosphate uridyl transferase, results in build-up of galactose-1-phosphate. If not diagnosed and treated it can lead to death within a few weeks.

One of the most sensitive methods to monitor enzymatic assays is to employ fluorometric techniques. Fluorescence is observed when a molecule emits light of one wavelength after absorbing light of a different wavelength. Fluorometric assays use a difference in the fluorescence of substrate from product to measure the enzyme reaction. An example of these assays is the use of the nucleotide coenzymes NADH and NADPH. Here, the reduced forms are fluorescent and the oxidised forms non-fluorescent. Oxidation reactions can therefore be followed by a decrease in fluorescence and reduction reactions by an increase. Synthetic substrates that release a fluorescent dye in an enzyme-catalyzed reaction are also available, such as biotin-6-aminoquinoline for screening of newborns for biotinidase deficiency.

Figure 8. Schematic diagram of a fluorescence assay for the enzyme, galactose-1-phosphate uridyl transferase (GALT). GALT in the blood sample catalyzes a reaction between galactose-1-phosphate and uridine diphosphoglucose contained in the assay substrate reagent. In the course of further reactions NADP (nicotinamide adenine dinucleotide phosphate) also contained in the assay substrate reagent is reduced to NADPH, a fluorescent substance that can be measured with excitation at 355 nm and emission detection at 460 nm.
Tandem Mass Spectrometry (MSMS)

In tandem mass spectrometry, electrospray ionization (ESI) is used to produce ions. These ions, or charged molecules, are extracted into the analyzer for separation on the basis of their mass-to-charge (m/z) ratios. The analyzer consists of three chambers (called quadrupoles; Q1, Q2, and Q3 respectively): Q1 and Q3 are mass spectrometers separated by a collision cell (Q2), which fragments the ions.

In the measurement of amino acids and acylcarnitines from dried blood spot samples it is first necessary to extract the amino acids and acylcarnitines. Dried blood spot disks of the samples are mixed with methanol and internal standards. The internal standard must match the chemical properties of the analyte molecule as closely as possible and, in general, a deuterium-labeled version of the analyte is used. For compounds where a radionuclide-labeled internal standard is unavailable a close chemical analog may be used.

The sample-standard mixtures may be analyzed directly or the compounds may be first converted to their butyl esters through a process known as derivatization. Derivatization helps to increase the strength of signal from some compounds. However, it is a long and laborious process and can also lead to inaccurate measurement of some acylcarnitines. The use of qualified and optimized reagents can compensate for reduced signal strength, and the new generation non-derivatized reagents and kits are widely considered to provide suitably high analytical performance.

The final samples are then introduced into the MSMS instrument, ionized, and analyzed. Signals corresponding to the selected range of mass-to-charge ratios are then detected and quantified by reference to the internal standards. For any particular analyte, the yield of product ions at the detector depends partly on the concentration of analyte in the original sample extract but also on the degree of “ion suppression” by other components in the mixture (salts for example) and by instrumental settings, particularly those affecting the ion source and conditions in the collision cell. Internal standards are used in order to correct for these effects and thus allow the concentrations of the analytes in question to be calculated. The analyte concentrations and ratios are then compared to pre-determined cut-off values.
Isoelectric Focusing

Electrophoresis is a well-established method for separating individual proteins in protein mixtures. Proteins are made from amino acids, and these amino acids have amphoteric properties, which means they can be either positively or negatively charged. Electrophoretic separation is on the basis of the net charge of the individual proteins, in other words the sum of the charges of the constituent amino acids. Expressed simply, a direct current is passed across a support membrane, and sample is applied close to the cathode. The individual proteins will separate out as they migrate towards the anode.

The pH of the solution in which a protein resides determines its net charge. The pH at which the protein has a net charge of zero is the isoelectric point (pI) of that protein.

The simple definition of isoelectric focusing (IEF) is electrophoresis in a pH gradient. It is an end-point separation method in which the proteins migrate to specific positions on the support membrane that correspond to their isoelectric points. In zone electrophoresis, on the other hand, a constant pH is implied.

The history of IEF began in 1964, when Olaf Vesterberg filed a Swedish patent on the synthesis of new chemicals called carrier ampholytes. In the process of making these new chemical species, Vesterberg took various types of polyamines and coupled them to specific acrylic acid derivatives. After an incubation period, the chemical solution produced an entirely new species of multi-branched chained molecules that had protonated amino groups and deprotonated carboxylic groups. These chemicals exhibited unique properties that could be used to generate pH gradients when subjected to direct electric current, and consequently they could be employed in an electrophoretic system.

In IEF a mixture of specific carrier ampholytes are blended into a purified high grade agarose support matrix. An anolyte solution and a catholyte solution are saturated onto paper wicks and then placed directly onto the surface of the gel. The sample is introduced anywhere onto the surface of the gel via a plastic sample mask, and direct current is applied by placing electrodes on
the electrode wicks. The ampholytes move in the direction of the current, and become sorted creating discrete pH zones when they reach their isoelectric points. A protein within the sample then migrates to the pH zone that represents its isoelectric point. At this point it will move no further but will accumulate to form a visible band.

**TR-FRET-Based End-Point PCR**

Genetic testing can be performed through nucleic acid analysis, which often utilizes the polymerase chain reaction (PCR), a basic tool in molecular biology for nucleic acid amplification. PCR generally involves four steps: sampling, sample preparation, amplification and detection. There are several applications that integrate the latter two stages to allow simple nucleic acid sequence analysis with minimal post-PCR handling. Unlike these conventional PCR methods, Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET)-based end-point PCR combines sample preparation, amplification and detection. After punching of sample disks all assay steps, including addition of amplification and detection reagents, thermal cycling, and fluorescence measurement, take place in the same vessel, making the method simple and easy to use.

**Figure 9.** With TR-FRET technology, donor (D) and acceptor (A) labels on unhybridized probes (upper left) are close to each other. This results in high energy transfer efficiency and intensive acceptor signal with short decay time (yellow curve on the graph). After hybridization (upper right), the rigid DNA chain forces the labels apart. This leads to reduced energy transfer efficiency and increased acceptor decay time (red curve).

TR-FRET–based end-point PCR involves a target sequence-specific probe, in which long-lifetime fluorescent lanthanide donor and acceptor fluorophores are coupled to opposite ends of a single probe molecule. When the probe is hybridized, it enables
direct detection of the target-hybridized probe population based on prolonged decay time of the energy-transfer induced acceptor signal. This probe approach also allows tailoring of both the wavelength and decay time of the induced acceptor signal enabling multianalyte assays. The method enables rapid homogeneous one-step DNA-detection requiring only one reagent per one target oligonucleotide. The use of long lifetime fluorescent lanthanides in the time-resolved measurement also effectively suppresses the nanosecond-lifetime assay background caused by the assay matrix and light scattering, and thereby increases the sensitivity of the assay. Moreover, the sensitivity for its part allows the use of small sample sizes thus diminishing the interference caused by the biological components and enabling the direct use of disk samples in homogeneous PCR.
Organizations Providing Information on Screening

There are a number of national and international organizations that are keen to help spread newborn screening in the world by sharing knowledge.

**ACMG**
The American College of Medical Genetics (ACMG) aims to improve health through medical genetics.

As part of its work to define and promote excellence in medical genetics practice in newborn screening ACMG has proposed a recommended uniform screening panel (RUSP).

The ACMG also promotes and provides medical genetics education, increases access to medical genetics services and integrates genetics into patient care.

[http://www.acmg.net/](http://www.acmg.net/)

**APHL**
The US-based Association of Public Health Laboratories (APHL) is also keen to help on a global scale and works with the International Society for Neonatal Screening (ISNS) to help countries that are introducing new programs. The association has broad experience, since public health laboratories perform newborn screening tests on more than 95% the four million babies born in the United States each year.


**CDC**
Centers for Disease Control and Prevention (CDC) collaborates in creating the expertise, information, and tools that people and communities need to protect their health – through health promotion, prevention of disease, injury and disability, and preparedness for new health threats.


**Climb - Children Living with Inherited Metabolic Diseases**
Climb is a national organization in the United Kingdom that provides metabolic disease specific information, advice and support to children, young
people, adults, families and professionals. It also provides information and support to families worldwide, funds educational and primary research programs and investigates treatments and medical services.

http://www.climb.org.uk/

EGAN
The European Genetic Alliances Network (EGAN) is a pan-European organization working for and on behalf of patients with disorders with a genetic or congenital nature. This includes both rare and common disorders.

http://www.egan.eu/

EPF
The European Patients’ Forum (EPF) is the umbrella organization of pan-European patient organizations active in the field of European public health and health advocacy.

EPF was founded in 2003 to become the collective patients’ voice at EU level. EPF currently represents 46 patient organizations. Included within this group are disease-specific patient organizations operating at EU level and national coalitions of patient organizations.

http://www.eu-patient.eu/

Genetic Alliance
Genetic Alliance is a not-for-profit organization in the United States whose purpose is to transform health through genetics, and promote openness about genetic diseases. It brings together diverse parties to create partnerships in advocacy, improve health systems; and revolutionize access to information to enable translation of research for health care services and individualized decision making.

http://www.geneticalliance.org/

Genetic Alliance UK
The Genetic Alliance UK is a national alliance of organizations with a membership of over 130 charities which support children, families and individuals affected by genetic disorders. The organization’s webpages include a comprehensive list of member organizations dedicated to individual disorders.

http://www.gig.org.uk/
**Genetics Society**
The Genetics Society was founded by William Bateson in 1919 and is one of the oldest “learned societies” devoted to Genetics in the world. Its membership of over 1700 consists of most of the UK’s active geneticists, including academics, researchers and students.

As a registered charity, it organizes meetings to promulgate genetics, supports students’ attendance at meetings, sponsors research through fieldwork grants and student bursaries, and promotes the public understanding of genetics.


**ISNS**
The International Society for Neonatal Screening (ISNS) in an organization with a membership of almost 400 specialists from screening programs all over the world.

The role of ISNS is to collect the opinions of the different people in the different countries and continents, and to spread these opinions as shared knowledge.


**March of Dimes**
The mission of the US-based March of Dimes organization is to improve the health of babies by preventing birth defects, premature birth, and infant mortality. The organization carries out this mission through research, community services, education and advocacy to save babies’ lives. March of Dimes researchers, volunteers, educators, outreach workers and advocates work together to give all babies a fair chance against the threats to their health: prematurity, birth defects, low birth weight.


**NNSGRC**
The National Newborn Screening and Genetics Resource Center (NNSGRC) is a cooperative agreement between the Maternal and Child Health Bureau (MCHB), Genetic Services Branch and the University of Texas Health Science Center at San Antonio (UTHSCSA), Department of Pediatrics. The project is funded by the Health Resources and Services Administration (HRSA). The mission of the NNSGRC is to provide a forum for interaction between consumers, health care professionals, researchers, organ-
izations, and policy makers in refining and developing public health newborn screening and genetics programs; and to serve as a national resource center for information and education in the areas of newborn screening and genetics.

http://genes-r-us.uthscsa.edu/

Rare Disease UK
Rare Disease UK is an alliance of key stakeholders brought together to develop strategic planning for rare diseases in the UK
http://www.raredisease.org.uk/

Save Babies Through Screening
The mission of the Save Babies Through Screening Foundation is to improve the lives of babies by preventing intellectual disability, disabilities and death from disorders detectable through newborn screening using routine heel-stick blood specimens.

http://www.savebabies.org/
http://www.savebabiescanada.org/
http://www.savebabiesuk.org/

SSIEM
The aim of the Society for the Study of Inborn Errors of Metabolism (SSIEM) is to foster the study of inherited metabolic disorders and related topics. The Society, founded in 1963, exists to promote exchange of ideas between professional workers in different disciplines who are interested in inherited metabolic disease.

http://www.ssiem.org/

UK Newborn Screening Programme Centre
The UK Newborn Screening Programme Centre has responsibility for developing, implementing and maintaining a high quality, uniform screening program for all newborn babies and their parents.

http://newbornbloodspot.screening.nhs.uk/

UK NSC
The UK National Screening Committee (UK NSC) advises Ministers and the NHS in the four UK countries about all aspects of screening and supports implementation of screening programs. Using research evidence, pilot
programs and economic evaluation, it assesses the evidence for programs against internationally recognized criteria.

The UK NSC also sets up practical mechanisms to oversee the introduction of new programs in the English NHS and monitors effectiveness and quality of these programs.

http://www.nsc.nhs.uk/

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACMG</td>
<td>American College of Medical Genetics</td>
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<td>APHL</td>
<td>Association of Public Health Laboratories</td>
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<tr>
<td>BIA</td>
<td>Bacterial inhibition assay</td>
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<td>BKT</td>
<td>β-ketothiolase deficiency</td>
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<td>BUN</td>
<td>Blood urea nitrogen</td>
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<tr>
<td>CAH</td>
<td>Congenital adrenal hyperplasia</td>
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<tr>
<td>CBS</td>
<td>Cystathionine β-synthase</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
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<tr>
<td>CFTR</td>
<td>Cystic fibrosis transmembrane conductance regulator</td>
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<tr>
<td>CH</td>
<td>Congenital hypothyroidism</td>
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<tr>
<td>CK</td>
<td>Creatine kinase</td>
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<tr>
<td>CUD</td>
<td>Carnitine Uptake Defect</td>
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<td>CYP21</td>
<td>Steroid 21-hydroxylase enzyme</td>
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<td>European Patients’ Forum</td>
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<td>FIA</td>
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<td>Free thyroxine</td>
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<td>G6PD</td>
<td>Glucose-6-phosphate dehydrogenase</td>
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<td>GA-I</td>
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<td>GALE</td>
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<td>Fetal hemoglobin</td>
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<tr>
<td>HCY</td>
<td>Homocystinuria</td>
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HELLP  Hemolysis, elevated liver enzymes, and low platelets
HMG  3-hydroxy-3-methylglutaric aciduria
HPA  Hyperphenylalaninemia
HRSA  Health Resources and Services Administration
hTSH  Thyroid stimulating hormone
IEF  Isoelectric focusing
IFMA  Immunofluorometric assay
IRMA  Immunoradiometric assay
IRT  Immunoreactive trypsin
ISNS  International Society for Neonatal Screening
IVA  Isovaleric acidemia
IVI  Intravenous
LCHAD  Long chain 3 hydroxyacyl-CoA dehydrogenase deficiency
MCAD  Medium chain acyl-CoA dehydrogenase deficiency
3-MCC  3-Methylcrotonyl-CoA carboxylase deficiency
MCD  Multiple carboxylase deficiency
MSMS  Tandem mass spectrometry
MSUD  Maple syrup urine disease
MUT  Methylmalonic acidemia
NADP  Nicotinamide adenine dinucleotide phosphate
NADPH  Nicotinamide adenine dinucleotide phosphate (reduced form)
NNSGRC  National Newborn Screening and Genetics Resource Center
17-OHP  17α-OH-progesterone
PCR  Polymerase chain reaction
pI  Isoelectric point
PKU  Phenylketonuria
PROP  Propionic Acidemia
RIA  Radioimmunoassay
RUSP  Recommended uniform screening panel
SCHAD  Short chain 3 hydroxyacyl-CoA dehydrogenase deficiency
SCID  Severe combined immunodeficiency
SIDS  Sudden infant death syndrome
SSIEM  Society for the Study of Inborn Errors of Metabolism
T4  Thyroxine
TBG  Thyroxine binding globulin
<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>TFP</td>
<td>Trifunctional protein deficiency</td>
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<tr>
<td>TREC</td>
<td>T-cell receptor excision circle</td>
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<td>TR-FRET</td>
<td>Time-resolved fluorescence resonance energy transfer</td>
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<td>TYR-I</td>
<td>Tyrosinemia type-1</td>
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<td>UK NSC</td>
<td>UK National Screening Committee</td>
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<td>VLCAD</td>
<td>Very long chain acyl-CoA dehydrogenase deficiency</td>
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</tbody>
</table>
REFERENCES

**General References on Newborn Screening and its History**


**Argininosuccinic Aciduria/Citrullinemia**


**Biotinidase Deficiency**


**Carnitine Uptake Defect**


CLSI. Newborn Screening by Tandem Mass Spectrometry; Approved Guideline. 1/LA32-A, Vol. 30 No. 16.


**Congenital Adrenal Hyperplasia**


### Congenital Hypothyroidism


### Cystic Fibrosis


**Galactosemia**

**Glutaric Acidemia Type I**


**Glucose-6-Phosphate Dehydrogenase Deficiency**


Homocystinuria


Isovaleric Acidemia


Maple Syrup Urine Disease


Medium Chain Acyl-CoA Dehydrogenase Deficiency


Methylmalonic Acidemias


**β-Ketothiolase Deficiency**


**Multiple Carboxylase Deficiency**


**Propionic Acidemia**


**Phenylketonuria**


**Severe Combined Immunodeficiency**


**Sickle Cell and other Hemoglobinopathies**


**Trifunctional Protein Deficiency**


**Tyrosinemia Type I**


**Very Long Chain Acyl-CoA Dehydrogenase Deficiency**


**Long Chain L-3 hydroxyacyl-CoA Dehydrogenase Deficiency**


**3-Hydroxy-3-Methylglutaric Aciduria**


3-Methylcrotonyl-CoA Carboxylase Deficiency


