CHAPTER

42

Disorders of Amino Acid Metabolism

Marc Yudkoff

OUTLINE

INTRODUCTION

An aminoaciduria usually results from the congenital absence of an enzyme needed for metabolism of an amino acid

Aminoacidopathies typically involve an inherited deficiency of an enzyme that is important for the metabolism of a particular amino acid (Table 42-1). The concentrations of that amino acid and its metabolites consequently rise sharply in blood, urine and body tissues, including the brain. When the enzymatic deficiency is nearly complete, the onset of disease tends to occur in infancy, often in the neonatal period. Partial enzyme deficiencies may not become apparent until later in life (Kahler & Fahey, 2003; Scriver et al., 2001).

Most untreated aminoacidopathies damage the brain. The pathophysiological factors that explain the encephalopathy remain unknown. Injury probably results from the accumulation of an amino acid or amino acid metabolite that is toxic to the nervous system, especially to the developing brain. Mental retardation and other manifestations of brain damage can result. Fortunately, these disorders often are amenable to therapy with a diet that is purposely low in the offending amino acid. In some instances, successful treatment may be possible by administration of a high dose of a vitamin that serves as a cofactor for the defective biochemical reaction (Table 42-1). Antidotes to the neurotoxicity have been developed for a few syndromes. Outcome often depends upon early institution of treatment. The advent of mass screening of newborns for metabolic diseases has greatly improved the prognosis.

The major metabolic fate of amino acids is conversion into organic acids; absent an enzyme to oxidize an organic acid, an organic aciduria results

Three features characterize the metabolism of essentially all amino acids: (1) incorporation into protein; (2) conversion into messenger compounds such as hormones and neurotransmitters; and (3) oxidation, which involves the conversion of amino acid nitrogen into ammonia and of amino acid carbon into organic acids that enter the tricarboxylic acid cycle, where they become oxidized.

Generalized defects of protein synthesis have not yet been described, presumably because they would be lethal early in development. Disturbances in the synthesis of messenger compounds such as thyroxine or neurotransmitters may occur, but they do not result in an aminoaciduria because relatively little amino acid is so disposed.

Many organic acidurias originate in the breakdown of the three branched-chain amino acids leucine, isoleucine and valine (Fig. 42-2). Metabolism of the organic acids requires the presence of specific enzymes, congenital deficiencies of which give rise to the organic acidurias. The clinical features of the organic acidurias are described in Table 42-2.

On rare occasions an organic aciduria occurs not because of an enzyme deficiency but from a failure to transport or activate a water-soluble vitamin that serves as a cofactor for the reaction in question. Thus, congenital deficiencies in the metabolism of vitamin B_{12} commonly give rise to methylmalonic aciduria (Fig. 42-1; Table 42-2). Similarly, defects in biotin metabolism can cause a severe organic aciduria (Table 42-2). It is very important to be aware of the defects of vitamin metabolism because the administration of large doses of these cofactors may completely prevent brain damage.

Untreated aminoacidurias can cause brain damage in many ways, often through impairing brain energy metabolism

Various biochemical changes occur in experimental models of these disorders. An adverse effect on brain energy metabolism has been observed in virtually all *in vitro* and animal models. *In vivo* evidence indicates that similar pathophysiological mechanisms probably occur in humans, particularly during metabolic decompensation. Thus, studies with ^{31}P-magnetic resonance spectroscopy in adults with phenylketonuria showed

TABLE 42-1 Disorders of Amino Acid Metabolism[a]

Disorder	Biochemical derangement	Classical findings	Treatment
I. Branched-Chain Amino Aciduria (Maple Syrup Urine Disease)	Defective branched-chain amino acid breakdown (Fig. 42-1)	Coma, convulsions, vomiting, respiratory failure in neonate	Diet low in branched-chain amino acids Thiamine for B_1-responsive disorders (rare) Hepatic transplantation
II. Glutaric Acidurias	Type I: Primary defect of glutarate oxidation	Type I: Severe basal ganglia/cerebellar disease with macrocephaly. Onset 1–2 years	Diet low in lysine and tryptophan
	Type II: Defect of electron transfer flavoprotein	Type II: Fulminant neurologic syndrome of the neonate. Often with renal/hepatic cysts. Usually fatal	Supplementation with coenzyme Q, riboflavin, carnitine
III. Phenylketonuria (PKU)	Usually defect of phenylalanine hydroxylase. In care cases, defect of biopterin metabolism (Fig. 42-3; Reaction 1)	Normal at birth. Mental retardation in untreated children. Avoidable with early institution of diet therapy. Prognosis less favorable in PKU secondary to defect of biopterin metabolism	Diet low in phenylalanine Carbidopa
IV. Non-ketotic Hyperglycinemia	Defect of the glycine cleavage system (Fig. 42-4)	Intractable seizures in neonate. Usually fatal in first few weeks of life	Diet low in glycine NMDA receptor blockers (variable efficacy) Sodium benzoate to lower blood glycine
V. Homocystinuria	Usually a failure of cystathionine synthase (Fig. 42-2; Reaction 6). Rarely associated with aberrant vitamin B-12 metabolism (Fig. 42-2)	Thromboembolic diathesis, marfanoid habitus, ectopia lentis. Mental retardation is frequent	Diet low in methionine Vitamin B_6 in pyridoxine-responsive syndromes Vitamin B_{12} in responsive syndromes Anti-clotting agents
VI. Urea Cycle Defects	Failure to convert ammonia to urea via urea cycle (Fig. 42-5)	Coma, convulsions, vomiting, respiratory failure in neonate. Often mistaken for sepsis of the newborn. Mental retardation, failure to thrive, lethargy, ataxia and coma in the older child. Associated with hyperammonemia and abnormalities of blood aminogram	Low protein diet Acylation therapy (sodium benzoate, sodium phenylacetate) Arginine therapy in selected syndromes Hepatic transplantation
VII. Disorders of Glutathione	Defective synthesis of glutathione, the major intra-cellular anti-oxidant	Spinocerebellar degeneration, mental retardation, cataracts, hemolysis. Severe acidosis in some cases	*N*-acetylcysteine (variable response)
VIII. Disorders of GABA	Vitamin B_6-dependent seizures often an absence of succinic semialdehyde dehydrogenase	Hypotonia, ataxia, mental retardation in older child. Increased urine 4-OH-butyric acid	Pyridoxine (B_6-dependent disorder) Inhibitors of GABA transaminase
IX. Canavan's Disease	Absence of N-acetylaspartate acylase	Rapidly progressive demyelinating disease of infancy	Gene therapy (experimental)

[a]For disorders of carbohydrate metabolism see Chapter 43.

increases of brain levels of ADP in the basal state (see Ch. 58 for detailed discussion of spectroscopy). Loading with phenylalanine caused slowing of the electroencephalogram (EEG) and a further increase in the concentration of ADP (Pietz et al., 2003). Similarly, an elevation of brain lactate has been observed during metabolic decompensation in the brains of patients with maple syrup urine disease (Jan et al., 2003). A variety of related factors probably are involved, including an uncoupling of oxidative metabolism, inhibition of the tricarboxylic acid cycle, impaired glucose homeostasis, alterations of the intracellular redox potential, changes in the metabolism of neurotransmitters and excessive stimulation of brain *N*-methyl-D-aspartate (NMDA)

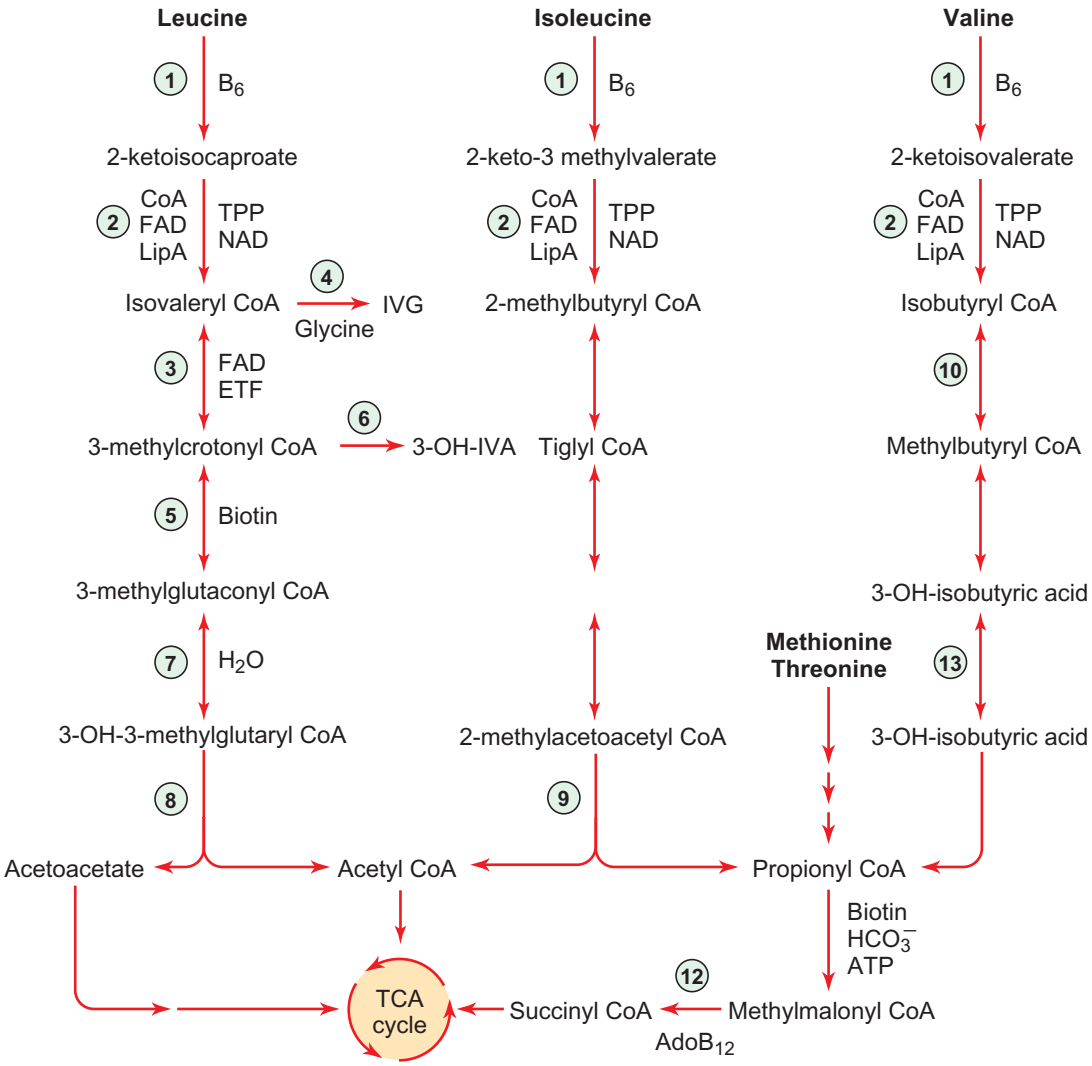

FIGURE 42-1 **Major pathways of branched-chain amino acid metabolism**. Maple syrup urine disease is caused by a congenital deficiency of reaction 2. Many of the primary organic acidurias, for example, isovaleric acidemia and methylmalonic acidemia, are referable to inherited defects of enzymes involved in the oxidation of organic acids derived from the branched-chain amino acids. *Enzymes*: *1*, branched-chain amino acid transaminase; *2*, branched-chain amino acid decarboxylase; *3*, isovaleryl-CoA dehydrogenase; *4*, glycine-*N*-acylase; *5*, 3-methylcrotonyl-CoA carboxylase; *6*, crotonase; *7*, 3-methylglutaconyl-CoA hydratase; *8*, 3-OH-3-methylglutaryl-CoA lyase; *9*, 2-ketothiolase; *10*, isobutyryl-CoA dehydrogenase; *11*, propionyl-CoA carboxylase; *12*, methylmalonyl-CoA mutase; *13*, 3-OH-isobutyryl-CoA deacylase. *TPP*, thiamine pyrophosphate; *LipA*, lipoic acid; *ETF*, electron transfer flavoprotein; *$AdoB_{12}$*, adenosylcobalamin; *IVA*, isovaleric acid; *IVG*, isovalerylglycine; *TCA*, tricarboxylic acid.

receptors, thereby leading to consumption of ATP via activation of Na^+, K^+-ATPase. (See pertinent topics of metabolism and neurotoxicity in Chs. 3, 11, 35 and 43). These mechanisms are not necessarily mutually exclusive. For example, during hyperammonemia more than one mechanism probably is operative (Felipo & Butterworth, 2002).

An imbalance of amino acids in the blood often alters the rate of transport of these compounds into the brain, thereby affecting levels of neurotransmitters and rates of protein synthesis

In almost all aminoacidopathies the blood concentrations of one or more amino acids increase markedly. This factor may competitively inhibit the transport of other amino acids across the blood–brain barrier (Wagner et al., 2000). Amino acids are transferred into the CNS via specialized transporters that mediate the uptake of neutral compounds. Excessive plasma levels of one compound may inhibit the transport of others. This phenomenon has been studied most carefully with respect to phenylketonuria, in which there occurs diminished entry into brain of tyrosine and tryptophan, the respective precursors of dopamine and serotonin (see Chs. 14, 15). *In vivo* studies with magnetic resonance spectroscopy support this hypothesis. When adults with phenylketonuria receive dietary supplements of neutral amino acids, the blood concentrations of tyrosine and tryptophan increase but blood levels of phenylalanine are unchanged. In contrast, the brain concentration of phenylalanine declines toward

TABLE 42-2 **The Organic Acidurias**

Organic Aciduria	Enzyme deficiency	Clinical findings	Treatment
Isovaleric Acidemia	Isovaleryl-CoA Dehydrogenase	Neonate: fulminant syndrome: coma, convulsions. Rancid odor of "sweaty socks". Older child: developmental delay, mental retardation, recurrent vomiting. Odor of "sweaty socks"	Low protein diet to restrict intake of leucine (precursor to isovaleric acidemia) Carnitine Glycine to promote acylation of isovalery-COA
3-Methylcrotonic Aciduria	3-Methylcrotonyl-CoA Carboxylase (biotin dependent)	Infancy: vomiting, metabolic acidosis, hyperlactatemia, convulsions, coma. Older child (2–5 years): recurrent vomiting, metabolic acidosis, hypoglycemia and progressive lethargy	Low protein diet to restrict intake of leucine
3-Methylglutaconic Aciduria	3-Methylglutaconyl-CoA Hydratase	Speech delay; developmental delay usually is mild	Low protein diet to restrict intake of leucine
3-Hydroxy-3-Methyl-Glutaric Aciduria	3-Hydroxy-3-Methyl-Glutaryl-CoA Lyase	Vomiting, lethargy, coma, convulsions, metabolic acidosis. Hypoglycemia without significant ketoaciduria	Low protein diet to restrict intake of leucine Avoidance of fasting to prevent hypoglycemia
2-Methylacetoacetyl-CoA Thiolase Deficiency	2-Methylacetoacetyl-CoA Thiolase	Recurrent acidosis, ketosis, vomiting, often with hypoglycemia. Prompt clinical response to intravenous glucose. Mental retardation not usual	Avoid prolonged fasting. Glucose infusion during acute episode
3-OH-Butyryl-CoA Deacylase Deficiency	3-OH-butyryl-CoA Deacylase	Multiple congenital anomalies, tetralogy of Fallot, facial dysmorphism, brain dysgenesis	None
Propionic Acidemia	Propionyl-CoA Carboxylase	Fulminant syndrome in neonate: coma, convulsions. Hyperglycinemia and hyperammonemia common. Milder, later-onset form: developmental retardation, failure to thrive, recurrent vomiting	Special diet low in isoleucine, valine, methionine, threonine. Carnitine supplementation. Liver transplantation may be beneficial
Methylmalonic Acidemia	Two Forms: 1. Methylmalonyl-CoA Mutase 2. Deficiency of metabolism of vitamin B_{12}, a cofactor for the reaction	Severe form: acidosis, hyperammonemia, hepatomegaly, hyperglycinemia, hypoglycemia, coma, convulsions, growth failure, psychomotor retardation. Basal ganglia damage ("metabolic stroke") is frequent, especially involving globus pallidus Defects of vitamin B_{12} metabolism may show homocystinemia	Special diet low in isoleucine, valine, methionine, threonine. Carnitine supplementation. Liver transplantation may be beneficial. Glucose and bicarbonate during acute episodes
Type I Glutaric Aciduria	Glutaryl-CoA Dehydrogenase	Macrocephaly common. Initially normal. Develop hypotonia, opisthotonus, seizures, rigidity, dystonia, facial grimacing. Atrophy of caudate and putamen	Special diet low in lysine and tryptophan
Type II Glutaric Aciduria	Deficiency of electron transport flavoprotein or of ETF:ubiquinone reductase	Hepatomegaly, hypoglycemia without ketonuria, lipid storage myopathy with hypotonia, metabolic acidosis, rancid urine odor ("sweaty socks"), enlarged and cystic kidneys, hepatic cysts, facial dysmorphism	Typically fatal in infancy. Profound developmental delay in rare surviving infant
Biotinidase Deficiency	Biotinidase	Eczema, seizures, deafness, lactic acidosis, hyptonia, lethargy, ataxia, neuropathy, immune deficiency, optic atrophy, alopecia	Biotin supplementation
Holocarboxylase Synthetase Deficiency	Holocarboxylase Synthetase	Profound acidosis with ketonuria, lactic acidosis, tachypnea, lethargy, hypotonia, seizures, unusual urinary odors. Usually severe neonatal onset	Biotin supplementation

the level observed in heterozygotes (Koch et al., 2003). Some patients reported that neutral amino acid supplementation afforded relief from depression, perhaps reflecting a relative increase in brain levels of dopamine and/or tryptophan (see Catecholamines in Depression, Ch. 60).

Distortion of the plasma aminogram in individuals with an aminoaciduria also may lead to a relative failure of brain protein synthesis. Thus, in mice with a deficiency of phenylalanine hydroxylase, the blood concentration of phenylalanine is more than 20 times greater than the control value, leading to partial saturation of the transport system and a diminution in the brain level of neutral amino acids other than phenylalanine. Rates of protein synthesis were concomitantly reduced (Smith & Kang, 2000).

Treatment of aminoacidurias with a low-protein diet may influence brain chemistry

It should be emphasized that the treatment of the patient with an aminoaciduria may affect brain chemistry, perhaps in an adverse manner. Nearly all patients receive a low-protein diet. Indeed, undiagnosed patients sometimes avoid consumption of protein, which they feel intuitively can cause lethargy, headache, nausea and mental confusion. As dietary protein declines, the intake of carbohydrate frequently increases. The concomitant rise of endogenous insulin secretion favors an increase in the ratio of the concentration of blood tryptophan to that of other amino acids, thereby promoting the entry of tryptophan to the brain. The latter amino acid is precursor to brain serotonin, which tends to increase. This physiology is known to be operative in patients with urea cycle defects.

Imbalances of brain amino acids may hinder the synthesis of brain lipids, leading to a diminution in the rate of myelin formation

Decreases of lipids, proteolipids and cerebrosides (Ch. 5) have been noted in several of these syndromes, e.g. maple syrup urine disease, when intra-myelinic edema is a prominent finding, particularly during the acute phase of metabolic decompensation (Morton et al., 2002). Pathological changes in brain myelin are common, especially in infants who die early in life. The fundamental lesion may involve a failure of myelin protein synthesis as a consequence of the imbalanced brain amino acid content. It also is probable that disturbances of energy metabolism lead to a failure of myelin synthesis during development, when lipid formation is very active (see myelin formation in (Chs. 10, 31). Amino acid metabolites may directly inhibit the synthesis of crucial lipids such as arachidonic acid and docosahexaenoic acid (see detailed discussion of these lipids in Ch. 36) (Infant & Huszagh, 2001). Similarly, excessive levels of phenylalanine appear to inhibit the synthesis of cholesterol (Shefer et al., 2000).

In many aminoacidurias, there may occur deficits in neurotransmitters and receptors, particularly the N-methyl-D-aspartate receptor

Brain damage and dysfunction in the aminoacidurias may reflect injury to neurotransmitter receptors and transporters (Hommes, 1994). Thus, the presence of a glycine-binding site on the NMDA glutamatergic receptor (see Ch. 17) prompted the hypothesis that the severe seizure disorder that typifies the congenital absence of the glycine cleavage system might be responsive to treatment with NMDA receptor antagonists (Deutsch et al., 1998). Excessive stimulation of the NMDA receptor presumably contributes to the intense convulsions (see Ch. 40). Similarly, hyperammonemia appears to impair the high-affinity uptake of glutamate into both astrocytes and neurons. A compensatory decrease of NMDA receptors may then occur as an adaptation to increased external glutamate (Chan et al., 2003).

Brain edema, often associated with increased intracranial pressure, may accompany the acute phase of metabolic decompensation in the aminoacidurias

Brain swelling and increased intracranial pressure may occur during acute illness. As indicated above, intramyelinic edema may occur in maple syrup urine disease. Swelling of brain astrocytes occurs with acute hyperammonemia because water is drawn into glia secondary to accumulation of glutamine, which is preferentially formed in astrocytes from glutamate and ammonia (see Ch. 17). Increased brain blood flow during hyperammonemia accentuates swelling (Willard-Mack et al., 1996).

DISORDERS OF BRANCHED-CHAIN AMINO ACIDS: MAPLE SYRUP URINE DISEASE

Maple syrup urine disease involves a congenital failure to oxidize the three branched-chain amino acids

The first congenital defect of branched-chain amino acid (BCAA) catabolism to be described was maple syrup urine disease (MSUD), a deficiency of mitochondrial branched-chain ketoacid dehydrogenase (Figure 42-1; reaction 2). The ketoacids are freely reaminated to the parent amino acids, the latter being readily measured in the blood and urine. The characteristic odor of the branched-chain ketoacids imparts to the urine a smell that resembles maple syrup or burned sugar.

The decarboxylase is composed of four subunits: E1-a, E1-b, E2 and E3. A specific kinase and phosphatase activate and deactivate the enzyme complex. Most MSUD patients have mutations involving the E1-a subunit that decarboxylates the ketoacid, although defects of the E1-b protein have been described (Chuang et al., 1995). The E1-a mutation usually causes faulty assembly of the heterotetrameric (a_2b_2) E1 protein. Mutations of either the E2 or E3 moieties are extremely rare. The E3 subunit is common to other decarboxylating systems, including pyruvate dehydrogenase and 2-oxo-glutarate dehydrogenase. Hence, mutations in this protein can cause lactic acidosis and deranged tricarboxylic acid cycle activity as well as an accumulation of the BCAAs.

Infants are protected during gestation because the placenta clears most potential toxins. The classical form of the disease

therefore does not become clinically manifest until a few days after birth. An initial phase of alternating irritability and lethargy progresses over a period of days to frank coma and respiratory embarrassment. Irreversible brain damage is common in babies who survive, particularly those whose treatment is delayed until after the first week of life.

Survivors may suffer a metabolic relapse at any time. The most common cause of relapse is intercurrent infection, which favors endogenous protein catabolism. As a consequence, the patient's limited capacity to oxidize BCAAs is overwhelmed and these compounds, together with their cognate ketoacids, accumulate to a toxic level. Relapse also can occur in association with surgery, trauma and emotional upset.

Patients with partial enzymatic deficiencies may present later in life with intermittent ketoacidosis, prostration and recurrent ataxia. The plasma concentrations of BCAA are elevated during these episodes but they may be normal or near normal during the periods when patients are metabolically compensated.

Rare patients respond to the administration of thiamine in large doses (10–30 mg/day). The clinical course is even milder than that of patients with intermittent disease. Thiamine is a cofactor for the branched-chain ketoacid dehydrogenase, and the presumed mutation involves faulty binding of the apoprotein to this vitamin.

In many localities, newborn screening has become standard for this disorder, which in the general population has an approximate incidence of 1/250,000 live births. Carrier detection as well as antenatal diagnosis are possible in most cases with gene sequencing.

Effective treatment of maple syrup urine disease involves the restriction of dietary branched-chain amino acids

Long-term treatment entails the dietary restriction of the BCAAs. This is accomplished by administration of a special formula from which these amino acids are removed. The outlook for intellectual development is favorable in youngsters in whom diagnosis is made early and who do not suffer recurrent, severe episodes of metabolic decompensation (Kaplan et al., 1991).

Gene therapy for this metabolic defect may become available within the next few years. *In vitro* studies have demonstrated the feasibility of retroviral-mediated gene transfer of both the E1-a and E2 subunits of the branched-chain decarboxylase complex (Chuang et al., 1995; Mueller et al., 1995).

DISORDERS OF PHENYLALANINE METABOLISM: PHENYLKETONURIA

Phenylketonuria usually is caused by a congenital deficiency of phenylalanine hydroxylase

Phenylketonuria (PKU) is among the more common aminoacidurias (≈1:20,000 live births). The usual cause is a nearly complete deficiency of phenylalanine hydroxylase, which converts phenylalanine into tyrosine (Fig. 42-2; reaction 1). In addition to 'classical' PKU, many youngsters have hyperphenylalaninemia caused by a partial deficiency of the enzyme. They do not suffer mental retardation, but they may have more subtle neurological problems (Cedarbaum, 2002; White et al., 2001).

The hydroxylase is a trimer (mol. wt ≈150,000) made up of identical subunits. It is located predominantly in the liver. The enzyme has been mapped to human chromosome 12q22–24.1, where the gene is made up of 13 exons extending over 90≈kb of genomic DNA.

Frank deletions of the gene are not common. A frequent mutation among northern Europeans (≈40%) is a G to A transition at the 5′ donor splice site in intron 12, resulting in absence of the C terminus. Another relatively common (≈20%) mutation in northern Europeans involves a C to T transition in exon 12, resulting in substitution of a tryptophan for an arginine residue (Eisensmith & Woo, 1991). Over

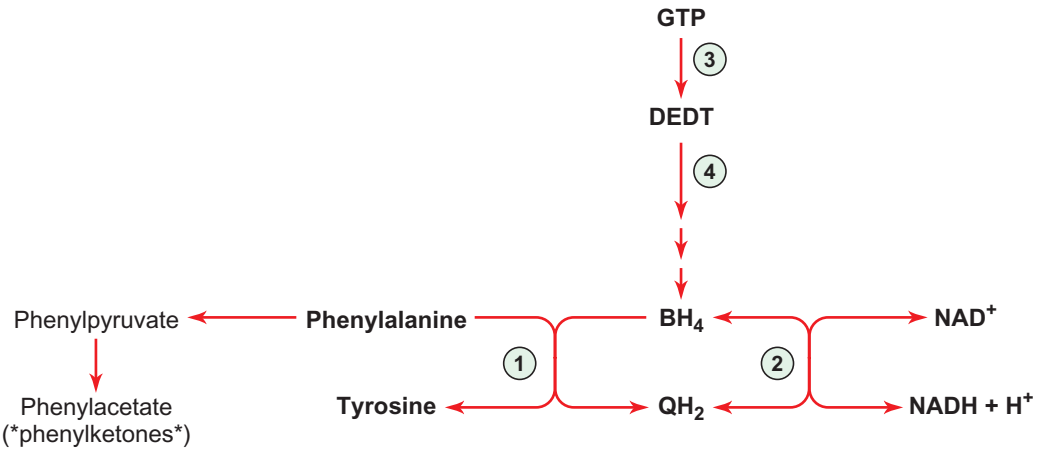

FIGURE 42-2 **The phenylalanine hydroxylase (PAH) pathway**. Phenylketonuria usually is caused by a congenital deficiency of PAH (reaction 1), but it also can result from defects in the metabolism of biopterin, which is a cofactor for the hydroxylase. *Enzymes*: *1*, phenylalanine hydroxylase; *2*, dihydropteridine reductase; *3*, GTP cyclohydrolase; *4*, 6-pyruvoyltetrahydrobiopterin synthase. QH_2, dihydrobiopterin; BH_4, tetrahydrobiopterin; *DEDT*, d-*erythro*-dihydroneopterin triphosphate.

70 different mutations have been described to date in the American population (Guldberg et al., 1996).

Specific mutations have been associated with specific haplotypes, the latter determined by analysis of restriction fragment length polymorphisms. This approach has been utilized for prenatal diagnosis. The study of haplotypes also has revealed that the majority (≈75%) of northern European patients are compound PKU heterozygotes.

The outlook for patients who are treated at an early age is favorable

Affected babies are normal at birth but almost all will be impaired unless they receive dietary restriction by age 3 months. Mass screening has largely eliminated the untreated PKU phenotype of eczema, poor growth, irritability, musty odor (caused by phenylacetic acid) and tendency to self-mutilation. Progressive motor dysfunction has been described in children with long-term hyperphenylalaninemia.

The clinical utility of dietary restriction of phenylalanine (200–500 mg/day of phenylalanine) is clear. Well-controlled patients have normal intelligence, although there may be an increased risk of perceptual learning disabilities, emotional problems and subtle motor difficulties (Diamond & Herzberg, 1996). Diet therapy must probably be maintained throughout adolescence and perhaps indefinitely. Performance may deteriorate after the diet is discontinued.

The genotypically normal offspring of an untreated mother may have microcephaly and irreversible brain injury as well as cardiac defects. Scrupulous monitoring of dietary phenylalanine intake in these women has improved outcome (Levy & Ghavami, 1996).

Rarely, phenylketonuria results from a defect in the metabolism of biopterin, a cofactor for the phenylalanine hydroxylase pathway

The electron donor for phenylalanine hydroxylase is tetrahydrobiopterin (BH4), which transfers electrons to molecular oxygen to form tyrosine and dihydrobiopterine (QH2; Fig. 42-2; reaction 2). BH_4 is regenerated from QH_2 in an NADH-dependent reaction that is catalyzed by dihydropteridine reductase (DHPR), which is widely distributed. In the brain, this enzyme and BH4 also are involved in hydroxylation of tyrosine and tryptophan (Chs 14 and 15). Human DHPR has been mapped to human chromosome 4p15.1–p16.1. The coding sequence shows little homology to other reductases, e.g., dihydrofolate reductase.

In rare instances, PKU is caused by defects in the metabolism of BH4, which is synthesized from GTP via sepiapterin (Fig. 42-2; reactions 3 and 4) (Kaufman, 1983). Even careful phenylalanine restriction fails to avert progressive neurological deterioration because patients are unable to hydroxylate tyrosine or tryptophan, the synthesis of which also requires tetrahydrobiopterin. Thus, neurotransmitters are not produced in sufficient amounts.

Patients sustain convulsions and neurological deterioration. The urine contains low levels of the metabolites of serotonin, norepinephrine and dopamine. The reductase also plays a role in the maintenance of tetrahydrofolate levels in brain, and some patients have had low folate levels in the serum and CNS. Treatment has been attempted with tryptophan and carbidopa to improve serotonin homeostasis and with folinic acid to replenish diminished stores of reduced folic acid. This therapy is sometimes effective. Diagnosis involves assay of DHPR in skin fibroblasts or amniotic cells. Phenylalanine hydroxylase activity is normal.

Other causes of PKU secondary to defective tetrahydrobiopterin synthesis include GTP cyclohydrolase deficiency and 6-pyruvoyltetrahydrobiopterin synthase deficiency. Patients with either defect have psychomotor retardation, truncal hypotonia with limb hypertonia, seizures and a tendency to hyperthermia. The intravenous administration of BH4 may lower blood phenylalanine levels but this cofactor may not readily cross the blood–brain barrier. Treatment with synthetic pterin analogs or supplementation with tryptophan and carbidopa may prove more efficacious, particularly if treatment is started early in life.

DISORDERS OF GLYCINE METABOLISM: NONKETOTIC HYPERGLYCINEMIA

Nonketotic hyperglycinemia results from the congenital absence of the glycine cleavage system, which mediates the interconversion of glycine and serine

Glycine catabolism proceeds primarily via the glycine cleavage system, a mitochondrial system that interconverts glycine and serine (Fig. 42-3; reaction 1). Pyridoxal phosphate and tetrahydrofolate are cofactors. This reaction also provides precursor to the "one-carbon pool" of folic acid intermediates that are pivotal to many synthetic reactions (Kikuchi, 1973).

Nonketotic hyperglycinemia causes a severe seizure disorder and profound brain damage

Infants affected by deficiency of the glycine cleavage system become ill with seizures by the first or second day of life. Intrauterine seizures may occur. The electroencephalogram often displays a hypsarrhythmia or a burst-suppression pattern. Patients display myoclonic jerks, hiccupping and a profound hypotonia. The few patients who survive past the first week usually sustain profound mental retardation and neurological disability. Brain imaging shows atrophy and a loss of myelin. Rarely, patients present later in life with psychomotor retardation and growth failure. Others have had initial normal development followed by a progressive loss of developmental milestones. Some patients have manifested spinocerebellar degeneration and other symptoms of motor dysfunction (Steiner et al., 1996).

In nonketotic hyperglycinemia, glycine is extremely high in the blood, often rising to >1 mmol/l (normal = 150–350 mmol/l). The concentration in the cerebrospinal fluid almost always exceeds 100 μmol/l (normal≈10 μmol/l). The cerebrospinal fluid

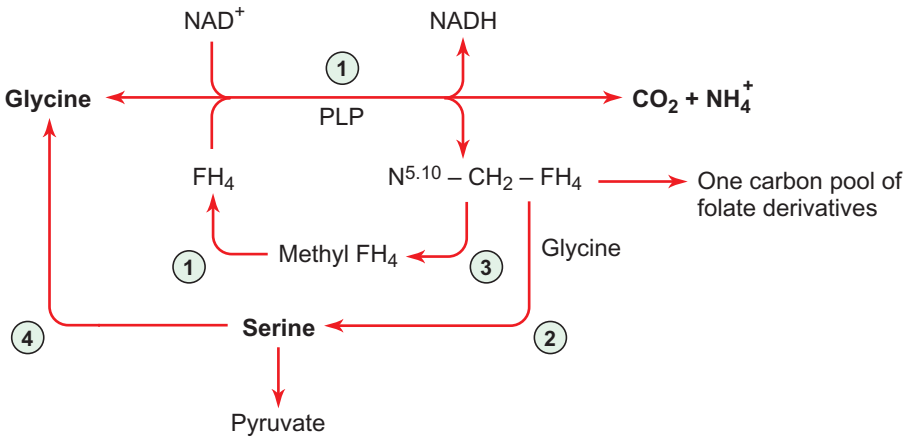

FIGURE 42-3 **Glycine cleavage system and some related reactions**. Glycine and serine are readily interchangeable. *Enzymes*: *1*, glycine-cleavage system; *2*, and *4*, serine hydroxymethyltransferase; *3*, $N^{5,10}$-methylenetetrahydrolate reductase. $N^{5,10}CH_2$-FH_4, $N^{5,10}$-methylene-tetrahydrolate; FH_4, tetrahydrofolic acid; *PLP*, pyridoxal phosphate.

(CSF):blood ratio of glycine usually is 5 to 10 times the control value (0.02), especially with the classical form of the disease. A transient form of nonketotic hyperglycinemia, probably reflecting delayed maturation of the glycine cleavage system, has been described in neonates with seizures but an otherwise normal neurological examination. The seizures ceased by 8 weeks of age and did not recur. Glycine concentrations of both the blood and the CSF were high. Urine organic acid analysis was normal. Prognosis is favorable in this situation.

Treatment for nonketotic hyperglycinemia is less effective than that available for other aminoacidurias

There is no specific therapy. Exchange transfusion and dialysis usually do not alter the progressive neurological deterioration. Sodium benzoate has been administered in the hope that glycine would react with it to form hippuric acid, but this approach is not helpful. It may be that a combination of benzoate and carnitine therapy is more effective (Van Hove et al., 1995). Similarly, the restriction of dietary protein and the administration of pyridoxine or choline have not proved useful.

Glycine is a neurotransmitter; it has a postsynaptic inhibitory activity in the spinal cord and in some central neurons (Box 12). Therapy with strychnine, which blocks the action of glycine at postsynaptic inhibitory receptors, has been unhelpful. Treatment with diazepam has been attempted because this drug displaces strychnine from its binding sites. The combination of benzoate and diazepam may be more effective, since high doses of the former reduce glycine levels in the CNS, thereby potentiating the ability of strychnine to block the glycine effect.

A few infants have been treated with antagonists of the NMDA receptor, an excitatory glutamatergic receptor for which glycine is a co-agonist (see Ch. 17) (Alemzadeh et al., 1996). Ketamine and dextromethorphan have been used with inconclusive results. Some infants may have had an improvement of their irritability and electroencephalogram. One infant, treated with both benzoate and dextromethorphan, was seizure-free by 12 months of age and had only moderately delayed development. However, this favorable experience has not always been duplicated. Treatment with dextromethorphan at the recommended dosage (maximum 5 mg/kg/day) seems to be well tolerated.

DISORDERS OF SULFUR AMINO ACID METABOLISM: HOMOCYSTINURIA

The transsulfuration pathway is the major route for the metabolism of the sulfur-containing amino acids

The transsulfuration pathway (Fig. 42-4) entails the transfer of the sulfur atom of methionine to serine to yield cysteine. The first step is activation of methionine, which reacts with ATP to form *S*-adenosylmethionine (Fig. 42-4; reaction 1). This compound is a key methyl donor and plays a prominent role in the synthesis of several neurotransmitters and of creatine (Fig. 42-4; reaction 2). A portion of the carbon of spermidine and spermine is formed by decarboxylation of *S*-adenosylmethionine.

Transfer of a methyl group from *S*-adenosylmethionine yields *S*-adenosylhomocysteine, which potently inhibits several methyltransferases; this may partially explain the pathology of homocystinuria. Tissue levels of *S*-adenosylhomocysteine ordinarily are very low, since this metabolite is rapidly cleaved by a specific hydrolase to homocysteine and adenosine (Fig. 42-4; reaction 3).

About half of the homocysteine so generated is remethylated to methionine, with either betaine or 5-methyltetrahydrofolic acid (methyl-FH_4) serving as methyl donor. The enzyme mediating remethylation, 5-methyltetrahydrofolate-betaine methyltransferase (Fig. 42-4; reaction 4), utilizes methylcobalamin as a cofactor. The kinetics of the reaction favor remethylation. Faulty remethylation can occur secondary to

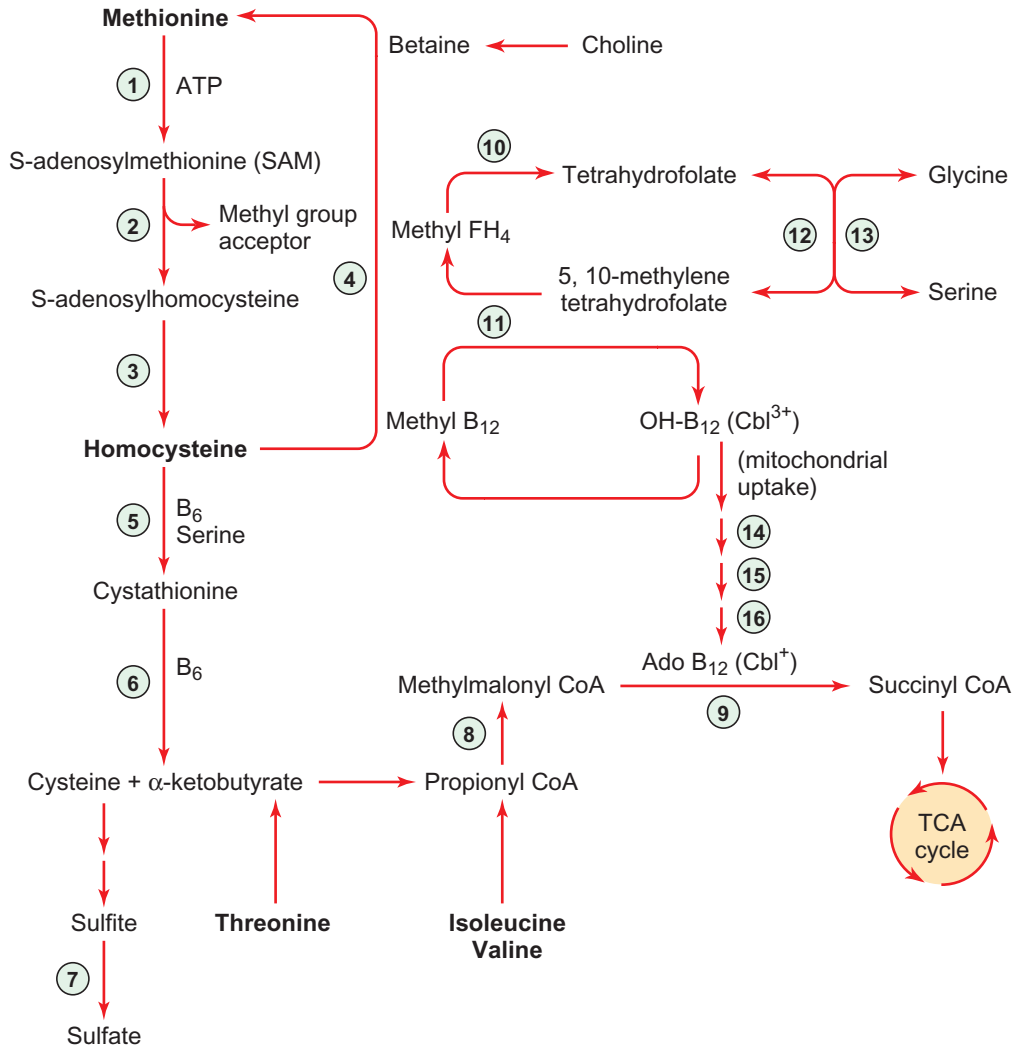

FIGURE 42-4 **The trans-sulfuration pathway and related metabolic routes.** Homocystinuria usually is caused by a congenital deficiency of cystathionine β-synthase (reaction 5). Sometimes homocystinuria is caused by a failure of the remethylation of homocysteine. This may occur because of a failure to generate methylfolate or methylcobalamin. If there is a generalized failure of cobalamin activation or absorption, methylmalonic aciduria as well as homocystinuria may result because cobalamin derivatives are essential to both pathways. *Enzymes*: *1*, methionine-activating enzyme; *2*, generic depiction of methyl group transfer from *S*-adenosylmethionine; *3*, *S*-adenosylhomocysteine hydrolase; *4*, homocysteine:methionine methyltransferase; *5*, cystathionine β-synthase; *6*, cystathionase; *7*, sulfite oxidase; *8*, propionyl-CoA carboxylase; *9*, methylmalonyl-CoA mutase; *10*, homocysteine:methionine methyltransferase, which is essentially the same as reaction 4, in which methyltetrahydrofolate (FH_4) is the methyl donor; *11*, $N^{5,10}$-methylenetetrahydrofolate reductase; *12* and *13*, glycine-cleavage system; *14* and *15*, hydroxycobalamin reductases; *16*, cobalamin adenosyltransferase. *OH-B_{12}*, hydroxocobalamin; *Ado B_{12}*, adenosylcobalamin; *Methyl-B_{12}*, methylcobalamin; *TCA*, tricarboxylic acid.

(1) dietary factors, e.g., vitamin B_{12} deficiency; (2) a congenital absence of the apoenzyme; (3) a congenital inability to convert folate or B_{12} to the methylated, metabolically active form (see below); or (4) the presence of a metabolic inhibitor, e.g., an antifolate agent that is used in an antineoplastic regimen.

The most common inherited cause of homocystinuria is a congenital deficiency of cystathionine-β-synthase, a pyridoxine-dependent enzyme that condenses homocysteine and serine to form cystathionine (Fig. 42-4; reaction 5). S-adenosylmethionine stimulates the forward reaction (Kluijtmans et al., 1996). This enzyme has been mapped to human chromosome 21. The equilibrium favors cystathionine synthesis. Thus, homocysteine levels normally are very low, since both the remethylation pathway and the cystathionine synthetase route efficiently dispose of this amino acid.

Cleavage of cystathionine is accomplished by cystathionase, another pyridoxine-dependent enzyme that is coded on human chromosome 16 (Fig. 42-4; reaction 6). The enzyme functions almost entirely to produce cysteine, there being virtually no reversal of the reaction.

Homocystinuria is the result of the congenital absence of cystathionine synthase, a key enzyme of the transsulfuration pathway

A variety of mutations have been described, including the synthesis of an unstable enzyme; a protein that loosely binds either pyridoxal phosphate, serine or homocysteine; or an enzyme differing in size from the wild strain (Kraus, 1994). Cystathionine synthase is present in many organs, including the brain, and homocystinuric patients typically manifest deficient enzyme activity in these tissues. Blood homocysteine levels are elevated (50–200 μmol/l; normal <10 μmol/l) and the blood cysteine concentration tends to be low, reflecting the failure of cysteine synthesis. Increased remethylation of homocysteine that is not converted to cystathionine results in elevated blood methionine, often in excess of 200 μmol/l (normal = 20–40 μmol/l).

Homocystinuria can be treated in some cases by the administration of pyridoxine (Vitamin B_6), which is a cofactor for the cystathionine synthase reaction

Some patients respond to the administration of pharmacological doses of pyridoxine (25–100 mg daily) with a reduction of plasma homocysteine and methionine. Pyridoxine responsiveness appears to be hereditary, with sibs tending to show a concordant pattern and a milder clinical syndrome. Pyridoxine sensitivity can be documented by enzyme assay in skin fibroblasts. The precise biochemical mechanism of the pyridoxine effect is not well understood but it may not reflect a mutation resulting in diminished affinity of the enzyme for cofactor, because even high concentrations of pyridoxal phosphate do not restore mutant enzyme activity to a control level.

About half of individuals who do not respond to pyridoxine will sustain *ectopia lentis* by age 5–10 years. Indeed, the diagnosis commonly is made by an ophthalmologist. The median IQ scores for B_6 -responsive and nonresponsive patients are 78 and 56, respectively. Some children present at 1–2 years with psychomotor retardation, convulsions (≈20% of cases) and psychiatric difficulties such as depression and personality disorders (≈50% of cases).

Patients with homocystinuria are at risk for cerebrovascular and cardiovascular disease and thromboses

The most striking feature is a thromboembolic diathesis that can occur in virtually any vessel, with thrombi common in the cerebral, cardiac and renal vasculature. Almost 25% of pyridoxine nonresponders sustain a major vascular insult during childhood. The comparable risk in untreated pyridoxine-responsive subjects is 25% by age 20 years. Vascular insults sometimes occur in association with dehydration secondary to vomiting and diarrhea. The stress of major surgery and anesthesia increases the risk of thrombosis by ≈5%. Homocystinuric patients who also have the relatively common Leiden mutation of clotting factor V are at sharply increased risk for developing a thrombosis (Mandel et al., 1996).

Patients commonly manifest a marfanoid habitus with arachnodactyly, high-arched palate, tall stature and pes cavus. Bony abnormalities are common, with osteoporosis and scoliosis being frequent findings. The orthopedic features are more common and severe in patients who do not respond to pyridoxine treatment. Demyelination and spongy degeneration of the white matter have been reported. Frank infarctions are relatively common in virtually all parts of the brain. The arterial wall shows thickening of the intima and splitting of the smooth musculature of the media. The changes are not dissimilar to those of atherosclerosis.

The probable cause of the pathology is hyperhomocysteinemia rather than hypermethioninemia. Homocysteine increases the adhesiveness of platelets *in vitro*, perhaps by favoring the synthesis of selected thromboxanes. The administration of homocysteine to rats or baboons can cause endothelial injury. Homocysteine may diminish the mean survival time of peripheral blood platelets, possibly by a direct toxic effect on the vascular endothelium, which becomes denuded and thereby provides an atherogenic nidus. A direct effect of homocysteine on the blood-clotting cascade also is possible. Thus, activation of factor V in cultured endothelial cells has been noted. This favors the conversion of prothrombin to thrombin. Homocysteine also promotes accumulation of copper in the vascular endothelium. This induces the oxidation of ceruloplasmin and the concomitant release of sufficient H_2O_2 to injure endothelial cells. Supplementation of the medium with catalase protects against such insult, thus confirming the role of oxidant injury.

High levels of homocysteine or one of its metabolites may directly affect brain function. The administration of homocysteine to rats induces grand mal convulsions, a phenomenon that is aggravated by either methionine or pyridoxine. Homocysteine-induced blockade of the γ-aminobutyric acid (GABA) receptor may be involved. In addition, brain can oxidize homocysteine to homocysteic acid, which has a glutamatergic activity.

A high intracerebral level of *S*-adenosylhomocysteine may inhibit methylation reactions involving *S*-adenosylmethionine. The metabolic repercussions would be extensive, including deficient methylation of proteins and of phosphatidylethanolamine as well as an inhibition of flux through catechol-*O*-methyltransferase and histamine-*N*-methyltransferase.

Prognosis is more favorable in the pyridoxine-responsive patients

Patients who respond to large doses of vitamin B_6 (250–500 mg/day for several weeks) have the best prognosis. Efficacy of treatment usually is reflected in a reduction of blood homocysteine and methionine to normal or near-normal levels. Since supplementation with pyridoxine can cause a deficiency of folic acid, the latter should be given (2–5 mg daily) at the same time. Any patient receiving pyridoxine should be monitored carefully for any signs of hepatotoxicity and for peripheral neuropathy (see Ch. 38).

Management of the pyridoxine-nonresponsive patient is difficult. Dietary restriction of methionine would seem logical,

but this often is unpalatable, especially to an adult patient who has adapted to a diet that has not been purposefully restricted in protein.

A valuable therapeutic adjunct is the administration of betaine (6–12 g daily), which lowers homocysteine levels by favoring remethylation (Dudman et al., 1996). A theoretical hazard of betaine treatment is increasing the blood methionine, sometimes to an extravagant degree (≈1 mmol/l). Experience to date indicates that betaine administration is safe, with no major side effects except for a "fishy" odor to the urine. Other therapeutic approaches have included the administration of salicylate to ameliorate the thromboembolic diathesis. Patients also have been treated with dietary supplements of L-cystine, since the block of the transsulfuration pathway could, in theory, diminish the synthesis of this amino acid.

Homocystinuria can occur when homocysteine is not remethylated back to form methionine

This situation is termed "remethylation deficiency homocystinuria". In the form of *S*-adenosylmethionine, methionine is the major methyl donor for biosynthetic reactions in the brain. Most remethylation defects result from aberrant metabolism of methylfolate or methylcobalamin. Patients often present early in life with lethargy, poor feeding, psychomotor retardation and growth failure. Hematological abnormalities are common, including megaloblastosis, macrocytosis, thrombocytopenia and hypersegmentation of the leukocytes. Occasional patients present later, when seizures, dementia, hypotonia, mental retardation, spasticity or a myelopathy become evident.

Biochemical findings are variable. The blood cobalamin and folate levels often are normal. Patients often have homocysteinemia with hypomethioninemia; the latter finding discriminates this group from homocystinuria secondary to cystathionine-β-synthase deficiency. Urinary excretion of methylmalonic acid may be high, reflecting the fact that vitamin B_{12} serves as a cofactor for the methyl-malonyl-CoA (coenzyme A) mutase reaction.

One form of remethylation deficit involves defective metabolism of folic acid, a key cofactor in the conversion of homocysteine to methionine

Methylenetetrahydrofolate reductase (Fig. 42-4; reaction 11) reduces $N^{5,10}$-methylenetetrahydrofolate to methyltetrahydrofolate in a reaction that is NADPH dependent and inhibited by *S*-adenosylmethionine. In brain this enzyme also is important for the reduction of dihydropteridines (see Disorders of Phenylalanine Metabolism, above).

Patients typically present by 6–12 months with severe developmental retardation, convulsions, microcephaly and homocysteinemia (≈50 μmol/l) with hypomethioninemia (<20 μmol/l). A few individuals have had psychiatric disturbances. The blood concentration of vitamin B_{12} is normal, and, unlike individuals with defects of cobalamin metabolism, these patients manifest neither anemia nor methylmalonic aciduria. The blood folic acid level is usually low.

A thromboembolic diathesis is not unusual and strokes have been reported. Other pathological changes have included microgyri, demyelination, gliosis and brain atrophy. Lipid-laden macrophages have been described.

A relatively large number of agents have been utilized to treat this intractable disorder: folinic acid (5-formyl-tetrahydrofolic acid), folic acid, methyltetrahydrofolic acid, betaine, methionine, pyridoxine, cobalamin and carnitine. Betaine, which provides methyl groups to the betaine:homocysteine methyltransferase reaction, is a safe treatment that lowers blood homocysteine and increases methionine.

Methionine synthase deficiency (cobalamin-E disease) produces homocystinuria without methylmalonic aciduria

This enzyme mediates the transfer of a methyl group from methyltetrahydrofolate to homocysteine to yield methionine (Fig. 42-4; reaction 4). A cobalamin group bound to the enzyme is converted to methyl-cobalamin prior to formation of methionine.

In cobalamin-E (cblE) disease there is a failure of methyl-B_{12} to bind to methionine synthase. It is not known if this reflects a primary defect of methionine synthase or the absence of a separate enzyme activity. Patients manifest megaloblastic changes with pancytopenia, homocystinuria and hypomethioninemia. There is no methylmalonic aciduria. Patients usually become clinically manifest during infancy with vomiting, developmental retardation and lethargy. They respond well to injections of hydroxocobalamin.

Cobalamin-c disease: remethylation of homocysteine to methionine also requires an 'activated' form of vitamin B_{12}

In the absence of normal B_{12} activation, homocystinuria results from a failure of normal vitamin B_{12} metabolism. Complementation analysis classifies defects in vitamin B_{12} metabolism into three groups: cblC (most common), cblD and cblF. Most individuals become ill in the first few months or weeks of life with hypotonia, lethargy and growth failure. Optic atrophy and retinal changes can occur. Methylmalonate excretion is excessive, but less than in methylmalonyl-CoA mutase deficiency, and without ketoaciduria or metabolic acidosis.

The fibroblasts do not convert cyanocobalamin or hydroxocobalamin to methylcobalamin or adenosyl-cobalamin, resulting in diminished activity of both N^5-methyltetrahydrofolate:homocysteine methyltransferase and methylmalonyl-CoA mutase. Supplementation with hydroxocobalamin rectifies the aberrant biochemistry. The underlying defect involves faulty intracellular processing of cobalamin because of a mutation in the MMACHC gene. Diagnosis should be suspected in a child with homocystinuria, methylmalonic aciduria, megaloblastic anemia, hypomethioninemia and normal blood levels of folate and vitamin B_{12}. A definitive diagnosis requires demonstration of these abnormalities in fibroblasts. Prenatal diagnosis is possible.

Treatment involves the administration of large doses (as much as 1 mg) of intramuscular hydroxocobalamin.

Administration of folate and betaine (see above) may be helpful, as well as a reduction of protein intake.

Hereditary folate malabsorption presents with megaloblastic anemia, seizures and neurological deterioration

Levels of folate in both the blood and the cerebrospinal fluid have been very low. The anemia is correctable with injections of folate, or with the administration of large oral doses, but the concentration in the CSF is still low, suggesting that a distinct carrier system mediates folate uptake into the brain and that this system is the same as that facilitating intestinal transport.

THE UREA CYCLE DEFECTS

The urea cycle is essential for the detoxification of ammonia

The urea cycle (Fig. 42-5) converts ammonia to urea (10–20 g N/day in the healthy adult). A urea cycle enzymopathy, whether associated with cirrhosis or an inherited metabolic defect, often causes a hyperammonemic encephalopathy and irreversible brain injury.

The cycle "begins" in hepatic mitochondria, where NH_3^-, HCO_3^- and ATP form carbamyl phosphate in a reaction catalyzed by carbamyl phosphate synthetase (CPS; Fig. 42-5; reaction 1). *N*-acetylglutamate (NAG), formed from glutamate and acetyl-CoA via *N*-acetylglutamate synthetase (Fig. 42-5; reaction 9), is an obligatory effector of CPS and an important regulator of ureagenesis. Various influences, including dietary protein, arginine and corticosteroids, augment the concentration of NAG.

Carbamyl phosphate condenses with ornithine to yield citrulline in the ornithine transcarbamylase (OTC) reaction. OTC is encoded on band p21.1 of the X chromosome, where the gene contains eight exons and spans 85 KB of DNA. The activity of this enzyme is directly related to dietary protein. There may be "tunneling" of ornithine transported from the cytosol to OTC, with the availability of intramitochondrial ornithine serving to regulate the reaction.

In the hepatic cytosol, citrulline reacts with aspartate to form argininosuccinate, catalyzed by argininosuccinate synthetase (AS) (Fig. 42-5; reaction 3). The mRNA for this enzyme is increased by starvation, corticosteroids or cyclic-AMP. Citrulline itself potently induces the mRNA.

Argininosuccinate lyase (AL) (Fig. 42-5; reaction 4) cleaves argininosuccinate to form fumarate, which is oxidized in the tricarboxylic acid cycle, and arginine, which is hydrolyzed to urea and ornithine via hepatic arginase. Both AL and

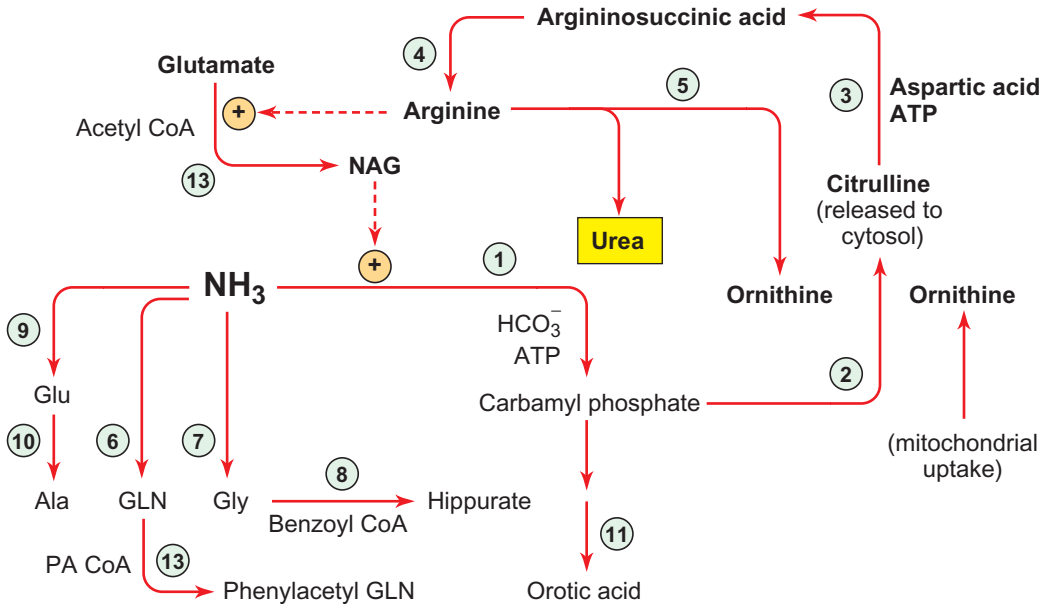

FIGURE 42-5 **The urea cycle and related reactions of ammonia metabolism.** Congenital hyperammonemia syndromes usually are caused by a deficiency of one of the enzymes of the urea cycle. Ammonia also can be metabolized to glutamate, alanine, glutamine and glycine. Administration of phenylacetate or of benzoate favors the formation of phenylacetylglutamine and hippurate, respectively, thereby providing an effective "antidote" to ammonia toxicity. *Enzymes*: *1*, carbamyl phosphate synthetase; *2*, ornithine transcarbamylase; *3*, argininosuccinate synthetase; *4*, argininosuccinate lyase; *5*, arginase; *6*, glutamine synthetase; *7*, glycine-cleavage system; *8*, glycine-*N*-acylase; *9*, glutamate dehydrogenase; *10*, alanine aminotransferase; *11*, cytosolic pathway of orotic acid synthesis, which becomes prominent when there is a block at the level of reaction 2, thus resulting in increased orotic acid excretion; *12*, *N*-acetylglutamate synthetase; *13*, phenylacetyl-CoA:glutamine transferase. *NAG*, *N*-acetylglutamate; *PA-CoA*, phenylacetyl-CoA; *GLN*, glutamine. The + symbols denote that arginine and NAG are positive effectors for reactions 12 and 1, respectively.

arginase are induced by starvation, dibutyryl cyclic-AMP and corticosteroids.

Urea cycle defects cause a variety of clinical syndromes, including a metabolic crisis in the newborn infant

Severe urea cycle defects become manifest in infants with a syndrome of coma, convulsions and vomiting during the first few days of life. Clinical confusion with septicemia is common, and many infants are treated futilely with antibiotics. Hyperammonemia is usually severe, even in excess of 1 mmol/l (normal in term infants $<100\,\mu$mol/l).

Diagnosis usually is made from the blood aminogram. The plasma concentrations of glutamine and alanine, the major nitrogen-carrying amino acids, are typically high and that of arginine is low. Patients with citrullinemia (deficiency of AS) or argininosuccinic aciduria (deficiency of AL) will manifest marked increases of the blood citrulline and argininosuccinate, respectively.

Urinary orotic acid generally is very elevated in babies with OTC deficiency and normal or even low in the infant with CPS deficiency. Patients with OTC deficiency have orotic aciduria because carbamyl phosphate spills into the cytoplasm, where it enters the pathway of pyrimidine synthesis.

Diagnosis of CPS or OTC deficiency may not be apparent from the blood aminogram. Ornithine levels typically are normal. The presence of hyperammonemia, hyperglutaminemia, hyperalaninemia and orotic aciduria in a critically ill infant affords presumptive evidence for OTC deficiency. The presence of this blood aminogram without orotic aciduria suggests carbamyl phosphate synthetase deficiency.

Diagnosis of a urea cycle defect in the older child can be elusive. Patients may present with psychomotor retardation, growth failure, vomiting, behavioral abnormalities, perceptual difficulties, recurrent cerebellar ataxia and headache. It is therefore essential to monitor the blood ammonia in any patient with unexplained neurological symptoms, but hyperammonemia is inconstant with partial enzymatic defects. Measurement of blood amino acids and urinary orotic acid is indicated. Molecular diagnosis by gene sequencing now is commercially available for virtually all urea cycle defects.

Hyperammonemia also occurs in some organic acidurias, particularly those that affect neonates. Therefore, the urine organic acids should be quantitated in all patients with significant hyperammonemia.

Except for patients with argininosuccinic aciduria, who may demonstrate varying degrees of hepatic fibrosis, there is little pathological change outside of the central nervous system.

Carbamyl phosphate synthetase deficiency

Carbamyl phosphate synthetase deficiency is rare. Neonates quickly develop lethargy, hypothermia, vomiting and irritability. The hyperammonemia typically is severe, even exceeding 1 mmol/l. Occasional patients with a partial enzyme deficiency have had a relapsing syndrome of lethargy and irritability upon exposure to protein. Brain damage can occur in both neonatal and late-onset groups.

N-Acetylglutamate synthetase deficiency

A deficiency of CPS also can arise because of the congenital absence of NAG synthetase, which catalyzes the formation of NAG from glutamate and acetyl-CoA. NAG is an obligatory effector of CPS. The few patients reported have had a malignant course of neonatal onset.

Ornithine transcarbamylase deficiency

This is the most common of the urea cycle defects. Presentation is variable, ranging from a fulminant, fatal disorder of neonates to a schizophrenic-like illness in an otherwise healthy adult. Males characteristically fare more poorly than do females with this X-linked disorder because of random inactivation (lyonization) of the X chromosome. If inactivation affects primarily the X chromosome bearing the mutant OTC gene, then a more favorable outcome can be anticipated. Conversely, the "unfavorably lyonized" female has a more active disease.

Gene sequencing is important to diagnosis. More than 80% of carriers can be detected, and antenatal diagnosis often is possible. Approximately one-third of the mothers of males and two-thirds of the mothers of females have been found to be noncarriers, reflecting the greater propensity for mutation in the male gamete.

Diagnosis of carriers (85–90%) can be made with measurement of urinary orotic acid following protein loading or administration of allopurinol. Loading studies with $^{15}NH_4Cl$ as metabolic tracer indicate that symptomatic female carriers for OTC produce less [^{15}N]-urea than a control population. Asymptomatic heterozygotes form urea at a normal rate but overproduce [5-^{15}N]-glutamine. Thus, whole body nitrogen metabolism is abnormal even in this group (Yudkoff et al., 1996).

Animal models for OTC deficiency include the "sparse fur" (*spf*) mouse (15% of control enzyme activity) and the "sparse fur–abnormal skin and hair" (*spf-ash*) mouse (5% of control). Both kinds of mutant mouse manifest hyperammonemia, orotic aciduria, growth failure and sparse fur.

OTC deficiency must be suspected in any patient, male or female, with unexplained neurological symptoms. The absence of hyperammonemia should not rule out the diagnosis, especially with a history of protein intolerance, a suggestive family history or an untoward reaction to infections. The blood amino acids and urinary orotic acid should be quantified in such individuals.

Citrullinemia

Neonates with AS deficiency may die, and most survivors suffer major brain injury. Patients with a partial deficiency may have a milder course, and a few individuals with citrullinemia have been phenotypically normal. The diagnosis usually is apparent from the hyperammonemia and the extreme hypercitrullinemia. The gene can be sequenced in amniocytes or chorionic villus samples, thus simplifying the problem of antenatal diagnosis.

Argininosuccinic aciduria

Patients manifest high levels of argininosuccinate in urine, blood and cerebrospinal fluid. Neonates have a stormy clinical course. Almost all have died or sustained severe brain injury. A peculiar finding is *trichorrhexis nodosa*, or dry brittle hair with nodular protrusions that are best visible with light microscopy. The precise cause is unknown.

Arginase deficiency

Most patients are thought to have psychomotor retardation during the first year of life, but the dominant presentation is a leukodystrophy with progressive spastic tetraplegia, especially in the lower extremities. Seizures and growth failure may occur, although some patients are of normal size. The motor dysfunction usually comes to clinical attention by age 2–3 years. Hyperammonemia is less severe than in neonatal-onset disorders. Plasma arginine is usually two to five times normal. Urine orotate is high, perhaps because arginine stimulates flux through the CPS reaction by favoring the synthesis of *N*-acetylglutamate.

Urea cycle defects sometimes result from the congenital absence of a transporter for an enzyme or amino acid involved in the urea cycle

Hyperornithinemia, hyperammonemia, homocitrullinuria

Affected neonates commonly suffer growth failure and varying degrees of mental retardation. Sometimes symptoms are deferred until adulthood. Vomiting, lethargy and hypotonia are noted after protein ingestion. Recurrent hospitalizations for hyperammonemia are the rule. Some patients have manifested a bleeding diathesis and hepatomegaly. Electron microscopy of the liver has shown irregularities of mitochondrial shape.

The underlying biochemical defect is a failure of mitochondrial uptake of ornithine. This results in a failure of citrulline synthesis and a consequent hyperammonemia. Urinary orotic acid is high, presumably because of underutilization of carbamyl phosphate. In contrast, excretion of creatine is low, reflecting the inhibition of glycine transamidinase by excessive levels of ornithine.

Lysinuric protein intolerance

Infants manifest growth failure, hepatosplenomegaly, vomiting, hypotonia, recurrent lethargy, coma, abdominal pain and, in rare instances, psychosis. Rarefaction of the bones is common, and both fractures and vertebral compression have been reported. Most patients are not mentally retarded. Patients have died with interstitial pneumonia, which may respond to corticosteroid therapy.

The cause is defective transport of dibasic amino acids by the proximal tubule and intestine. The transport defect occurs at the basolateral rather than the luminal membrane. Hyperammonemia reflects a deficiency of intra-mitochondrial ornithine. An effective treatment is oral citrulline supplementation, which corrects the hyperammonemia by allowing replenishment of the mitochondrial pool of ornithine.

Successful management of urea cycle defects involves a low-protein diet to minimize ammonia production as well as medications that enable the excretion of ammonia nitrogen in forms other than urea

Protein restriction is the mainstay of therapy. In patients with severe disease, tolerance for dietary protein may be so limited that it is not possible to support growth.

Treatment with sodium benzoate and sodium phenylacetate represents an important therapeutic advance. Benzoyl-CoA reacts in the liver with glycine to form hippurate and phenylacetyl-CoA reacts with glutamine to yield phenylacetylglutamine, thereby allowing waste nitrogen elimination not as urea but as conjugates of benzoate and phenylacetate (Brusilow et al., 1980; Maestri et al., 1991; Maestri et al., 1996). The clinical utility of phenylacetate is limited by its objectionable odor. Sodium phenylbutyrate, which is less malodorous and is converted to phenylacetate, has been used with success. Acylation therapy has greatly improved survival and morbidity. Thus, the outlook for female heterozygotes with OTC deficiency is favorable for those who are treated from an early age (Maestri et al., 1996), although careful cognitive profiling may disclose subtle defects in performance even in ostensibly normal female heterozygotes (see Box, below).

Patients who survive the neonatal period can be maintained with a low-protein diet and sodium benzoate. A useful therapeutic adjunct for citrullinemia and argininosuccinic aciduria is dietary arginine supplementation, which enhances the ability to eliminate nitrogen as either citrulline or argininosuccinate. Maintaining normal arginine levels also facilitates protein synthesis.

Liver transplantation, if successful, affords complete metabolic correction, although relatively minor deviations of amino acid concentration may persist postoperatively. The morbidity of organ transplantation and the availability of suitable donors restrict the utility of this approach. Hepatic transplantation is technically difficult—and potentially dangerous—in an affected neonate. Most metabolic centers require that a baby reach a threshold weight (usually about 10 kg) before proceeding with liver transplantation.

Hemodialysis relieves acute toxicity during fulminant hyperammonemia. Exchange transfusions also have been performed, but this technique has not been equally useful in removing ammonia.

The possibility of gene therapy for these disorders is a subject of intense scrutiny (Ye et al., 1996). An adenoviral vector containing a cDNA for the OTC gene has been given to mice with a congenital deficiency of OTC. The result was complete correction of hepatic OTC activity over a two-month period. Transient correction of serum glutamine and urine orotic acid was reported. This experimental approach holds enormous promise for the management of this enzymopathy and other inborn errors of intermediary metabolism.

COGNITION IN UREA CYCLE DEFECTS: ROLE OF AMMONIA, GLUTAMINE AND MYOINOSITOL

Marc Yudkoff

Dietary restriction of protein and selected amino acids usually improves clinical outcome in disorders of amino acid metabolism, but subtle defects in cognitive performance may occur even with scrupulous dietary treatment. Thus, in ostensibly asymptomatic female carriers for ornithine transcarbamylase deficiency (OTCD), an X-linked form of a urea cycle defect (see in chapter text) , significant weaknesses occur in fine motor dexterity and speed as well as nonsignificant weaknesses in nonverbal intelligence, visual memory, attention/executive skills, and math (Gyato et al., 2004). The degree of cognitive disability correlates with the rate of residual ureagenesis, as determined by measurement of $^{15}NH_4Cl$ conversion to [^{15}N]urea. Studies with 1H MRS (magnetic resonance spectroscopy) in female carriers suggest a diminution of the brain myoinositol concentration and an increase in the level of glutamine (Gropman et al., 2008), the latter occurring because of increased entry of NH_3 from blood into astrocytes, the site of the glutamine synthetase reaction. Accumulation of glial glutamine favors cell swelling and consequent release of myoinositol in an attempt to restore water balance. Future research may determine whether the loss of myoinositol is large enough to affect second-messenger signaling through the phosphoinositide pathway.

References

Gropman, A. L., Fricke, S. T., Seltzer, R. R., Hailu, A., Adevemo, A., Sawyer, A., et al., and Urea Cycle Disorders Consortium. (2008). 1H MRS identifies symptomatic and asymptomatic subjects with partial ornithine transcarbamylase deficiency. *Molecular Genetics and Metabolism*, *95*, 21–30.

Gyato, K., Wray, J., Huang, Z. J., Yudkoff, M., & Batshaw, M. (2004). Metabolic and Neuropsychological Phenotype in Women Heterozygous for Ornithine Transcarbamylase Deficiency. *Annals of Neurology*, *55*, 80–86.

DISORDERS OF GLUTATHIONE METABOLISM

The tripeptide glutathione is the major intracellular antioxidant

The tripeptide glutathione (γ-glutamyl- cysteinyl-glycine) is the major intracellular antioxidant. It is synthesized via these reactions (Fig. 42-6):

1. Glutamate + cysteine + ATP → γ-glutamylcysteine + ADP + P_i
2. γ-glutamylcysteine + glycine + ATP → glutathione + ADP + P_i

Glutathione is subsequently metabolized in the γ- glutamyl cycle:

3. Glutathione + amino acid → γ-glutamyl-amino acid + cysteinylglycine
4. γ-glutamyl-amino acid → 5-oxoproline + amino acid
5. 5-oxoproline + ATP + $2H_2O$→glutamate + ADP + P_i
6. Cysteinylglycine → cysteine + glycine.

The cycle is renewed after the cysteine formed in reaction 6 and the glutamate derived from reaction 5 are converted to γ-glutamylcysteine via γ-glutamylcysteine synthetase (reaction 1).

5-Oxoprolinuria: glutathione synthetase deficiency

Patients have metabolic acidosis caused by excessive formation of 5-oxoproline (pyroglutamic acid; Fig. 42-6, reaction 4). This occurs because the diminution of intracellular glutathione relieves the feedback inhibition on the γ-glutamylcysteine synthetase pathway (reaction 1), thereby augmenting the concentration of γ-glutamylcysteine and the subsequent conversion of this dipeptide to cysteine and 5-oxoproline in the cyclotransferase pathway (reaction 4).

Clinical findings include mental retardation, severe metabolic acidosis, and evidence of a spastic quadriparesis and cerebellar disease. Some patients develop normally until late childhood, when a progressive loss of intellectual function became appreciated. Patients also may manifest a mild hemolysis. Pathological changes have included atrophy of the cerebellum and lesions in the cortex and thalamus. There is no specific therapy.

γ-Glutamylcysteine synthetase deficiency

Patients with this rare disorder (Fig. 42-6, reaction 1) have spinocerebellar degeneration, peripheral neuropathy, myopathy and an aminoaciduria secondary to renal dysfunction. Psychosis and a hemolytic anemia have been noted.

γ-Glutamyltranspeptidase deficiency

These patients display glutathionuria and varying degrees of mental retardation (Fig. 42-6, reaction 3). The enzyme is present in the brain, primarily in the capillaries. No specific treatment is available.

5-Oxoprolinase deficiency

These patients excrete increased amounts of oxoproline and have a somewhat elevated plasma concentration (Fig. 42-6, reaction 5). They have not had significant neurological symptoms.

DISORDERS OF γ-AMINOBUTYRIC ACID METABOLISM

Congenital defects in the metabolism of γ-aminobutyric acid have been described

GABA is formed via the action of glutamate decarboxylase (see Ch. 18). Metabolism of this neurotransmitter is mediated first by uptake into neurons and glia and subsequent

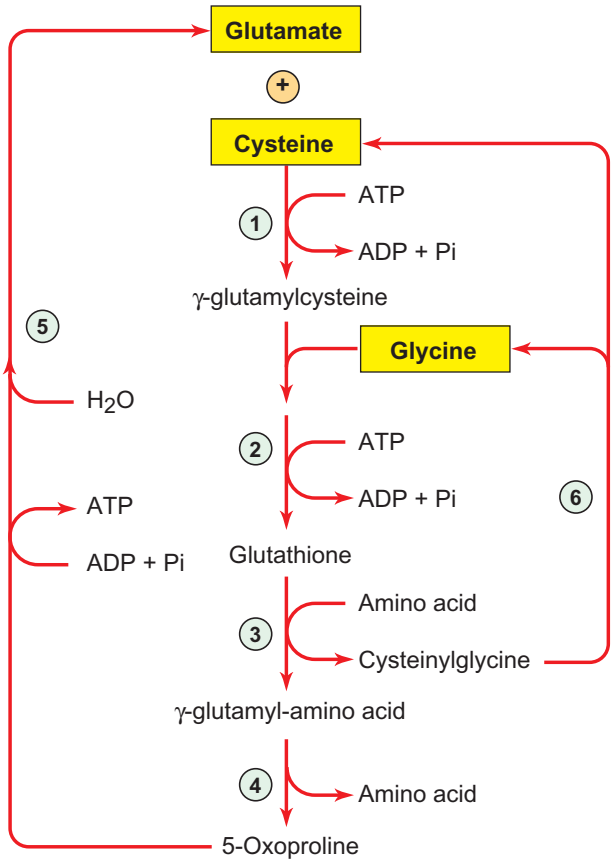

FIGURE 42-6 **Metabolism of glutathione**. Deficiency in reaction 2 leads to severe metabolic acidosis caused by excessive formation of 5-oxoproline from γ-glutamylcysteine in reaction 4. Deficiencies in reactions 1 and 3 also have neurological effects. Deficiencies in reaction 5 are known, but these patients have no significant neurological symptoms. Enzymes: (1) γ-glutamylcysteine synthetase; (2) Glutathione synthetase; (3) γ-glutamyltranspeptidase; (4) Cyclotransferase; (5) 5-oxoprolinase; (6) Peptidase.

transamination to succinic semialdehyde via γ-aminobutyric acid transaminase (GABA-T). The semialdehyde is oxidized to succinate via succinic semialdehyde dehydrogenase.

Pyridoxine dependency

Pyridoxine dependency is characterized by severe seizure activity of early onset, perhaps even *in utero*. Patients respond dramatically to parenteral administration of pyridoxine (10–100 mg). The cause often is a deficiency of α-amino adipic semialdehyde dehydrogenase and consequent accumulation of α-amino adipic semialdehyde, a metabolite of lysine that forms a complex with pyridoxal phosphate (vitamin B6). Some individuals have pyridoxine-responsive seizures but without a deficiency of this enzyme.

γ-Aminobutyric acid transaminase deficiency

Patients with this rare disorder have severe psychomotor retardation and hyperreflexia. The concentrations of GABA and β-alanine are high in cerebrospinal fluid and blood. GABA-T activity is much diminished in blood lymphocytes and in the liver. A curious finding is increased stature, perhaps reflecting the ability of GABA to evoke release of growth hormone.

Succinic semialdehyde dehydrogenase deficiency

Patients have mental retardation, cerebellar disease, and hypotonia. They excrete large amounts of both succinic semialdehyde and 4-hydroxybutyric acid. There is no known therapy.

DISORDERS OF *N*-ACETYL ASPARTATE METABOLISM

Canavan's disease is the result of a deficiency of the enzyme that breaks down *N*-acetylaspartate, an important donor of acetyl groups for brain myelin synthesis

Infants seem normal at birth, but developmental delay is apparent by age three months. Increased head circumference (>98th percentile) is common, and hydrocephalus sometimes is suspected. Neurological function deteriorates rapidly over a period of months. Optic atrophy leads to blindness. Infants manifest minimal interest in their environment. Spasticity is frequent and seizures may occur. Imaging of the brain shows demyelination and brain atrophy with enlargement of the ventricles and widening of the sulci. Pathological examination shows swelling of the astrocytes with elongation of the mitochondria. Vacuoles appear in the myelin sheets.

Urinary excretion of *N*-acetylaspartate is elevated and the cerebrospinal fluid concentration may be 50 times control values. The cause is a deficiency of aspartoacylase, which cleaves *N*-acetylaspartate to form aspartate and acetyl-CoA. The enzyme occurs primarily in the white matter, but *N*-acetylaspartate is most abundant in gray matter. The defect is expressed in skin fibroblasts.

N-acetylaspartate is among the most abundant amino acids in the brain, although its precise function remains elusive. Putative roles have included osmoregulation and the storage of acetyl groups that subsequently are utilized for myelin synthesis. The relationship of the enzyme defect to the clinical findings remains problematic. No specific therapy is yet available.

References

Alemzadeh, R., Gammeltoft, K., & Matteson, K. (1996). Efficacy of low-dose dextromethorphan in the treatment of nonketotic hyperglycinemia. *Pediatrics*, *97*, 924–926.

Brusilow, S., Tinker, J., & Batshaw, M. L. (1980). Amino acid acylation: A mechanism of nitrogen excretion in inborn errors of urea synthesis. *Science*, *207*, 659.

Cedarbaum, S. (2002). Phenylketonuria: An update. *Current Opinion in Pediatrics*, *14*, 702–706.

Chan, H., & Butterworth, R. F. (2003). Cell-selective effects of ammonia on glutamate transporter and receptor function in the mammalian brain. *Neurochemistry International*, *43*, 525–532.

Chuang, D. T., Davie, J. R., Wynn, R. M., Chuang, J. L., Koyatam, H., & Cox, R. P. (1995). Molecular basis of maple syrup urine disease and stable correction by retroviral gene transfer. *The Journal of Nutrition*, *125*, 1766S–1772S.

Deutsch, S. I., Rosse, R. B., & Mastropaolo, J. (1998). Current status of NMDA antagonist interventions in the treatment of nonketotic hyperglycinemia. *Clinical Neuropharmacology*, *21*, 71–79.

Diamond, A., & Herzberg, C. (1996). Impaired sensitivity to visual contrast in children treated early and continuously for phenylketonuria. *Brain*, *119*, 523–538.

Dudman, N. P., Guo, X. W., Gordon, R. B., Dawson, P. A., & Wilcken, D. E. (1996). Human homocysteine catabolism: Three major pathways and their relevance to development of arterial occlusive disease. *The Journal of Nutrition*, *126*(Suppl. 4), 295s–300s.

Eisensmith, R. C., & Woo, S. L. (1991). Phenylketonuria and the phenylalanine hydroxylase gene. *Molecular Biology and Medicine*, *8*, 3–10.

Felipo, V., & Butterworth, R. (2002). Neurobiology of ammonia. *Progress in Neurobiology*, *67*, 259–279.

Guldberg, P., Levy, H. L., Hanley, W. B., et al. (1996). Phenylalanine hydroxylase gene mutations in the United States: Report from the Maternal PKU Collaborative Study. *American Journal of Human Genetics*, *59*, 84–94.

Hommes, F. A. (1994). Loss of neurotransmitter receptors by hyperphenylalaninemia in the HPH-5 mouse brain. *Acta Paediatrica Supplement*, *407*, 120–121.

Infant, J. P., & Huszagh, V. A. (2001). Impaired arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acid synthesis by phenylalanine metabolites as etiological factors in the neuropath-ology of phenylketonuria. *Molecular Genetics and Metabolism*, *72*, 185–198.

Jan, W., Zimmerman, R. A., Wang, Z. J., Berry, G. T., Kaplan, P. B., & Kaye, E. M. (2003). MR diffusion imaging and MR spectroscopy of maple syrup urine disease during acute metabolic decompensation. *Neuroradiology*, *45*, 393–399.

Kahler, S. G., & Fahey, M. C. (2003). Metabolic disorders and mental retardation. *American Journal of Medical Genetics*, *117C*, 31–41.

Kaplan, P., Mazur, A., Field, M., Berlin, J. A., Berry, G. T., Heidenreich, R., et al. (1991). Intellectual outcome in children with maple syrup urine disease. *Journal of Pediatric*, *119*, 46–50.

Kaufman, S. (1983). Hyperphenylalaninaemia caused by defects in biopterin metabolism. *Journal of Inherited Metabolic Disease*, *8*(Suppl. 1), 20–27.

Kikuchi, G. (1973). The glycine cleavage system: Composition, reactin mechanism and physiological significance. *Molecular and Cellular Biochemistry*, *1*, 169–175.

Kluijtmans, L. A., Boers, G. H., Stevens, E. M., et al. (1996). Defective cystathionine beta-synthase regulation by *S*-adenosylmethionine in a partially pyridoxine responsive homocystinuria patient. *The Journal of Clinical Investigation*, *98*, 285–289.

Koch, R., Moseley, K. D., Yano, S., Nelson, M., Jr., & Moats, R. A. (2003). Large neutral amino acid therapy and phenylketonuria: A promising approach to treatment. *Molecular Genetics and Metabolism*, *79*, 110–113.

Kraus, J. P. (1994). Molecular basis of phenotype expression in homocystinuria. Komrower Lecture. *Journal of Inherited Metabolic Disease*, *17*, 383–390.

Levy, H. L., & Ghavami, M. (1996). Maternal phenylketonuria: A metabolic teratogen. *Teratology*, *53*, 176–184.

Maestri, N. E., Hauser, E. R., Bartholomew, D., & Brusilow, S. W. (1991). Prospective treatment of urea cycle disorders. *The Journal of Pediatrics*, *119*, 923.

Maestri, N. E., Brusilow, S. W., Clissold, D. B., & Bassett, S. S. (1996). Long-term treatment of girls with ornithine transcarbamylase deficiency. *The New England Journal of Medicine*, *335*, 855–859.

Mandel, H., Brenner, B., Berant, M., et al. (1996). Coexistence of hereditary homocystinuria and factor V Leiden–effect on thrombosis. *The New England Journal of Medicine*, *334*, 763–768.

Morton, D. H., Strauss, K. A., Robinson, D. L., Puffenberger, E. G., & Kelley, R. I. (2002). Diagnosis and treatment of maple syrup disease: A study of 36 patients. *Pediatrics*, *109*, 999–1008.

Mueller, G. M., McKenzie, L. R., Homanics, G. E., Watkins, S. C., Robbins, P. D., & Paul, H. S. (1995). Complementation of defective leucine decarboxylation in fibroblasts from a maple syrup urine disease patient by retrovirus-mediated gene transfer. *Gene Therapy*, *2*, 461–468.

Pietz, J., Rupp, A., Ebinger, F., et al. (2003). Cerebral energy metabolism in phenylketonuria: Findings by quantitative *in vivo* ^{31}P MR spectroscopy. *Pediatric Research*, *53*, 654–662.

Scriver, C. R., Beaudet, A. L., Sly, W. S., & Valle, D. (2001). *The Metabolic and Molecular Basis of Inherited Disease*. New York: McGraw Hill.

Shefer, S., Tint, G. S., Jean-Guillaume, D., et al. (2000). Is there a relationship between 3-hydroxy-3-methylglutaryl coenzyme a reductase activity and forebrain pathology in the PKU mouse? *Journal of Neuroscience Research*, *61*, 549–563.

Smith, C. B., & Kang, J. (2000). Cerebral protein synthesis in a genetic model of phenylketonuria. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 11014–11019.

Steiner, R. D., Sweetser, D. A., Rohrbaugh, J. R., Dowton, S. B., Toone, J. R., & Applegarth, D. A. (1996). Nonketotic hyperglycinemia: Atypical clinical and biochemical manifestations. *The Journal of Pediatrics*, *128*, 243–246.

Van Hove, J. L., Kishnani, P., Muenzer, J., et al. (1995). Benzoate therapy and carnitine deficiency in non-ketotic hyperglycinemia. *American Journal of Medical Genetics*, *59*, 444–453.

Wagner, M., Coelho, D. M., Barschak, A. G., et al. (2000). Reduction of large neutral amino acid concentrations in plasma and CSF of patients with maple syrup urine disease. *Journal of Inherited Metabolic Disease*, *23*, 505–512.

White, D. A., Nortz, M. J., Mandernach, T., Huntington, K., & Steiner, R. D. (2001). Deficits in memory strategy use related to prefrontal dysfunction during early development: Evidence from children with phenylketonuria. *Neuropsychology*, *15*, 221–229.

Willard-Mack, C. L., Koehler, R. C., Hirata, T., et al. (1996). Inhibition of glutamine synthetase reduces ammonia-induced astrocyte swelling in rat. *Neuroscience*, *71*, 589–599.

Ye, X., Robinson, M. B., Batshaw, M. L., Furth, E. E., Smith, I., & Wilson, J. M. (1996). Prolonged metabolic correction in adult ornithine transcarbamylase-deficient mice with adenoviral vectors. *The Journal of Biological Chemistry*, *271*, 3639–3646.

Yudkoff, M., Daikhin, Y., Nissim, I., Jawad, A., Wilson, J., & Batshaw, M. B. (1996). *In vivo* nitrogen metabolism in ornithine transcarbamylase deficiency. *The Journal of Clinical Investigation*, *98*, 2167–2173.