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CLINICAL AND LABORATORY APPROACH TO A NEONATE SUSPECTED OF AN INBORN ERROR OF METABOLISM*

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Foreword

Metabolic and molecular advances have led to the identification of hundreds of inborn errors of metabolism, too many for most to recall from memory alone without referral to authoritative compilations of published works, often assembled in formidable (in size and scope) books weighing many pounds. Those encountering such disorders in neonates face unique and special challenges because of the fragility of their patients, the rapidity with which these conditions progress, and the fact that many of the expressions of inherited metabolic disturbances mimic other serious conditions, thereby delaying diagnosis and placing the newborn at serious risk for survival.

A simple algorithm to quickly establish the diagnosis of the numerous inborn errors of metabolism that have been reported would be helpful. However, because of the diversity of the conditions and defects that can occur, numerous subsets would have to be developed to cover the full spectrum of conditions that may be encountered.

In this report, conditions to consider when confronted with a newborn suspected of an inborn error of metabolism are grouped, based primarily on the presenting clinical findings in the neonate together with laboratory tests that can be performed by most clinical laboratories. Further workup to narrow the possibility should follow, confirming the diagnosis by special tests with consultation from specialists who are more familiar with these conditions. Establishing the diagnosis by specific enzymatic assay would require the involvement of specialists with the laboratory resources to perform the tests. The approach suggested is by no means exhaustive. It does not include genetic conditions that are unlikely to be expressed in the newborn period, nor those that are extremely rare in occurrence.

Only an abbreviated historical account is presented, omitting many important citations to individual papers from a large family of dedicated investigators. Many citations are to chapters in the three volumes of The Metabolic and Molecular Bases of Inherited Disease by Scriver, Beaudet, Sly and Valle, with the

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expectation that the reader would pursue other details cited in these works and those published since then, as may be clinically indicated, in their workup of any neonate suspected of an inborn error of metabolism.

Background

Garrod's[1] conceptualization of our chemical individuality proposed in 1908 stemmed from his study of alkaptonuria. Plumbing was not in use and the affected was readily identified as the urine turned dark upon standing. The constancy of this feature and its expression in only some members of families with higher rates of consanguineous marriages led Garrod to ascribe the trait to a recessively inherited inborn error of the metabolism of homogentisic acid, which he had identified as the compound in urine that darkened after oxidation[2,3].

It took 50 more years before the absence of homogentisic acid oxidase activity was demonstrated as the only defect in the degradation of phenylalanine and tyrosine in alkaptonuric liver biopsies[4]. In the intervening years, important advances leading to our understanding of the genetic basis of clinical diseases were made, including the proposal that the synthesis of enzymes was determined by genes, one gene for one enzyme[5] and that the primary amino acid sequence of a mutant protein would be altered as was found with the hemoglobin in sickle cell anemia accounting for the abnormal function of the protein[6]. Until recently, more than 300 inborn errors of metabolism, or IEMs, have already been identified.

Most arise from the inheritance of mutant alleles that are autosomally recessive. A few are due to a defective dominant allele or to maternally inherited mutant mitochondrial DNA or X chromosome. The majority are single base-pair substitutions that can affect transcription, translation, mRNA splice junctions, processing and translation, and regulatory sites. Less frequent mutations include gene deletions with or without abnormal insertions, duplications, inversions, and expansion or contraction of unstable repeat sequences[7].

Mutations lead to the synthesis of mutant proteins that either fail to function at all, function partially, with altered interactions with cofactors, or with increase in activity. Total absence of enzyme protein synthesis; abnormal protein processing, assembly, and secretion; or defective importation of normally synthesized enzymes into various intracellular compartments can also occur[8].

Collective data are available on the frequency of 8 IEMs in the US[9] (Table I). The most frequent is cystic fibrosis, which occurs once in every 2000 live births. Phenylketonuria (PKU) occurs once in 10-25.000 live births in the US while the incidence is almost three times higher in Turkey[10]. Galactosemia and maple syrup urine disease also occur with higher frequency in Turkey than in the US, perhaps as a result of a higher rate of consanguineous marriages in Turkey[11,12].

Table I: Frequency of IEMs by Newborn Screening

IEM	Frequency	
	USA[1]	Turkey[2]
Cystic fibrosis	1:2.000	
Congenital hypothyroidism	1:3.600-5.000	1:2,736
Congenital adrenal hyperplasia	1:12.000	
Phenylketonuria	1:10.000-25.000	1:4,500
Galactosemia	1:60.000-80.000	higher
Biotinidase deficiency	1:72.000-126.000	
Homocystinuria	1:50.000-150.000	
Maple syrup urine disease	1:250.000-40.000	higher

Live births.
1 from reference (9) and 2 from references (10) and (11).

Various strategies are available for treatmenat of many IEMs, the commonest being the restriction of intake of the substrate that cannot be metabolized. As this may not be sufficient in some cases, compounds have been identified that can link with specific metabolites through alternative pathways to produce nontoxic conjugates that can be eliminated by the kidneys[13-15]. Synthetic hormones and blood proteins can also replace those that may not be synthesized. Sometimes, dysfunctional enzymes can be activated by increasing the concentrations of the cofactors involved. Experience with organ transplantation is limited but promising for some storage disorders and cystic fibrosis. Somatic gene therapy may become available for other conditions in the future, as in adenosine deaminase deficiency[8].

Many of the signs and symptoms exhibited by newborns with IEMs are also seen after hypoxic injury to the brain, intracranial hemorrhage, or infection (Table II). Differentiating an IEM from such conditions could, therefore, be very challenging, as even a rash, an enlarged liver, an odor, or discoloration of the urine can occur in newborns suffering from certain infections as well as an IEM.

Table II: Some Presenting Symptoms of IEMs in Neonates

Neurological	Gastrointestinal	Other
poor sucking reflex	poor feeding/vomiting	dysmorphia
lethargy -> coma	vomiting/diarrhea	organomegaly
hypotonia/hypertonia		rash
opisthotonus	Cardiopulmonary	odor
tremors/myoclonus/seizures	hyper/hypoventilation	cataracts
hypothermia	apnea	jaundice
	bradycardia	

According to Dr. Özalp and her colleagues[11], the odds that an infant with such symptoms would have an IEM is almost four percent in Turkey. Thus, the possibility of an IEM should be entertained whenever an infant with symptoms suggestive of hypoxic injury to the brain, intracranial hemorrhage, or systemic infection is encountered.

Recommended Approach

Once alerted that an IEM might be possible, quickly assess the urgency of the clinical status of the newborn regarding the possibility of imminent decompensation from a cardiopulmonary or neurological standpoint. Stabilization with fluids, intubation and assisted ventilation may be necessary.

Whether symptoms developed after a normal period following birth is important to establish and would be known at the time of examination of the newborn. Similarly, maternal, familial and perinatal factors of importance would be known. If the neonate exhibits dysmorphic features, check the appearance of the parents.

Then, samples of blood, urine and, if possible, cerebrospinal fluid (CSF) should be obtained, setting aside aliquots, including the cells, for specific diagnostic tests. Table III lists the tests that would ordinarily be obtained from a clinical laboratory. Table IV lists the preferred manner of processing the various samples for specific diagnostic tests that could be done to confirm and establish the diagnosis of the specific IEM involved. Processing of blood samples for lactate and pyruvate and the ketone bodies should be done at the bedside to prevent interconversions that can occur if the blood is allowed to stand long before deproteinization.

Table III: Recommended Initial Laboratory Tests

Sample	Clinical Laboratory Tests	Special Laboratory Tests
Blood	Complete blood count, glucose, electrolytes, pH, arterial O_2/CO_2, BUN, lactate/pyruvate, β-hydroxy-butyrate/acetoacetate, uric acid, bilirubin, ammonia, liver enzymes	Amino acids, organic acids, fatty acids, carnitine and carnitine esters, enzyme assays on red blood cells (rbcs) or white blood cells,
Urine	Urinalysis including test for both reducing substances (Clinitab) and glucose (Gluco-strix), ketones-acetone (Acetab), ketoacids (dinitrophenylhydra-zine test), urobilinogen	Orotic acid, amino acids, organic acids, pterin forms, porphyrins, sulfite, as indicated
CSF	Protein, glucose, amino acids, lactate/pyruvate	
Other		CT of the head for possible brain edema and radiological exam of organs/tissues, as indicated to r/o infection, calcifications, abnormal size, or malformations

Table IV: Processing of Samples for Specific Diagnostic Studies

Sample	Procedure
Whole blood	Blot on filter paper, dry, store at room temperature
Plasma	EDTA, heparin, or Ca^{++} but not citrate, transport cold, or freeze and transport frozen
Serum	Transport cold, or freeze and transport frozen
Urine and CSF	Freeze, no additions
Red blood cells/ white blood cells	Remove plasma, refrigerate and transport cold, freeze only if washed with saline first and transport frozen
Skin biopsy	In culture media, or keep cold and transport cold, or freeze in liquid nitrogen and transport frozen
Postmortem tissues	Treat skin as above; other tissues freeze and transport frozen

Personally examine a fresh specimen of urine, or a recently wet diaper, smelling it for odors. The urinalysis should be complete and include a test for reducing substances (Clinitab), for glucose, and for ketoacids. The latter is usually done using an acidic 2,4-dinitrophenylhydrazine solution. A computed tomography (CT) of the head may be necessary to assess for brain edema depending on the clinical findings, and other radiological tests should be obtained as indicated following the initial clinical assessment.

After samples have been collected, a vitamin cocktail would be infused consisting of selected vitamins that serve as cofactors for enzymes that may be involved in some IEMs. These include vitamins B1, 2, 6 and 12, biotin and folate, mixed with 10% glucose and infused at 60-70 calories/kg over several hours (Table V). Improvement implies one of the ingredients in the cocktail was correcting an enzymatic step, in which case the treatment may have to be continued. Needless to say, monitoring the fluid and electrolyte balance and the status of the body fluids must continue throughout therapy.

Table V: Vitamin Cocktail for Initial Infusion*

Vitamin	Dose	Associated IEM
Thiamine (B_1)	10 mg	MSUD, Lactic acidemias
Riboflavin (B_2)	100-300 mg	Electron transport defects
Pyridoxine (B_6)	250-500 mg	B_6-dependent seizures, homocystinuria
Cobalamin (B_{12})	1-2 mg	Methylmalonic acidemia, Homo-cysteine methyltransferase defects
Biotin	20 mg	Organic acidurias
Folate	70-120 mg	Homocystinuria, Hyperphenyl-
or Folinic acid	12.5 mg	alaninemia

Definitive diagnostic tests are available for most of the known IEMs, but no single test covers all possible conditions. Often, tests are run by different laboratories, requiring undue volumes of blood and numbers of samples to be drawn. Furthermore, most tests take days to weeks before results become available. Therefore when dealing with a newborn suspected of an IEM one must rely on one's clinical judgement to narrow the differential diagnosis to a few conditions by taking into account the presenting clinical symptoms and several key laboratory findings.

In view of the large number and diversity of IEMs, are there clinical clues that can be used to narrow the possible conditions to a manageable few? Or, how would a neonate with an IEM present? Unlike older children with storage defects demonstrating the effect of time on the accumulation of unmetabolizable substrates that distort the face, body and intellect, most neonates with IEMs are not readily identifiable, with a few exceptions.

The majority will appear normal and referral may be because of a positive screening test or a family history of a previous sibling with such a disorder. Since the IEMs with the highest frequency are cystic fibrosis, cystinuria, and classical PKU, where no physical stigmata are present at birth, most newborns with IEMs will appear normal. However, 10-20 percent of newborns with cystic fibrosis present with meconium ileus[16], and vomiting suggestive of pyloric stenosis occurs in many PKUs[17].

IEMs can also appear as an acute metabolic disturbance following poor milk intake or vomiting, with the development of hyperventilation and the onset of progressive neurological abnormalities.

Some babies show predominantly neurological symptoms of a progressive nature, as though intoxicated either following or before the initiation of feeding.

Signs of other organ or tissue dysfunction could also be the manner of presentation, including severe, explosive diarrhea related to feeding, nonphysiological jaundice, or discoloration of the urine-which may turn dark as in alkaptonuria, or reddish as in the porphyrias.

Rarely, dysmorphia or other overt physical abnormalities can actually be present at birth as a result of in utero effects that impact organogenesis or growth which cannot be compensated for by maternal factors. Such prenatal effects can result in prematurity, smallness in size for the gestational age of the newborn, macrocephaly, a characteristic dysmorphia, gross body distortions, cataracts, and hepatomegaly or other organ size derangements.

A) Examples of A Few IEMs Presenting with Dysmorphia or Other Overt Physical Abnormalities Include

1. Neonates with hyperphenylalaninemia, not classical PKU, but due to cofactor recycling or cofactor synthesis defects, who may be small for their gestational age[18]. In addition, these newborns would exhibit various neurological abnormalities.

2. Newborns with glutaryl-CoA dehydrogenase deficiency or glutaric acidemia Type I may present with macrocephaly and only later develop a progressive dystonia and dyskinesia. The macrocephaly is associated with CT findings showing dilatation of the lateral ventricles and widening of the cortical sulci. Bouts of sudden onset of neurological deterioration with episodic ketotic hypoglycemia, hyperammonemia and liver abnormalities with a characteristic organic aciduria may also develop. This defect involves a mitochondrial matrix enzyme which transfers electrons to ubiquinone in the respiratory chain[19].

3. Abnormal facies occur in a number of IEMs, including:

a) Multiple acyl-CoA dehydrogenase deficiency or glutaric acidemia Type II, where the neonate presents with a high forehead, low set ears, hypertelorism, hypoplastic midface, rocker bottom feet, abdominal wall muscle defects, hypospadias and hepatomegaly. Metabolically, they exhibit severe hypoketotic or nonketotic hypoglycemia and metabolic acidosis, with fatty degeneration of the liver parenchymal, renal tubular epithelial, and myocardial cells. In this disorder, the metabolism of several amino acids and fatty acids is blocked, leading to the accumulation of specific organic acids in the urine including isovaleric acid in some patients, giving off the odor of sweaty feet[20].

b) Pyruvate dehydrogenase or PDH deficiency, where the neonate presents with the fetal alcohol facies. Presumably this resemblance stems from the effects of acetaldehyde found in alcohol toxicity which inhibits PDH, leaving these infants to develop severe, intractable lactic acidemia[21].

c) The cerebro-hepato-renal syndrome of Zellweger, where a Down syndrome-like facies with deformities is found. This disorder is due to defective targeting of enzymes bound for the peroxisomes, single membrane enclosed intracellular organelles, which lay empty due to failure of the enzymes, normally housed in the peroxisomes, to function. Another striking finding in these infants is the profound hypotonia they exhibit[20].

d) In another peroxisomal defect, rhizomelic chondrodysplasia punctata, gross abnormalities of the limbs, are exhibited right from birth. There is considerable shortening of the proximal portions of the limbs with vertebral and control nervous system (CNS) abnormalities. Unlike Zellweger, in this syndrome, some peroxisomal bodies can be found with some enzymes present but defects in plasmalogen biosynthesis and phytanic acid oxidation are selectively deficient[20].

e) In I-cell disease or mucolipidosis II, the neonate may be small with the characteristic clinical features already apparent at birth. These include coarse facial features with epicanthal folds, a flat nasal bridge, anteverted nostrils, macroglossia, restricted joint movement, hypotonia, hernias, talipes equinovarus, and congenital hip dislocations. There is abnormal lysosomal enzyme transport

in mesenchymal cells with the lysosomal enzymes secreted into the extracellular space. Targeting of lysosomal enzymes to the lysosomes is receptor-mediated through mannose 6-P04 markers on the enzymes. Of two enzymes that synthesize the mannose 6-P04 on the lysosomal enzymes in the Golgi complex, phosphotransferases appear to be defective in I-cell disease. Diagnosis is by assay of the serum for the activities of lysosomal enzymes with verification by assay of the enzymes in cultured fibroblasts and the ratio of extracellular as opposed to the intracellular activities of the enzymes[22].

4. In the X-linked oculocerebrorenal syndrome of Lowe, congenital cataracts are always found. It takes several weeks before the proximal tubular acidosis with bicarbonate wasting leading to the Fanconi syndrome is expressed by increase in phosphate, amino acid and glucose excretion. Failure to thrive, recurrent infections and polyuria with low urine osmolarity would be expressed later. Any infant with congenital cataracts should be checked for this syndrome by examination of the urine and blood for these abnormalities.

5. In Wolman's disease, hepatosplenomegaly of massive proportions and calcification with enlargement of both adrenal glands are observed in the first week of life. Persistent and forceful vomiting associated with marked abdominal distension and steatorrhea occurs in the first weeks. Diarrhea, jaundice or a persistent low-grade fever can also occur. Neurologically, no specific signs are detectable at birth, but a progressive loss of alertness develops within a few weeks after the onset of the other symptoms.

Deficient lysosomal acid lipase activity is the cause of this disease resulting in massive accumulation of cholesteryl esters and triglycerides in the tissues of the body. Liver function tests may be abnormal but other laboratory tests are not diagnostic. Calcifications of the adrenal glands and depressed adrenal responses are suggestive of the diagnosis. Biopsy of tissue is needed for confirmation. Diagnosis would then be established by demonstration of the deficiency of acid lipase activity in cultured skin fibroblasts, lymphocytes, or other tissues. Suppression of cholesterol and apolipoprotein B synthesis by 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors and cholestyramine treatment has not yet been tried in this fatal disorder[23].

6. Ceramidase deficiency of Farber's disease, a deficiency of a lysosomal acid ceramidase, can result in several phenotypic expressions that differ in severity and sites of the major tissue involvement but all of which result in a unique triad of findings: subcutaneous nodules, arthritis, and laryngeal abnormality frequently first noted as a hoarseness which may progress to aphonia and feeding difficulties.

Ceramides are intermediates in sphingolipid metabolism and are produced in the synthesis and degradation of gangliosides. In addition, ceramides are components of myelin and cellular membranes. Ceramidases degrade ceramide,

and acid, neutral, and alkaline ceramidases have been detected. In all phenotypes, granulomatous infiltrations are found in the subcutaneous, joint, and lung tissues, with parenchymal cells also accumulating storage material and being frequently involved in granulomatous reaction. The storage material is ceramides, a significant proportion of which are 2-hydroxy fatty acids that can be found in biopsied tissues. Also, ceramides accumulate in body fluids and deficiency of lysosomal acid ceramidase activity is detectable in white blood cells and cultured skin fibroblasts[24]. No specific therapy is available.

7. In galactosidosis, Type I, affected infants present with hydrops, edema, coarse facies, inguinal hernias, and telangiectases. Ocular abnormalities including a cherry red spot may also be present. Cardiac and renal dysfunction with proteinuria and hepatosplenomegaly occur.

This disorder is due to a combined deficiency of lysosomal β-galactosidase and neuraminidase because of a deficiency of a protective lysosomal protein that functions uniquely. With the β-galactosidase, the protective protein prevents the rapid proteolytic degradation of the enzyme. With the neuraminidase, association with the protective protein is a requirement for activation, while association with β-galactosidase confers stability. Affected individuals excrete an excessive amount of sialyloligosaccharides in their urine which can be detected by thin-layer chromatography. Confirmation would be by enzyme assay in white blood cells or cultured skin fibroblasts[25]. No specific therapy is available.

8. Three inherited defects of the connective tissues can be readily detected in the newborn period: osteogenesis imperfecta, Ehlers-Danlos syndrome and the dystrophic form of epidermolysis bullosa[26].

a) Osteogenesis imperfecta, dominantly inherited, can result in perinatal fractures, particularly of the long bones of the arms and legs, the ribs, or the small bones of the hands and feet. Blue sclerae are also present. There is defective production of Type I procollagen in this disease which can be demonstrated in cultured dermal fibroblasts. No treatment is available.

b) Ehlers-Danlos syndrome is characterized by marked skin fragility and hyperextensibility of both the skin and joints: More than 10 mutations are known but the exact defect that results is not known. In Type I, an autosomal dominant disorder, infants are often born prematurely because of premature rupture of the membranes. In Type VII, multiple joint dislocations and bilateral congenital hip dislocations occur. No treatment is available.

c) Epidermolysis bullosa is characterized by blistering of the skin, often present at birth, occurring within the epidermis at the dermal-epidermal junction or within the dermis below the basemet membrane, and is inherited by either dominant or recessive mechanisms. Progressive syndactyly from scarring of the skin and

mucosal involvement leading to esophageal strictures can lead to profound disability. Survival is limited both by infection and derangements of the gastrointestinal tract. No treatment is available.

Thus, some affected newborns can present with distinguishing features at birth. Careful physical examination could lead to the early recognition of these as well as other inherited disorders of metabolism.

B) Examples of IEMs Presenting with Predominantly Neurological Symptoms of A Progressive Nature Suggestive of Intoxication Include

1. Maple syrup urine disease or MSUD where the newborn develops increasing lethargy, hypertonia, and intermittent opisthotonus. Breast-feeding may delay onset of symptoms. The disorder results from failure to process the keto acids from the branched chain amino acids (BCAA), leucine, isoleucine and valine, to their respective CoA derivatives by a single enzyme complex which is a combined decarboxylase-dehydrogenase. The respective keto acids and amino acids accumulate and spill over into the urine[27] causing the urinary DNPH test to be strongly positive. (detected by dinitrophenylhydrazine (DNPH) test by adding an equal volume of 0.1% of DNPH in 2 N HCl to urine).

If this is found along with the characteristic odor, reduce the BCAA intake and provide high levels of thiamine until the aminogram is completed. Detection of increases in the BCAA and the presence of alloisoleucine in the aminogram of the blood should confirm the diagnosis of MSUD. (the nonprotein amino acid, L-alloisoleucine, is formed endogenously in MSUD from isoleucine through 3-methyl-2-oxo-pentanoic acid undergoing nonenzymatic racemization and transamination to L-alloisoleucine).

Computed tomography (CT) scans are normal at three days of age in affected asymptomatic MSUD infants identified by sibship advantage. By nine days, however, CT abnormalities can develop despite treatment, showing generalized brain edema. A unique localized intense edema involving the cerebellar deep white matter, the dorsal brain stem and cerebral peduncles is seen by imaging studies, suggestive of dysmyelination. The EEG is abnormal in newborns with classic MSUD with comb-like rhythms of 5 to 9 Hz spindle-like sharp waves over the cortical regions[28].

During acute decompensation, dialysis may be necessary. Leucine is responsible for the abnormal neurological signs and isoleucine is responsible for intensifying the odor. Therefore, leucine is key, and its levels should be monitored above all else. Treatment for MSUD is to avoid the substrates that cannot be metabolized using a BCAA free formula offering 2-3 grams of amino acids or protein equivalent/kg/day. Remember that the BCAA are essential, required for protein synthesis, and must be provided in the diet through natural foods to complement the amonut of protein ingested in the special formula. In cow's milk, isoleucine and valine contents are much lower than leucine. During correction of an acute

state, it is important to correct the isoleucine and valine levels to normal despite elevated leucine levels, or else catabolism will not reverse. Should a catabolic state result, insulin can be judiciously used to reverse catabolism. A trial of thiamine therapy giving 50-300 mg/d for three weeks is advisable[27].

2. Nonketotic hyperglycinemia. With a rapid progression of neurological abnormalities characterized by hypotonia, in contrast to MSUD, together with hypoventilation and abnormal eye movements and seizures, consider nonketotic hyperglycinemia. The neurological abnormalities are profound and intractable, developing after a brief normal period after birth with the hypoventilation leading to progressive respiratory failure. Increasing lethargy, poor feeding, intermittent ophthalmoplegia, segmental myoclonic jerks, hiccups, and coma also occur. The only laboratory finding of significance is a raised glycine level in the blood, urine and CSF. A CSF to blood ratio for glycine of > 0.08 is diagnostic. Many other IEMs are associated with elevated glycine levels due to accumulated metabolites that interfere with the hepatic glycine cleavage enzyme, but their CSF glycine content is usually normal.

Nonketotic hyperglycinemia is due to a defect in the glycine cleavage system, a four-peptide complex located in the inner mitochondrial membrane of the major organs which incorporates the carbon skeleton of glycine into purines, glutathione, creatine, and the precursor of heme and porphyrins[29]. Glycine is a neurotransmitter, inhibitory in the spinal cord and brain stem causing apnea and hiccupping early in the disease. It is an excitatory agonist of the N-methyl-D-aspartate (NMDA)-type glutamate receptor channel complex in the cortex explaining intractable seizures. Glycine binding enhances glutamate binding to the NMDA site, increasing the frequency of channel openings once glutamate is bound to the receptor. This promotes sustained stimulation of excitatory impulses and also blocks normal impulse traffic. Thus, neurological defects found in this disorder are due to both the inhibitory and stimulatory roles of glycine in the CNS.

Without the amino acid profile, a trial of protein intake reduction and benzoate to remove glycine as hippurate by an alternate pathway could be tried. Seizure management is difficult, and valproate must be avoided as it interferes with the synthesis of what little amounts of the glycine cleavage enzyme proteins that occur. Dextromethorphan, an antitussive whose major metabolites are moderately potent antagonists of the NMDA sites, has been used together with benzoate with some results on seizure control and reduction of glycine levels[29].

3. Sulfite oxidase deficiency or molybdenum cofactor deficiency. Where neurological abnormalities develop almost immediately after birth with intractable, tonic-clonic seizures, axial hypotonia, and peripheral hypertonia (a pyramidal syndrome with spastic tetraplegia, feeding difficulties, ± dislocated lenses), consider sulfite oxidase deficiency or molybdenum cofactor deficiency, which are almost universally fatal.

For diagnosis of sulfite oxidase deficiency, an increase in sulfite and low sulfate with increased sulfocysteine accumulation must be documented. Urinary sulfite

should be elevated as detected with a dipstick test (manufactured in the US, called Merckoquant Sulfite Test). Fresh urine is used as sulfite will be oxidized to sulfate at room temperature. Quantitative tests for sulfite (elevated), sulfate (diminished) and thiosulfate can be done by anion column chromatography, and S-sulfocysteine (increased) can be identified by amino acid analysis. Restriction of sulfur-containing amino acid intake, binding of sulfite with compounds like cysteamine, and the administration of thiamine may be tried[29].

Three enzymes require molybdenum as a component of a cofactor necessary for activity: sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase. Molybdenum cofactor deficiency is also fatal. Symptoms appear within the first or second weeks of life, resembling sulfite oxidase deficiency, as described above. Diagnosis should show high levels of sulfite, thiosulfate and S-sulfocysteine, but near normal levels of sulfate, an absence of urothion (degradation product of molybdopterin) and elevation of xanthine and hypoxanthine. The severe neurological abnormalities seen with molybdenum cofactor deficiency are presumed to result from tha absence of sulfite oxidase activity. Measures to diminish sulfite production by restriction of the intake of the sulfur-containing amino acids plus the use of thiol compounds with the potential to bind sulfite, such as penicillamine or mercaptoethanesulfonate, have been tried without effect. Addition of cysteamine to absorb excess sulfite, thiamine, and inorganic sulfate to avoid a potential sulfate deficiency should also be tried in this disorder[30].

4. Infantile gangliosidosis. Affected patients exhibit neurological abnormalities shortly after birth with poor sucking and subnormal weight gain. Neurological deterioration develops with an exaggerated startle response and increased deep tendon reflexes and progresses to severe brain damage. Dysmorphia develops with time with hirsutism and joint deformities. Macular cherry-red spots are pathognomonic and hepatosplenomegaly also occurs. Storage of ganglioside G_{M1} occurs in the brain and visceral organs, and keratan sulfate, keratan sulfate-derived oligosaccharides and mucopolysaccharides accumulate in the liver and spleen. Mucopolysaccharides and various forms of galactose-containing oligosaccharides are markedly increased in the urine. The genetic defect is due to deficiency of β-galactosidase activity which can be detected in leukocytes and cultured fibroblasts[31]. No treatment is available, as of yet.

5. Tetrahydrobiopterin (BH_4) deficiencies. If the newborn is small for his gestational age and the neurological findings are mild but progressive, consider a cofactor defect in the enzymatic conversion of phenylalanine (PA) to tyrosine (TY), which is not PKU. The cilinical laboratory findings will be normal but the PKU test would return positive.

Widespread screening for PKU has identified a minority of patients with high PA levels who have normal liver PA hydroxylase or PAH activity. Their defect is at the level of the cofactor that is required by PAH.

The conversion of PA to TY requires not only the hydroxylase itself, but a cofactor which is produced in our bodies from folic acid (Table VI). Folate itself is not the cofactor but must be enzymatically converted to the active factor, tetrahydrobiopterin or BH_4, to serve as the cofactor required by PAH. The cofactor function of tetrahydrobiopterin in the hydroxylating reaction with aromatic amino acids relates to its ability to reduce molecular oxygen, provide electrons and oxidize to the quinonoid form of dihydrobiopterin. Consumption of tetrahydrobiopterin is stoichiometric during hydroxylation of phenylalanine. It is regenerated from the quinonoid form of dihydrobiopterin by dihydropteridine reductase.

Table VI: Phenylalanine Conversion to Tyrosine

Phenylalanine	Phenylalanine hydroxylase or PAH tetrahydrobiopterin or BH_4	Tyrosine
Dihydropteridine reductase recycles BH_4 after PAH reaction		
GTP cyclohydrolase + 6-pyruvoyltetrahydropterin synthase		BH_4 available for PAH and other enzymes involved in neurotransmitter synthesis

Tetrahydrobiopterin or BH_4 is made available for PAH by the actions of three enzymes: one that recycles it after it is altered during the conversion of PA to TY, and two separate enzymes that actually synthesize the cofactor from precursors such as guanosine-triphosphate (GTP). GTP is the major precursor of the pterin nucleus. The initial step in its conversion towards the pterin nucleus is catalyzed by GTP-cyclohydrolase I. Deficiency of GTP-cyclohydrolase I impairs BH_4 synthesis, leading to hyperphenylalaninemia. 6-pyruvoyltetrahydropterin synthase deficiency is the most prevalent form of hyperphenylalaninemia not due to PAH deficiency. Defects in each have been reported and differences amongst these result in differences in their neurological symptomatology (Table VII).

Table VII: Disorders of BH_4 Synthesis

A) GTP cyclohydrolase deficiency (shunts GTP to pterin pathway)
   1. *Clinical features*: progressive truncal hypotonia and limb hypertonia with seizures and intermittent hyperthermia.
   2. *Laboratory findings*: elevated serum phenylalanine levels, low blood and urinary neopterin and biopterin, low neurotransmitter levels, and normal liver PAH activity.

B) 6-pyruvoyltetrahydropterin synthase deficiency
   1. *Clinical features*: difficulty swallowing, oculogyric spasms, truncal hypotonia, limb hypertonia, hyperthermia, seizures, low birth weight, and abnormal white brain matter by MRI.
   2. *Laboratory findings*: elevated serum phenylalanine levels, high neopterin levels in plasma and urine, low biopterin levels, and normal liver PAH activity.

The same form of the cofactor is also required by other enzymes that synthesize important neurotransmitters resulting in diminished levels of these agents, accounting for the neuropathy. The neurological abnormalities found in defects of the enzyme that regenerates the cofactor and of the two enzymes that are needed to synthesize the specific cofactor are not seen in classical PKU, where the cofactor is available and recycled appropriately[32].

Distinction of these conditions from PKU can be made by checking the blood PA levels before and after an IV or oral dose of BH_4 (7.5 mg/kg). If the PA level drops, a cofactor enzyme problem may be the cause of the high PA levels and the neurological symptoms. Distinction between the enzymatic defects of BH_4 synthesis rests in the urinary pterin forms that are found (Table VII).

Treatment of these disorders by reducing phenylalanine intake, while ensuring adequate tyrosine intake, as in PKU, is not sufficient. Three other measures are necessary: 1. provision of folate, the precursor of the cofactor or folinic acid which crosses the blood-brain barrier more readily, 2. provision of certain amines that can be converted to the neurotransmitters by other routes, and 3. prescribing an inhibitor of enzymes that catabolize the neurotransmitters, such as carbidopa.

Each of these enzymes, unlike phenylalanine hydroxylase, is expressed in peripheral blood cells as well as by the liver (Table VIII), making it easier to collect a sample for enzymatic assay to confirm the diagnosis without a liver biopsy.

Table VIII: Tissues for Enzymatic Differentiation of the Hyperphenylalaninemias

Enzyme	Tissues
Phenylalanine hydroxylase	Liver
Dihydropteridine reductase	Liver, skin fibroblasts, red blood cells white blood cells platelets, amniocytes
GTP-cyclohydrolase	Liver, monocytes after phytohemagglutinin
6-pyruvoyl tetrahydropterin synthase	Liver, red blood cells

C) Examples of Disorders That Develop After A Brief Normal Period with Either Vomiting or Poor Feeding with Increasing Hyperventilation and Lethargy and Progressing to Coma, Which Necessitates Distinction of the Basis for the Hyperventilation. One of Several Patterns May be Encountered

1. Hyperventilation due to central drive resulting in respiratory alkolosis. Urea cycle defects. If there is no metabolic acidosis, but rather a respiratory alkalosis with the blood pH > 7.4, glucose is normal or slightly low, the ketones are not elevated and the BUN is low, check the blood ammonia and the urine for orotic

acid. With a low BUN, consider a urea cycle defect (above arginase, either carbamoyl phosphate synthase or CPS, ornithine transcarbamoylase or OTC, argininosuccinic acid or ASA synthetase or ASA lyase defects, or the hyperornithinemia, homocitrullinemia and hyperammonemia syndrome, or triple H syndrome) where the hyperventilation is centrally driven secondary to brain edema, leading to the respiratory alkalosis. (remember that with time, a metabolic acidosis and lactic acidemia could develop).

The clinical presentation of CPS, OTC, AS and AL deficiencies are very similar. The similarity of the clinical presentation relates to the hyperammonemia. All are inherited as autosomal recessive traits except for OTC deficiency which is sex-linked. Neonatal onset of symptoms is usually upon a perfectly normal, full-term pregnancy and delivery with no physical abnormalities upon birth, for at least the first 24 hours. Then, lethargy and poor feeding occur with the progressive addition of other symptoms, including vomiting, progression of lethargy, hypothermia, and hyperventilation, which can rapidly progress to a fatal outcome.

The urea cycle defects are treatable, but identification of the specific defect is necessary for proper therapy of the several defects that are included. Immediately, reduce protein intake. In the acute crisis, hemodialysis and the administration of phenylacetate with benzoate may be necessary to reduce the total ammonia load. For CPS and OTC, hemodialysis plus intravenous benzoate (0.25 g/kg) and phenylacetate (0.25 g/kg) diluted in 10 percent glucose would be infused over 24 hours. Hemodialysis should be repeated until ammonia is only three to four times normal, at which point treatment with oral sodium phenylbutyrate could follow. In ASA lyase defects, parenteral arginine could be used, with or without hemodialysis. For ASA synthetase deficiency, hemodialysis plus benzoate and phenylacetate with 10 percent arginine HCl at a dose of 0.66 g/kg/d would be used, as recommended[33]. For argininosuccinase deficiency, hemodialysis and 10 percent arginine HCl at a dose of 0.66 g/kg/d may be sufficient.

Chronically, dietary protein intake should be reduced to 1.5-2 g/kg/d. For CPS, OTC and AS deficiencies, 0.5 g/kg/d of phenylbutyrate could be given for conversion to phenylacetate, conjugation with CoA, and linkage with glutamine to form phenylacetylglutamine for excretion, which removes two nitrogens per molecule excreted. For ASA lyase defects, 0.5 g/kg/d of arginine enables removal of waste nitrogen as ASA for which renal clearance is the same as the GFR.

The triple H syndrome can present in neonates who appear normal with an uneventful course, if they are breast-fed. When fed a high protein formula, refusal to eat, vomiting, lethargy and even episodes of coma develop. Protein restriction to less than 1.2 g/kg/d with the possible addition of ornithine for enhancement of ornithine transport into the mitochondria is recommended.

Ammonia is toxic and hyperammonemia can produce severe brain edema where ammonia appears to be the only cause for the acute encephalopathy. Studies in normal, awake primates where concentrations of ammonia were progressively increased to five times normal resulted in progressive behavioral, physiological, biochemical and neurological findings similar to patients with hyperammonemia. As the ammonia levels rose, intracranial pressures rose with hyperventilation and the development of respiratory alkalosis. Brain edema with flattening of the cortical gyri and herniation of the cerebellar tonsils occurred[34].

Once the neonate is over the acute crisis, the long-term management would consist of a low protein diet together with other compounds that would help to reduce the nitrogen load, depending on the site of the enzyme block. For both ASA synthase and lyase, arginine would be limiting, and thus addition of arginine to the diet (0.4-0.7 g/kg/d) is recommended. Brusilow[33] recommends that arginine be provided to all patients with defects of the urea cycle except for those with an arginase defect, as arginine is necessary for various proteins in growth and turnover, as well as being a substrate for nitric oxide production. Normal plasma ammonia levels are important but the plasma glutamine can be used as a useful guide since it rises before the plasma ammonia does.

A reasonably accurate diagnosis can be made with the amino acid profile and the urinary orotic acid level. Clinically, differentiation between the first two steps in the urea cycle would depend on the urinary orotic acid levels. These would be higher in OTC deficiency, because the substrate which cannot be metabolized, carbamoyl phosphate, accumulates and is channelled to the formation of the pyrimidine nucleotides, of which orotic acid is an intermediate. The presence or absence of ASA should differentiate the synthase from the lyase defects for ASA. Urea cycle defects would also result in increased glutamine levels and decreased ornithine and arginine concentrations.

Long term, the components to be monitored in the blood would also depend on the site of the block. Should seizures be a problem, valproic acid should be avoided as it can aggravate the hyperammonemia by reducing the formation of N-acetylglutamate, which is required for activation of CPS.

2. Hyperventilation due to metabolic acidosis with hypoglycemia and lactic acidosis.

Following a similar catastrophic, life-threatening progression of symptoms after a brief normal period with increasing hyperventilation and increasingly progressive neurological abnormalities, the basis for the hyperventilation may be a metabolic acidosis where pH is < 7.3. If so, carefully consider the blood glucose, lactic acid, pyruvate and ketone body levels and the anion gap while a waiting the amino acid and organic acid results, as a large number of different disorders can do this and their tentative diagnosis may be possible upon recognition of the implications of these findings. Whether or not there is ketoaciduria would also be helpful to know.

Under a fed state, glucose should be in abundance with excess stored in the liver as glycogen for later release and utilization, between feedings and during a short fast. The two main organs that store glucose as glycogen are the liver and muscle.

Under fasting conditions, whether due to disinterest in feeding, lethargy and somnolence, or vomiting, because of an IEM, intracranial hemorrhage, or infection, the fuel status is strained. Sensors tigger the uncoupling of glucose from glycogen by phosphorolysis, which is the cleavage of the glycogen bond by orthophosphate or Pi, to form glucose-1-PO4. This form of glucose cannot diffuse out of the cell but must undergo several steps before release by the action of glucose-6 phosphatase located on the luminal side of the smooth endoplasmic reticulum. The enzymes that process glycogen to free glucose are present in the liver, but the critical final one, glucose 6-phosphatase, is absent in muscle (Fig. 1). Thus, despite its larger store of glycogen (due to the sheer number of muscles that we have), glucose cannot be released from glycogen by muscle, while it can from the liver. Instead, the phosphorylated glucose from glycogen in muscle is converted to lactate and pyruvate, which are released and transported to the liver for conversion to glucose by gluconeogenesis.

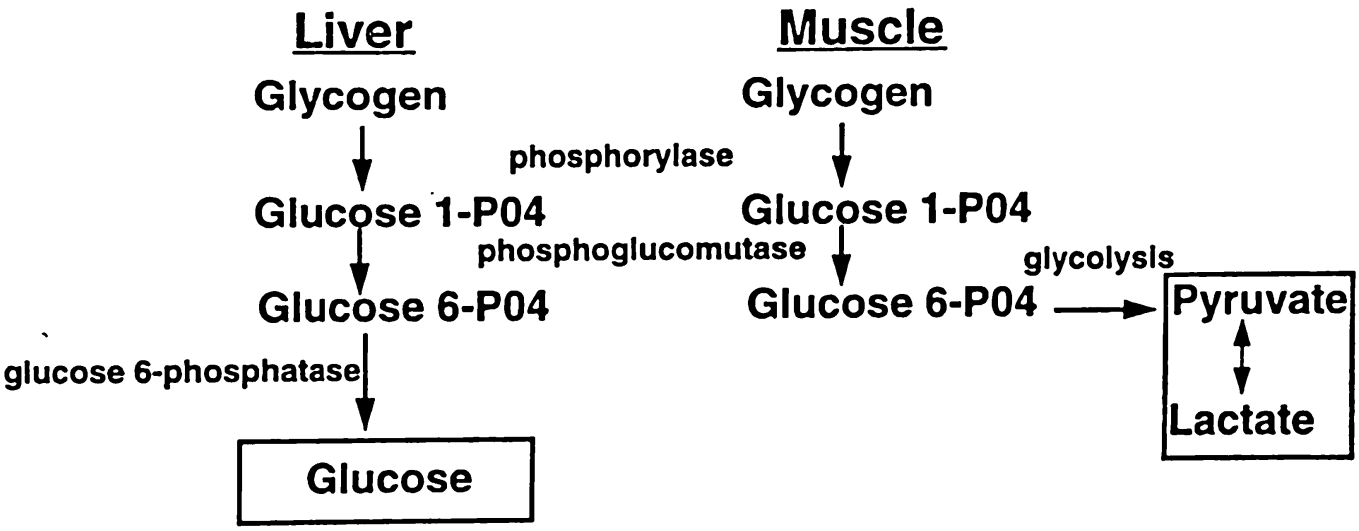

Fig. 1: The key enzymatic steps regulating glycogenolysis in the liver and muscle are in small letters. End-products of the muscle and liver are enclosed.

As fasting continues, lactate, pyruvate, and alanine released from peripheral tissues, particularly from muscle, are processed at an accelerated rate in the liver by gluconeogenesis (Fig. 2). Any block in gluconeogenesis could lead to back up of these intermediates, depending on the site of the block. Ketosis would occur under prolonged fasting or, if caloric needs are not met, as the fatty acids released by adipose tissue are also brought to the liver for oxidation and conversion to the ketone bodies for export and use by the brain, as well as by the heart and muscles.

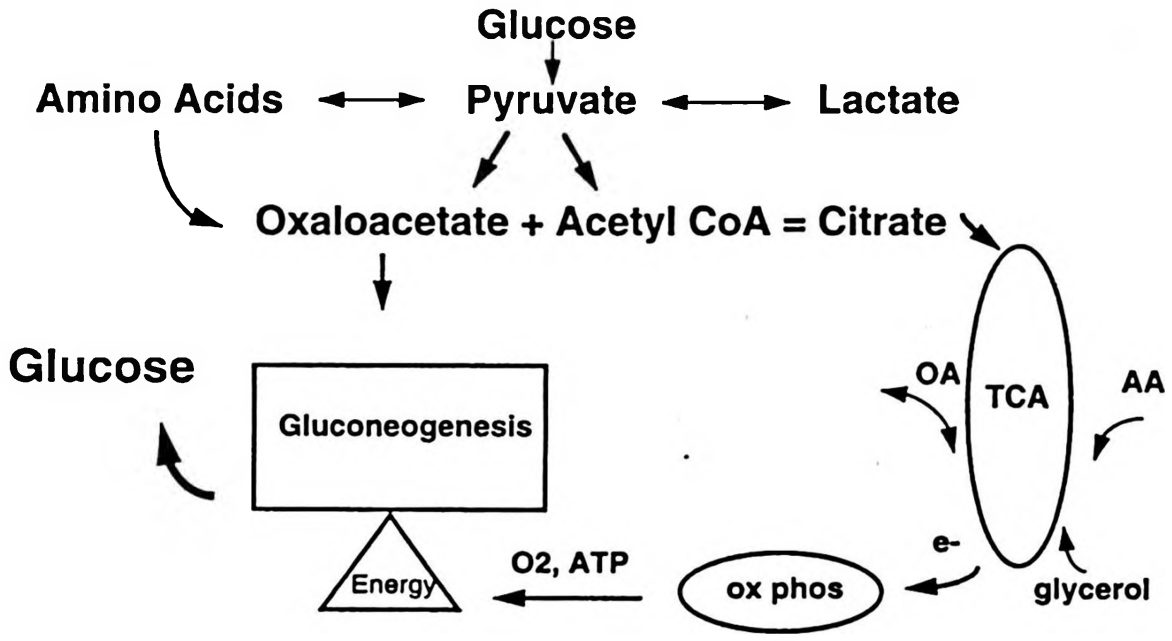

Fig. 2: Pyruvate entering the mitochondria can be converted to acetyl-CoA or oxaloacetate. Citrate, formed from oxaloacetate and acetyl-CoA, is metabolized in the tricarboxylic acid or TCA cycle, generating electrons that are transported by carriers, $FADH_2$ and NADH, to the electron transport complex for oxidative phosphorylation, generating ATP that is used as fuel in various cellular processes, including gluconeogenesis.

Gluconeogenesis is not the reversal of glycolysis or glycogenolysis, but requires its own specific enzymes. Pyruvate, or lactate and alanine after conversion to pyruvate, is sequentially acted upon enzymatically to form glucose 6-P04. The same terminal enzyme, glucose 6-phosphatase, is required to release free glucose, just as in glycogenolysis. Since this enzyme is present in the liver but not in muscle, the process of gluconeogenesis is a major function of the liver. Thus, under fasting conditions, glucose would be released by the liver by two processes, glycogenolysis and gluconeogenesis, whereas only lactate and pyruvate would be released by muscle.

Defect of glucose 6-phosphatase. Therefore, between feeding or during a short fast, defects of the enzymes of glycogenolysis result in mild to moderate hypoglycemia without a rise in lactic acid or pyruvic acid levels, as long as gluconeogenesis continues to occur in the liver. However, a defect of glucose 6-phosphatase results in profound hypoglycemia because glucose 6-P04, from either glycogenolysis or gluconeogenesis, would not be cleaved to free glucose. Lactic acid and pyruvic acidemia would occur as these alternative fuels continue to be released by muscle but cannot complete the cycle of gluconeogenesis. A defect at this site is widely referred to as von Gierke's disease or Type I glycogen storage disease.

Enzymes along key steps in gluconeogenesis include: pyruvate carboxylase or PC, phosphoenolpyruvate carboxykinase or PEPCK, fructose 1.6 biphosphatase, glucose-6 phosphatase, enzymes of the tricarboxylic acid or TCA cycle, or the electron transport chain. Defects with any of the rate-limiting enzymes of gluconeogenesis lead to severe hypoglycemia, metabolic acidosis, and severe lactic acidosis and pyruvic acidemia as these alternative fuels continue to be released by muscle but cannot be processed to glucose, causing them to accumulate. Ketosis would, of course, occur as fatty acids continue to be mobilized and oxidized by the liver at an accelerated pace.

Fructose 1.6 biphosphatase deficiency deserves special mention. This is a severe disorder of gluconeogenesis causing life-threatening episodes. Half of the patients exhibit symptoms by day one to four with hyperventilation, profound acidosis, irritability, somnolence or coma, apneic spells, dyspnea, tachycardia, muscular hypotonia, moderate hepatomegaly, and hypoglycemia. Lactate, ketones, alanine and uric acid levels are elevated in blood and urine. Liver function is usually within normal limits, as are renal tubular function and coagulation. This enzymatic lesion prevents the endogenous formation of glucose from the precursors lactate, glycerol and gluconeogenic amino acids such as alanine. For survival, the patient depends on exogenous glucose; therefore, treatment is with glucose and sodium bicarbonate to correct the acidosis. Oral glycerol provokes abnormalities like oral fructose, but the response to galactose is normal. Avoidance of fasting is critical for this autosomal recessively inherited disorder[35].

The normal lactate to pyruvate ratio is 25. That is, there should be 25 moles of lactate for every mole of pyruvate in the blood, under normal conditions. If both are increased but their ratio is about 25, there must be excessive production of both by the muscles with normal processing of lactate and pyruvate by the liver, as may occur after excessive muscular contractions during a seizure with no block in gluconeogenesis. If both are increased but the ratio is < 25, there must be a block in the processing of pyruvate.

In order for gluconeogenesis to occur, sufficient ATP must be produced. This requires a functioning tricarboxylic acid or TCA cycle linked to an electron transport mechanism. Pyruvate from various sources must be converted first to acetyl-CoA by PDH in the mitochondria, and then to citrate, an intermediate of the TCA cycle. This cycle provides intermediates for the biosynthesis of a number of compounds, including various amino acids and the porphyrins, while generating electrons that are carried by special carriers, NADH and $FADH_2$, the nicotinamide and flavin adenine dinucleotides that carry the electrons from the TCA cycle to the electron transport complex (Fig. 2). There, the electrons are transferred to oxygen by a process coupled to the phosphorylation of ADP producing ATP[21, 36, 37].

Glucose oxidation results in the synthesis of the 3-carbon intermediate, pyruvate, in the cytosol, which is further metabolized in the mitochondrial compartment via the TCA cycle (Fig. 2). Pyruvate entering the mitochondria is acted upon by PDH or pyruvate carboxylase, leading to the formation of acetyl-CoA or oxaloacetate, respectively.

In PDH deficiency, there is severe lactic acidemia as pyruvate can only be processed through pyruvate carboxylase to form oxaloacetate. With a block in PDH, pyruvate backs up, forming lactate, which is released in abundance in this disorder. The severity of the disease is a function of the severity of the lactic acidemia. The most severely affected infants who die before six months have intractable lactic acidemia, low residual activity of PDH complex with cystic lesions of the cerebral hemispheres, or cerebral atrophy.

Treatment for PDH is a high fat, low carbohydrate diet that is ketogenic to provide an alternative source of acetyl-CoA from the breakdown of fatty acids, which can still occur in the liver. The acetyl-CoA can also be converted to β-OH butyrate and acetoacetate, the ketone bodies, for ready transport to the brain, where it could be used by the brain mitochondria as acetyl-CoA and react with oxaloacetate to form citrate to enter the TCA cycle. Thiamine may also be beneficial. Dichloroacetate to activate residual PDH activity may be of some benefit in an acidotic crisis[21].

Pyruvate carboxylase deficiency. Pyruvate can also be acted upon by pyruvate carboxylase, a biotin-requiring enzyme, which converts pyruvate to oxaloacetate, important in several ways, including the formation of citrate by linking to acetyl-CoA and as substrate for gluconeogenesis (Fig. 2). Thus, defective pyruvate carboxylase or PC activity is associated with limits on 1 (gluconeogenesis and 2) the availability of oxaloacetate for conversion to citrate with consequences on the TCA cycle.

Gluconeogenesis can go on in PDH defects as PC is unaffected and there is another source of acetyl-CoA for interaction with oxaloacetate to form citrate, from the oxidation of fatty acid metabolism, which also occurs in the mitochondria. Thus, if fatty acid oxidation is adequate, sufficient citrate would be produced to run the TCA cycle despite the presence of a PDH defect[21].

TCA cycle or oxidative phosphorylation defects. Abnormalities due to mutations that affect the TCA cycle or the generation of ATP by oxidative phosphorylation cause various degrees of hypoglycemia and lactic acidemia, depending on the residual enzymatic activity present. Obviously, total blocks are incompatible with life. Distinction among disorders of the TCA cycle depend on the organic acid profiles in the urine. The genes for the five enzymes of oxidative phosphorylation are both nuclear and mitochondrial. Therefore, some defects can be acquired by maternally transmitted mutant mitochondrial DNA[37].

All of the defects with hypoglycemia would benefit from the administration of glucose and appropriate salts to correct the acidosis. Depending on the defect suspected, other measures could also be tried, including the administration of vitamins that serve as cofactors for the enzymes involved until the organic acid profiles are completed and the specific diagnosis is confirmed.

3. Hyperventilation with metabolic acidosis and severe anionic gap.

In another group of conditions where the degree of hypoglycemia is milder, there is no ketoaciduria, and the degree of the ketosis and lactic acidosis cannot account for the anionic gap, consider such organic acidemias as isovaleric acidemia with the odor of sweaty feet and elevated leucine levels, propionic acidemia, methylmalonic acidemia, or multiple carboxylase defects. Each would be distinguished by the specific abnormal organic acid accumulations found in the plasma and urine. Hyperammonemia can also occur in these disorders. Clinically, thrombocytopenia, neutropenia, and pancytopenia are also found during acute episodes of acidosis.

Immediately reduce the protein intake, and reverse the hypoglycemia and the acidosis. After the organic acid findings are in, take whatever specific steps are needed to restrict the intake of substrates that cannot be metabolized, removing offending metabolites through alternative pathways and augmenting residual enzyme activity by administration of appropriate cofactors that are involved, depending on the defect.

For isovaleric acidemia due to deficiency of isovaleryl-CoA dehydrogenase activity[38], L-leucine intake must be reduced. As leucine is an essential amino acid, too severe reduction can lead to undesirable consequences. Adjunctive therapy with glycine and L-carnitine have been used to reduce the plasma levels of isovaleric acid through the formation of isovalerylglycine[39] and isovalerylcarnitine[40] (250 mg/kg by gavage of glycine and 25 mg/kg/6h of carnitine). Follow up on 1.5-2 g/kg of protein plus glycine and carnitine. Isovalerylglycine is nontoxic and readily excreted; isovalerylcarnitine is less well excreted compared to isovalerylglycine. Diagnosis requires analysis of the organic acids. Isovaleric acid should be elevated in the absence of elevation of other short-chain acids[41, 42]. A pancytopenia can develop due to the isovaleric acidemia.

For propionic acidemia, patients present with dehydration, severe ketoacidosis, lethargy and coma developing within the first day of life. The profound metabolic acidosis is most striking. If the neonate survives the initial episode, intermittent bouts would occur in association with infection, constipation, and increased protein intake. Neurologic sequelae including dystonia, severe chorea, and pyramidal signs are frequently seen.

This disorder is due to a defect in the catabolism of several essential amino acids (isoleucine, valine, methionine and threonine), the odd carbon-chain fatty acids, cholesterol, uracil, and thymine by propionyl-CoA carboxylase activity.

Intake of the amino acids involved must be reduced without limiting growth. Catabolism of isoleucine, methionine and threonine accounts for 50 percent of the propionyl-CoA accumulating in affected patients. Other metabolites also contribute to the accumulation of propionyl-CoA, but gut bacteria may account for 20 percent or more. The serum propionic acid level can rise 100-fold. Pancytopenia can occur, which appears to be due to inhibition of bone marrow proliferation and maturation and, possibly, to a shortened red blood cell survival due to the propionic acidemia[43]. This enzyme is one of the biotinylated carboxylases, and while biotin administration can reduce the degree of propionic acidemia in some cases, improvement in an isolated propionyl-CoA carboxylase deficency has yet to be reported[44].

A low protein diet (0.5-1.5 g/kg/d) or selective reduction of the content of propionate precursors is used. Avoidance of fasting, biotin administration, and L-carnitine (100 mg/kg/d) may help to reduce the excretion of propionic acid[45]. To reduce gut bacteria production of propionic acid, metronidazole (10 mg/kg) has been used[46].

For methylmalonic acidemia, a primary block in the mutase fails to explain the acidosis, hypoglycemia, hyperglycinemia and the hyperammonemia that occur. It has been suggested that these features result from the effects of accumulated organic acids and esters in the mitochondria that inhibit other specific intramitochondrial steps. A pancytopenia can occur due to the methylmalonic acidemia[47]. For treatment, address the life-threatening problems of ketoacidosis, hypoglycemia, hyperammonemia and dehydration. Institute a restricted protein diet, restricting the amino acid precursors of methylmalonic acid and give 1-2 mg CN-cobalamin. L-carnitine supplements could be used to replete stores of free carnitine and enhance the reduction of methylmalonic acid[45], and the use of metronidazole[46] could have beneficial effects on reducing the gut as a source of methylmalonic acid.

Multiple carboxylase or biotin defects. Symptoms that are seen include tachypnea and hyperventilation, irritability, hypotonia, skin rash, etc. These disorders are associated with metabolic acidosis, mild to moderate hyperammonemia, and organic aciduria involving the substrates for the individual enzymes involved.

While the majority of IEMs involve one metabolic pathway, the biotin defects involve enzymes across all three classes of metabolites carbohydrate, fats, and amino acids resulting in defects in gluconeogenesis, fatty acid synthesis, the catabolism of the odd carbon length fatty acids, and the catabolism of leucine. Four carboxylases known to require biotin, a water soluble member of the vitamin B family as a prosthetic group, catalyze these pathways, including pyruvate carboxylase, propionyl-CoA carboxylase, β-methylcrotonyl-CoA carboxylase and acetyl-CoA carboxylase. Each carboxylase is synthesized as an inactive apoenzyme requiring biotinylation catalyzed by the enzyme, holocarboxylase

synthase. Three of the carboxylases are mitochondrial and one is cytosolic. When these enzymes are ready for normal catabolism, the biotin that is attached is conserved by recycling by a number of enzymes, the biotinidases, that are detectable in the blood. Mammals cannot synthesize biotin, which must be obtained from the diet. There is some evidence, however, that the gut bacteria also contributes as a source. The defects that can arise may involve the holocarboxylase synthase, one of each of the individual carboxylases, or the recycling enzymes, the biotinidases. If the holocarboxylase is involved, each of the individual carboxylases would also be affected. Treatment with biotin can reverse symptoms, if the apoenzymes are not affected[48].

4. Metabolic acidosis without ketonemia, the nonketotic hypoglycemias.

As fasting is prolonged, more and more fatty acids are released by adipose tissue and are taken up by the liver, enzymatically linked to CoA and then to carnitine for transport into the mitochondria, where the machinery for β-oxidation of the fatty acids are located, before reconversion back to the fatty CoA for β-oxidation in the mitochondria. Two-carbon acetyl-CoAs are enzymatically removed for conversion to the ketone bodies for export and use as the fuels, acetoacetate and β-OH butyrate, by the brain and muscle. In the blood, acetoacetate will spontaneously convert to acetone, noticed as the familiar sweet odor on the breath of patients in ketoacidosis. The acetyl-CoA can also be used in the TCA cycle after formation of citrate with oxaloacetate.

In the presence of metabolic acidosis and hypoglycemia, the presence of ketone bodies indicates that the enzymes of fatty acid β-oxidation and ketone body formation are present and working hard. In the presence of metabolic acidosis and hypoglycemia, the absence of ketone bodies signifies a block in either 1. the supply of acetyl-CoA for ketone body formation, as in PDH deficiency, or a fatty acid oxidation defect, or in 2. the processing of the acetyl-CoA for the exportable fuels, acetoacetate and β-OH butyrate.

Defects at selected enzymatic steps in the formation of the ketone bodies lead to nonketotic types of metabolic acidosis with hypoglycemia during fasting.

Fatty acid oxidation defects. With a defect in the steps that move the fatty acids into the mitochondria, the availability of glucose and of short chain fatty acids which do not require the addition and switching of CoA for carnitine and back to CoA, should allow sufficient acetyl-CoA for the TCA cycle and activation of PC for gluconeogenesis. A defect in the β-oxidation of a longer chain fatty acid would leave the short and medium-length fatty acids available for both the TCA cycle and gluconeogenesis to occur, as long as fasting is avoided. Also, the peroxisomal system uses an oxidase, not a dehydrogenase, on long-chain fatty acids, which could reduce long-chain ones to shortened fatty acids that may then by-pass the defect in the mitochondria. Depending on the chain length of

the fatty acid involved in fatty acid oxidation defects, ketosis may be absent or present. A medium chain length fatty acid oxidation defect is the most frequent derangement you are likely to see and is the one fatty acid oxidation with the highest chance of death on prolonged fasting. Long-chain fatty acids would still be broken down to provide some acetyl-CoA moieties, and PDH and PC could still function to provide some back up with gluconeogenesis and function of the TCA cycle, accounting for the mild hypoglycemia and mild ketosis that can be seen. Frequently, these children are misdiagnosed as a case of sudden infant death or Reye's syndrome. With each type of defect, the organic acids in the blood and urine and the carnitine status would have to be analyzed to pinpoint the lesion[49].

3-hydroxy-3-methylglutaryl-CoA lyase deficiency. If there is a metabolic acidosis with nonketotic hypoglycemia with hyperammonemia, increased liver size and liver enzymes, consider a defect involving 3-hydroxy-3-methylglutaryl-CoA lyase, which occurs with increased frequency in Arabic populations. Patients present with vomiting, hypotonia, and increasing lethargy, and coma develops in 10 percent. A bimodal age distribution is reported with 30 percent of cases presenting between two to five days of age, and 60 percent between three and 11 months. All have metabolic acidosis and 90 percent have hypoglycemia, both of which are very severe. Half of the patients have severe hyperammonemia and hepatomegaly with increase in the liver enzymes. The death rate is rather high at 20 percent. Patients are often misdiagnosed as Reye's syndrome until the characteristic organic acids are found in the urine. This defect leads to an inability to form acetoacetic acid and 3-hydroxybutyric acid, so patients show no ketosis, despite their acidosis[50].

As this is a block in the formation of the ketone bodies, fasting must be avoided. Treatment of acute episodes consists of glucose to control the hypoglycemia and appropriate electrolytes. A protein-restricted diet should be followed offering 1.5-2 g/kg/day, with restriction of fat to about 25 percent caloric intake.

Other nonketotic defects. A similar clinical presentation can be found in glutaric acidemia. Type I, in association with extrapyramidal symptoms and macrocephaly at birth due to glutaryl-CoA dehydrogenase deficiency, as well as in glutaric acidemia. Type II, which is associated with dysmorphia, prematurity and the odor of sweaty feet.

For glutaric acidemia Type I, treatment consists of reduction of protein or a lysine and tryptophan-low formula plus L-carnitine and riboflavin. Valproic acid has been tried to selectively increase GABA in synaptic areas by inhibiting GABA transaminase or succinic semialdehyde dehydrogenase, or by inhibiting GABA uptake by glial cells and nerve endings. Most importantly, one must rapidly treat the acidosis and hypoglycemia during intercurrent infections and catabolic states.

For glutaric acidemia Type II, or multiple acyl-CoA dehydrogenase deficiency with a neonatal onset, the patient is often premature, presenting by 24^{th}-28^{th} hour of life with hypotonia, hepatomegaly, severe hypoglycemia, metabolic acidosis, and the odor of isovaleric acidemia. Some neonates present with palpable kidneys due to cysts, facial dysmorphism, rocker bottom feet, muscular defects of the anterior abdominal wall, and anomalies of the external genitalia, including hypospadias. Most die in the first few weeks. Those who survive die within a few months after hypoketotic or nonketotic hypoglycemia and metabolic acidosis, with fatty degeneration of the liver parenchymal, renal tubular epithelial, and myocardial cells, and with accumulation of metabolites of compounds that are oxidized by enzymes that transfer electrons to electron transfer flavoproteins. Generally, treatment has not been very successful and has consisted of the use of diets low in fat and protein with supplementation of carnitine and riboflavin. The parenteral administration of methylene blue (2 g/kg/d) has also been tried as an artificial electron acceptor to remove flavin-bound electrons from residual acyl-CoA dehydrogenase with equivocal results[37].

D) Examples of Other Defects in the Newborn Based on Their Clinical Presentation

1. Jaundice without Severe Anemia

a) Galactosemia can arise from defects of one of three enzymes along the metabolic pathway that converts galactose to glucose: galactose 1-P04 uridyltransferase, galactokinase, and uridine diphosphate galactose 4-epimerase. Defects with these enzymes result in the accumulation of galactose and the development of toxicity symptoms such as failure to thrive, vomiting, and liver disease, beginning a few days after the ingestion of milk. Jaundice due to elevated unconjugated bilirubin often appears within a few weeks of life. Mild hemolysis suggestive of erythroblastosis fetalis can occur. Abnormal liver function tests and hepatomegaly with the full blown picture of liver disease with ascites, in the absence of portal hypertension or severe hypoalbuminemia, can also occur. Cataracts can be present within days of birth. Delay in development, neurological symptoms, and ovarian failure are late consequences of galactosemia.

As the primary dietary source of carbohydrate for the newborn is lactose, the principal disaccharide in mammalian milk which consists of glucose and galactose, the action of lactase in the intestinal tract would release sufficient galactose to produce symptoms in vulnerable newborns. The major metabolic pathway for galactose is its conversion to glucose without disruption of the carbon skeleton. Several enzymatic steps are involved. First, galactose is phosphorylated to form galactose1-P04 by galactokinase. Then, galactose 1-P04 reacts with UDP-glucose, producing UDP-galactose and glucose 1-P04, catalyzed by galactose 1-P04 uridyltransferase. A defect at this step is responsible for classic galactosemia. The UDP-galactose is finally acted upon by the epimerase, which inverts the hydroxyl group of the galactose moiety at the fourth carbon of the hexose chain to form UDP-glucose.

Laboratory findings include abnormal liver function tests, elevated blood and urine galactose, reducing substance in the urine, hyperaminoaciduria, albuminuria, and hyperchloremic metabolic acidosis, which could be due to the gastrointestinal disturbance that occurs in galactosemia, poor food intake, and/or as renal tubular defect in urine acidification that occurs in this disease. There is also an increase in the galactose 1-P04 content of the red blood cells. The diagnosis can be made in the circulating red blood cells (rbcs) by increase in galactose 1-P04 and by enzymatic assay for the specific enzymes involved.

Treatment is to restrict galactose intake by avoiding proprietary formulas as well as both human and cow's milk. Soybean based milks or a casein hydrolysate, Nutramigen, can be used instead[51].

b) Bile and bilirubin metabolic defects. Newborns have increased bilirubin levels due to the decreased erythrocyte half-life, diminshed hepatic capability to dispose of bilirubin, and diminished intestinal bacteria that degrade bilirubin to urobilinogen, with a greater surface-to-volume ratio of the bowel enhancing the intestinal absorption of unconjugated bilirubin compared to adults[52]. As a result, about half of all newborn become clinically jaundiced during the first five days of life. The serum bilirubin, largely unconjugated, can reach 10 mg/dl or more before decreasing to normal levels in seven to 10 days[53]. Plasma bilirubin concentrations are higher in breast-fed compared to formula-fed infants and will promptly fall upon discontinuation of breast-feeding. Levels as high as 15-24 mg/dl have been reported within 10-19 days of age without the development of kernicterus[54].

1. Crigler-Najjar syndrome Type I, due to the absence of hepatic bilirubin UDP-glucuronosyltransferase activity, manifests itself within the first few days of life with jaundice due to increased plasma concentrations of indirect-reacting bilirubin, which can lead to kernicterus and death. Apart from the jaundice and neurological impairment, other findings are not abnormal. Stool color, bilirubin production, the hematocrit, red blood cell (rbc) morphology and kinetics, as well as the liver function tests are normal. Aggressive phototherapy and plasmapheresis can avert kernicterus, if instituted in a timely manner, but long-term outlook is still very bleak. Liver transplantation appears to result in long-term survival.

2. Bile acid biosynthesis defects and cholestasis. Manifestations of cholestatic liver disease at birth with pale stools, dark urine, and progressive jaundice can occur because of defects in either 3β-hydroxysteroid-Δ^5-oxidoreductase/isomerase or 3-oxo-Δ^4-steroid 5β-reductase activity.

With the former derangement, 3β-hydroxystreid-Δ^5-oxidoreductase/isomerase, there is increased circulating conjugated bilirubin, liver transminases, alkaline phosphatase and low levels of vitamin E. Cholate and chenodeoxycholate are not detectable in the plasma. Diagnosis can be made by negative ion fast atomic

bombardment-mass spectrometry of a urinary bile acid fraction where a pattern corresponding to characteristic sulfated esters is found. A urinary test for the accumulating bile acids in urine can be made by the Lifschutz reaction, and enzymatic assay can be done on cultured skin fibroblasts. Treatment with chenodeoxycholic acid (250 mg/d) inhibits the rate-limiting enzyme in bile acid biosynthesis, cholesterol 7α-hydroxylase, and diminishes the production of toxic metabolites from cholesterol[55].

With 3-oxo-Δ^4-steroid 5β-reductase deficiency, jaundice, pale stools and dark urine are also seen within the first day or so of life. A marked increase in conjugated bilirubin and coagulopathy also occur. Fasting bile acids are elevated in the serum in contrast to the other defect leading to cholestasis in newborns. In this disorder, there is failure to convert two bile acid intermediates, 7α-hydroxy and 7α, 12α-dihydroxy 4-cholesten-3-one, to the corresponding 3-oxo-5β satured derivatives toward chenodeoxycholic and cholic acid formation. However, the side chain of the accumulated 3-oxo-Δ^4 bile acid precursors may be oxidized despite incomplete transformation of the ring nucleus, accounting for the increase in the serum bile acids that occurs. In affected newborns, the liver transaminases and the serum alkaline phosphatase are normal, in contrast to the former disorder. Definitive evidence for this diagnosis has yet to be obtained from enzymatic study. Treatment with exogenous bile acids inhibits cholesterol 7α-hydroxylase activity, preventing the accumulation of potentially toxic bile acid intermediates, while exerting a choleretic effect and improving intestinal fat absorption[55].

3. Copper transport defect or Menkes disease. Newborns with the X-linked classic Menkes disease with widespread disturbance in the intracellular transport of copper are often born prematurely and exhibit hypothermia and hyperbilirubinemia as neonates. Most appear normal but some can exhibit trichorrhexis nodosa, monilethrix, and unusual facies as neonates. The cerebral degeneration, vascular complications (subdural hematoma, arterial rupture, and thrombosis), and bone changes develop later. Serum copper and ceruloplasmin levels are low with marked differences from normal by two weeks of age. Liver copper content is low whereas gut tissue content is high. Treatment to reverse the copper deficiency can restore hepatic and serum levels to normal without reversal of the neurological damage that occurs. Copper histidinate treatment has been the usual form of copper administered[56].

2. Jaundice with Anemia

a) Glucose 6-P04 dehydrogenase deficiency. A number of defects can result in hemolytic anemia and jaundice in the neonate. Glucose 6-P04 dehydrogenase (G6PD) deficiency resulting from many allelic mutations of the gene is the commonest known enzymopathy affecting people worldwide. G6PD produces NADPH, the coenzyme that serves as a hydrogen donor in numerous enzymatic reactions, some of which are catalyzed by glutathione reductase, an enzyme

important in protection against oxidative damage. A defect in G6PD would reduce this ability and allow peroxide to accumulate, exceeding the ability of catalase to detoxify the peroxide, leading to hemolysis. Red cell morphology is affected and Heinz boides appear due to denatured protein adhering to the celluar membrane. Reticulocytosis and impaired liver function can also appear. There is striking variability in its expression, but kernicterus occurs with increased frequency amongst affected newborns in both Africa and Southeast Asia. Avoidance of oxidant drugs, treatment of hypoxia, sepsis and acidosis, prophylactic administration of phenobarbital to improve hepatic conjugation of bilirubin, and phototherapy or exchange transfusion could be used[57].

b) Pyruvate kinase deficiency. Severe neonatal anemia and jaundice occur. Red blood cell morphology is usually not affected in the neonate except in severe cases when macrocytosis and echinocytes can be detected. Erythrocyte osmotic fragility is normal, the Coombs test is negative, and cold agglutinins are not found. Red blood cell (rbc) life span is shortened and cells are selectively sequestered and destroyed in the spleen as well as the liver. Indirect bilirubinemia and decreased haptoglobin concentrations reflect the severity of the hemolytic process. Fecal urobilinogen excretion is increased. Splenectomy permits the newly formed cells to survive longer, and transfusions of red blood cells (rbcs) may be necessary from time to time. Enzyme assay on red blood cells (rbcs) must be done without contamination with leukocytes[58].

c) Intrinsic red blood cell (RBC) structural membranopathies. Hereditary spherocytosis is caused by the progressive loss of red blood cell (rbc) membrane surface. Defects of spectrin, ankyrin, pallidin and band 3 of the red blood cell (rbc) membrane have been found in this disorder. Affected infants develop jaundice and anemia within the first few days of life, requiring exchange transfusion to prevent kernicetrus. Once the hemoglobin is corrected by the transfusion, the progression of the anemia is much slower and the course of the disease can be reduced. Later, the spleen may enlarge. The presence of spherocytes and increased osmotic fragility are key diagnostic findings. Besides the anemia, the reticulocytes are increased and there is increase in unconjugated bilirubin in the blood. Fecal excretion of urobilinogen is increased. Splenectomy is the treatment for this disease[59].

3. Congenital Cyanosis or Methemoglobinemia

Over 400 human hemoglobin variants have been demonstrated, the majority of them due to single amino acid substitutions in one of the globin chains. Variants resulting in sickle cell and the thalassemias may express abnormalities in the first year of life, but rarely in the neonate. Among the clinical diseases resulting in the neonatal period are variants where 1) the hemoglobin levels are normal but cyanosis is noted at birth, such as in hemoglobin Bart, observed in Southeast

Asians or people of Mediterranean origin, which results in a syndrome that is fatal in utero, or soon after birth, or 2) there is an accumulation of methemoglobin due to hemoglobin M[60].

a) Hemoglobin Bart presents in a newborn who is pale and edematous with signs of cardiac failure and intrauterine hypoxia. Hepatosplenomegaly and other organ abnormalities may also be found. For diagnosis, the hemoglobin level, hematocrit, red blood cell (rbc) indices, hemoglobin electrophoresis, and estimation of the fetal hemoglobin, hemoglobin A_2 and hemoglobin Bart levels should be made, not only on the patient but also on family members. Further analysis by a hematological laboratory would be necessary to pinpoint the variant.

b) Congenital cyanosis can result from an inherited structural defect of the hemoglobin or due to a defect in one of the enzymes involved in maintaining the level of reduced hemoglobin within the normal range. The structural defect of hemoglobin resulting in congenital methemoglobinemia, a benign condition, is due to a dominant pattern of inheritance of hemoglobin M. Individuals with the enzymatic derangements are cyanotic from birth. Ten to 15 percent of total hemoglobin as methemoglobin produces the cyanosis, whereas 2.5-3 times that amount of deoxygenated hemoglobin results in a comparable degree of cyanosis. The degree of methemoglobinemia found in patients is well tolerated and patients are asymptomatic. Treatment is unnecessary, and the methemoglobinemia clears with parenteral methylene blue. Oral ascorbic acid and riboflavin are also reported to keep the methemoglobin level at five percent.

4. Hemophilia

Hemophilia presents as bruising and hematoma formation unaccountable by trauma, or as continued bleeding after circumcision or needle puncture in a male neonate. Various mutations of X-linked factor VII deficiency interfere with the production of normal levels of factor VIII. A history of other affected males in the extended family may be obtained. A prolonged partial thromboplastin time, normal prothrombin time, normal bleeding time, normal platelet count with a specific defect in factor VIII clotting activity, and no antibodies to factor VIII are diagnostic. Treatmenat is by replacement therapy using either blood derived factor VIII or recombinant products that are available, recognizing that the half-life of factor VIII is only about 12 hours[61].

5. Diarrhea

Disaccharidase deficiencies or intolerance cause severe diarrhea starting within hours or days of life, with dehydration occurring after ingestion of the disaccharide that is not cleaved by specific intestinal enzymes which are either diminished or absent (lactase or sucrase-isomaltase). Flatulence, borborygmi, and abdominal distension also occur. Reducing substances can be found in the feces. Breath

hydrogen is increased after ingestion of the suspected disaccharide, and chromatographic analysis of the stool would identify the disaccharide that is not cleaved. Avoidance of the offending disaccharide-containing formula is key[62].

6. Hormone Deficiencies

a) Congenital hypothyroidism. Inadequate production of thyroid hormone is due to a number of causes, only a few of which are genetic, but all of which can result in mental retardation, growth failure, deafness, and neurological abnormalities. The majority of affected newborns will be normal in appearance because of placental transfer of maternal thyroid hormone. Unless there is a prior history of an affected sibling, or persistence of neonatal jaundice, the clinician may not be aware of this diagnosis. Only with diligence and care can a low metabolic rate, poor peripheral circulation, bradycardia, constipation, or a goiter be noted. A free T_4, TSH, or both, tests can be done using the dried filter paper spot used in many screening programs. The free T_4 would be low and the TSH would be elevated. Treatment is with synthetic thyroid.

b) Congenital adrenal hyperplasia[63]. A number of enzymatic defects can affect the biosynthesis of the adrenal corticosteroids. The commonest is a 21-hydroxylase deficiency which causes decreased synthesis of cortisol. Corticotropin-releasing factor and ACTH levels increase, and the high ACTH levels increase the levels of the precursors of cortisol, of which 17 α-OH progesterone is a major component. The 17 α-OH progesterone has a sodium loss and potassium retention effect with water loss. The increased ACTH increases the production of androgens. This increased androgen production leads to the somatic and sexual precocity seen in affected newborns. In females, an incorrect sex assignment could result. In three-fourths of affected males, a salt-losing syndrome can develop. Danger of life-threatening adrenal crises arises which can result in nine percent mortality in the newborn period. This defect can be detected by neonatal screening tests run on blood spots collected on filter paper for increased 17 α-OH progesterone[9]. Treatment is by administration of cortisone with careful monitoring. Females may require surgery to correct ambiguity of the genitalia in the future.

Other inherited conditions that are readily detectable by physical examination (Down syndrome, albinism, etc.), some that are difficult to detect in the newborn (Marfan), and others that express themselves after the first few months of life have not been included in this report on a suggested clinical and laboratory approach to the diagnosis of an IEM in the neonate.

Would new screening tests be available to identify all affected neonates in the future? Newborn screening tests rely on time-dependent changes in the concentrations of substances in the blood for identification [phenylalanine for PKU,

leucine for MSUD, methionine for homocystinuria, galactose for galactosemia, 17-hydroxyprogesterone for congenital adrenal hyperplasia and thyroxine (T4) and thyrotropin (TSH) for congenital hypothyroidism]. In the US, it is recommended that screening be done after 24 hours of age but before day five or, if infant is discharged before at 24 hours of age, that the initial specimen be collected in the hospital and follow-up specimen be sent in by day seven to 21[9]. Recent policies on early discharge of postpartum mothers and their infants as a cost-saving strategy raise concerns of missing cases. Thus, the feasibility of genotyping from dried blood spots was explored for phenylalanine hydroxylase.

Dried blood spots as a source of genomic DNA for polymerase chain reaction (PCR) gave a low yield of PCR products, necessitating a two-step amplification procedure to be used with three percent different oligonucleotide primers. Ninety-six percent of mutant alleles were identified, not 100 percent[64]. For he work entailed and the four percent positive error rate, genotyping may not be suitable for widespread use as a screening tool for PKU. Whether genotyping for other disorders would result in tolerable positive error rates, or none at all, remains to be seen. (Interestingly, PKU appears to be caused by more than 200 mutations at the PAH locus, most of which are strongly associated with a specific restriction fragment length polymorphism or a variable number of tandem repeat haplotypes; five novel substitutions in the PAH genes were also found).

But, for the tools currently in use, a separate test is done for each disorder screened and only a few IEMs are included. Advances in the design of mass analyzers, new ionization techniques and efficient computing systems have led to tandem mass spectrometers (MS) which can analyze specific compound groups such as the amino acid and acylcarnitine mixtures without prior separation. Automated electro-spray ionizatilon tandem mass spectrometers can now be used for the diagnosis of amino acid and fatty acid IEMs from dried blood spots[65]. While this approach is bound to become more widespread in use, the range of disorders that can be identified is still limited.

A combination gas chromatography/MS (GC/MS) approach extending the disorders to a total of 22 IEMs is in use in Japan[66]. This approach requires pretreatment of the urine sample to remove urea with urease plus derivatization. The time required for analysis is still a bit long, allowing only 80 samples to be analyzed per instrument in a working day, but development is proceeding to automate and thus decrease the time required for GC/MS.

With families at risk, prenatal DNA analysis can be helpful. Amniocytes and chorionic villus samples can be used, but contamination of the chorionic villus by maternal decidua is possible when PCR amplification for restriction fragment length polymorphism (RFLP) or mutational analysis is used.

Finally, even if the diagnosis is readily apparent, or a screening test returns as positive, consult your local experts early, encourage parental adherence to the recommendations of a multidisciplinary team of professionals, and be prepared for a life-long relationship with your patient, complete with genetic counseling with the family and the patient as well.

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