SECTION III

Metabolism of Proteins & Amino Acids

Biosynthesis of the Nutritionally Nonessential Amino Acids

28

Victor W. Rodwell, PhD

BIOMEDICAL IMPORTANCE

All 20 of the amino acids present in proteins are essential for health. While comparatively rare in the Western world, amino acid deficiency states are endemic in certain regions of West Africa where the diet relies heavily on grains that are poor sources of amino acids such as tryptophan and lysine. These disorders include kwashiorkor, which results when a child is weaned onto a starchy diet poor in protein; and marasmus, in which both caloric intake and specific amino acids are deficient.

Humans can synthesize 12 of the 20 common amino acids from the amphibolic intermediates of glycolysis and of the citric acid cycle (Table 28–1). While *nutritionally* nonessential, these 12 amino acids are not "nonessential." All 20 amino acids are *biologically* essential. Of the 12 nutritionally nonessential amino acids, nine are formed from amphibolic intermediates and three (cysteine, tyrosine and hydroxylysine) from nutritionally essential amino acids. Identification of the twelve amino acids that humans can synthesize rested primarily on data derived from feeding diets in which purified amino acids replaced protein. This chapter considers only the biosynthesis of the twelve amino acids that are synthesized in human tissues, not the other eight that are synthesized by plants.

NUTRITIONALLY NONESSENTIAL AMINO ACIDS HAVE SHORT BIOSYNTHETIC PATHWAYS

The enzymes glutamate dehydrogenase, glutamine synthetase, and aminotransferases occupy central positions in amino acid biosynthesis. The combined effect of those three enzymes is to transform ammonium ion into the α-amino nitrogen of various amino acids.

Glutamate and Glutamine. Reductive amination of α-ketoglutarate is catalyzed by glutamate dehydrogenase (Figure 28–1). Amination of glutamate to glutamine is catalyzed by glutamine synthetase (Figure 28–2).

Alanine. Transamination of pyruvate forms alanine (Figure 28–3).

Aspartate and Asparagine. Transamination of oxaloacetate forms aspartate. The conversion of aspartate

Table 28–1. Amino acid requirements of humans.

Nutritionally Essential	Nutritionally Nonessential
Arginine[1]	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Hydroxyproline[2]
Tryptophan	Hydroxylysine[2]
Valine	Proline
	Serine
	Tyrosine

[1]"Nutritionally semiessential." Synthesized at rates inadequate to support growth of children.
[2]Not necessary for protein synthesis but formed during post-translational processing of collagen.

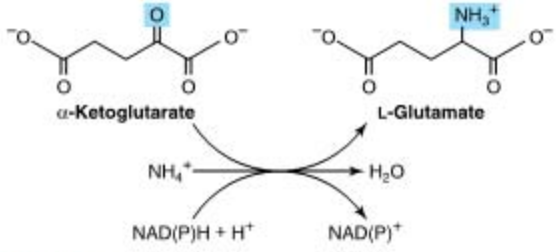

Figure 28–1. The glutamate dehydrogenase reaction.

to asparagine is catalyzed by asparagine synthetase (Figure 28–4), which resembles glutamine synthetase (Figure 28–2) except that glutamine, not ammonium ion, provides the nitrogen. Bacterial asparagine synthetases can, however, also use ammonium ion. Coupled hydrolysis of PP_i to P_i by pyrophosphatase ensures that the reaction is strongly favored.

Serine. Oxidation of the α-hydroxyl group of the glycolytic intermediate 3-phosphoglycerate converts it to an oxo acid, whose subsequent transamination and dephosphorylation leads to serine (Figure 28–5).

Glycine. Glycine aminotransferases can catalyze the synthesis of glycine from glyoxylate and glutamate or alanine. Unlike most aminotransferase reactions, these strongly favor glycine synthesis. Additional important mammalian routes for glycine formation are from choline (Figure 28–6) and from serine (Figure 28–7).

Proline. Proline is formed from glutamate by reversal of the reactions of proline catabolism (Figure 28–8).

Cysteine. Cysteine, while not nutritionally essential, is formed from methionine, which is nutritionally essential. Following conversion of methionine to ho-

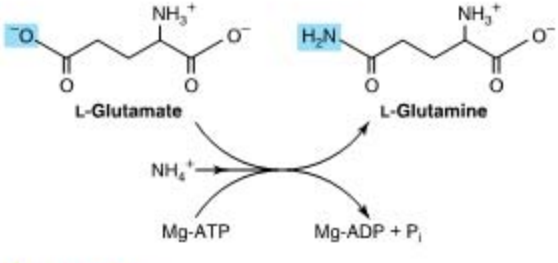

Figure 28–2. The glutamine synthetase reaction.

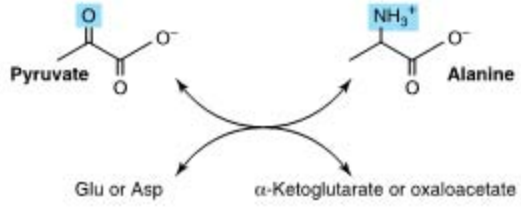

Figure 28–3. Formation of alanine by transamination of pyruvate. The amino donor may be glutamate or aspartate. The other product thus is α-ketoglutarate or oxaloacetate.

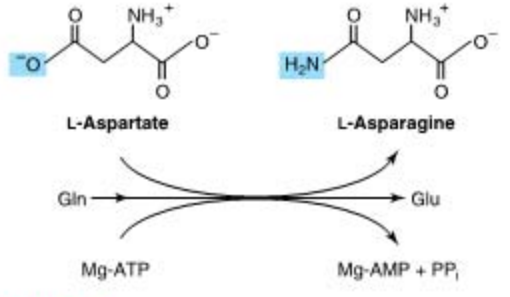

Figure 28–4. The asparagine synthetase reaction. Note similarities to and differences from the glutamine synthetase reaction (Figure 28–2).

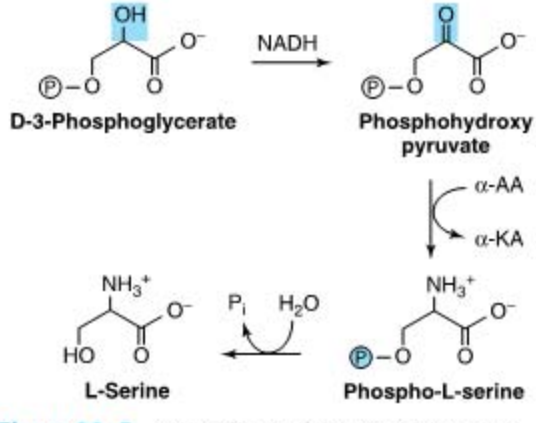

Figure 28–5. Serine biosynthesis. (α-AA, α-amino acids; α-KA, α-keto acids.)

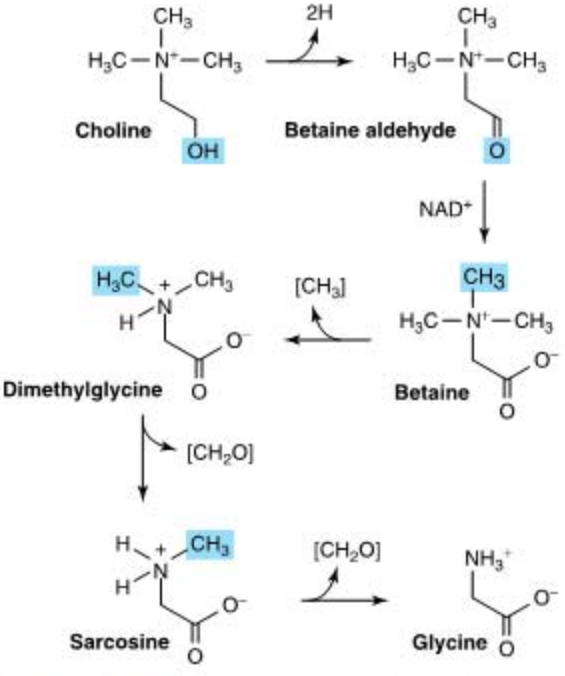

Figure 28–6. Formation of glycine from choline.

mocysteine (see Chapter 30), homocysteine and serine form cysteine and homoserine (Figure 28–9).

Tyrosine. Phenylalanine hydroxylase converts phenylalanine to tyrosine (Figure 28–10). Provided that the diet contains adequate nutritionally essential phenylalanine, tyrosine is nutritionally nonessential. But since the reaction is irreversible, dietary tyrosine cannot replace phenylalanine. Catalysis by this mixed-function oxygenase incorporates one atom of O_2 into phenylalanine and reduces the other atom to water. Reducing power, provided as tetrahydrobiopterin, derives ultimately from NADPH.

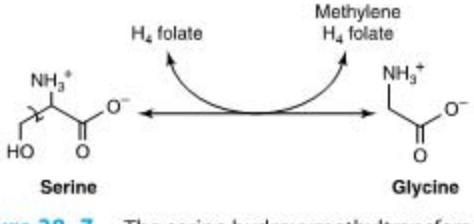

Figure 28–7. The serine hydroxymethyltransferase reaction. The reaction is freely reversible. (H_4 folate, tetrahydrofolate.)

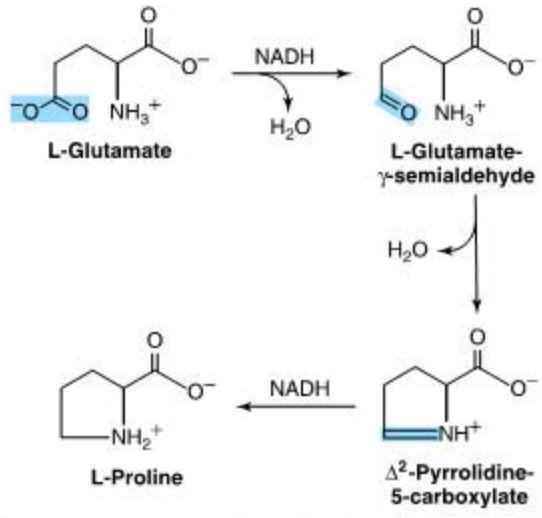

Figure 28–8. Biosynthesis of proline from glutamate by reversal of reactions of proline catabolism.

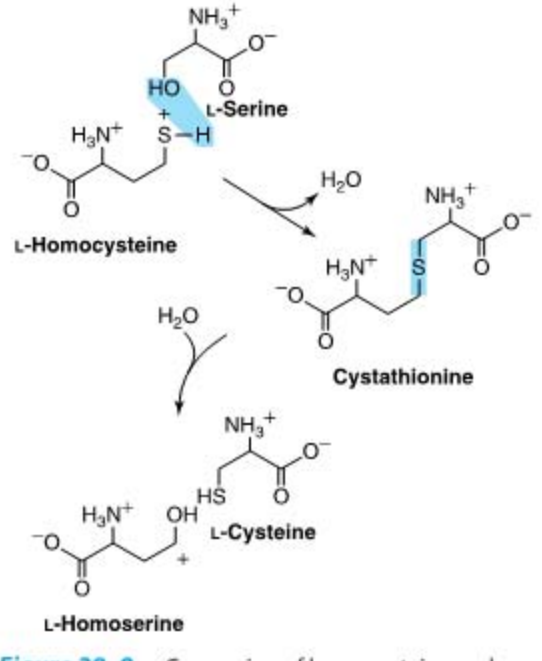

Figure 28–9. Conversion of homocysteine and serine to homoserine and cysteine. The sulfur of cysteine derives from methionine and the carbon skeleton from serine.

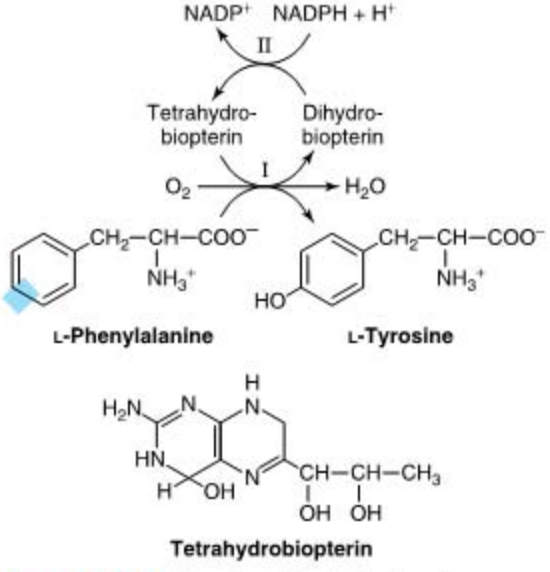

Figure 28–10. The phenylalanine hydroxylase reaction. Two distinct enzymatic activities are involved. Activity II catalyzes reduction of dihydrobiopterin by NADPH, and activity I the reduction of O_2 to H_2O and of phenylalanine to tyrosine. This reaction is associated with several defects of phenylalanine metabolism discussed in Chapter 30.

Hydroxyproline and Hydroxylysine. Hydroxyproline and hydroxylysine are present principally in collagen. Since there is no tRNA for either hydroxylated amino acid, neither dietary hydroxyproline nor hydroxylysine is incorporated into protein. Both are completely degraded (see Chapter 30). Hydroxyproline and hydroxylysine arise from proline and lysine, but only after these amino acids have been incorporated into peptides. Hydroxylation of peptide-bound prolyl and lysyl residues is catalyzed by prolyl hydroxylase and lysyl hydroxylase of tissues, including skin and skeletal muscle, and of granulating wounds (Figure 28–11). The hydroxylases are mixed-function oxygenases that require substrate, molecular O_2, ascorbate, Fe^{2+}, and α-ketoglutarate. For every mole of proline or lysine hydroxylated, one mole of α-ketoglutarate is decarboxylated to succinate. One atom of O_2 is incorporated into proline or lysine, the other into succinate (Figure 28–11). A deficiency of the vitamin C required for these hydroxylases results in scurvy.

Valine, Leucine, and Isoleucine. While leucine, valine, and isoleucine are all nutritionally essential

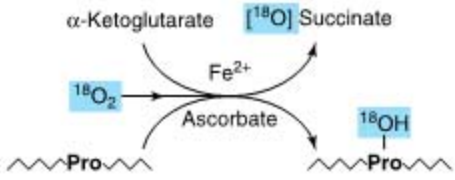

Figure 28–11. The prolyl hydroxylase reaction. The substrate is a proline-rich peptide. During the course of the reaction, molecular oxygen is incorporated into both succinate and proline. Lysyl hydroxylase catalyzes an analogous reaction.

amino acids, tissue aminotransferases reversibly interconvert all three amino acids and their corresponding α-keto acids. These α-keto acids thus can replace their amino acids in the diet.

Selenocysteine. While not normally considered an amino acid present in proteins, selenocysteine occurs at the active sites of several enzymes. Examples include the human enzymes thioredoxin reductase, glutathione peroxidase, and the deiodinase that converts thyroxine to triiodothyronine. Unlike hydroxyproline or hydroxylysine, selenocysteine arises co-translationally during its incorporation into peptides. The UGA anticodon of the unusual tRNA designated $tRNA^{Sec}$ normally signals STOP. The ability of the protein synthetic apparatus to identify a selenocysteine-specific UGA codon involves the selenocysteine insertion element, a stem-loop structure in the untranslated region of the mRNA. Selenocysteine-$tRNA^{Sec}$ is first charged with serine by the ligase that charges $tRNA^{Sec}$. Subsequent replacement of the serine oxygen by selenium involves selenophosphate formed by selenophosphate synthase (Figure 28–12).

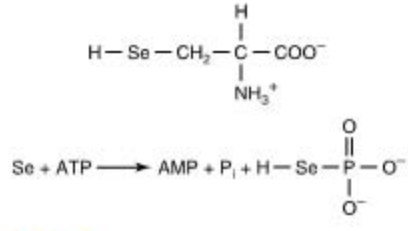

Figure 28–12. Selenocysteine *(top)* and the reaction catalyzed by selenophosphate synthetase *(bottom)*.

SUMMARY

- All vertebrates can form certain amino acids from amphibolic intermediates or from other dietary amino acids. The intermediates and the amino acids to which they give rise are α-ketoglutarate (Glu, Gln, Pro, Hyp), oxaloacetate (Asp, Asn) and 3-phosphoglycerate (Ser, Gly).
- Cysteine, tyrosine, and hydroxylysine are formed from nutritionally essential amino acids. Serine provides the carbon skeleton and homocysteine the sulfur for cysteine biosynthesis. Phenylalanine hydroxylase converts phenylalanine to tyrosine.
- Neither dietary hydroxyproline nor hydroxylysine is incorporated into proteins because no codon or tRNA dictates their insertion into peptides.
- Peptidyl hydroxyproline and hydroxylysine are formed by hydroxylation of peptidyl proline or lysine in reactions catalyzed by mixed-function oxidases that require vitamin C as cofactor. The nutritional disease scurvy reflects impaired hydroxylation due to a deficiency of vitamin C.
- Selenocysteine, an essential active site residue in several mammalian enzymes, arises by co-translational insertion of a previously modified tRNA.

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Catabolism of Proteins & of Amino Acid Nitrogen

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BIOMEDICAL IMPORTANCE

This chapter describes how the nitrogen of amino acids is converted to urea and the rare disorders that accompany defects in urea biosynthesis. In normal adults, nitrogen intake matches nitrogen excreted. Positive nitrogen balance, an excess of ingested over excreted nitrogen, accompanies growth and pregnancy. Negative nitrogen balance, where output exceeds intake, may follow surgery, advanced cancer, and kwashiorkor or marasmus.

While ammonia, derived mainly from the α-amino nitrogen of amino acids, is highly toxic, tissues convert ammonia to the amide nitrogen of nontoxic glutamine. Subsequent deamination of glutamine in the liver releases ammonia, which is then converted to nontoxic urea. If liver function is compromised, as in cirrhosis or hepatitis, elevated blood ammonia levels generate clinical signs and symptoms. Rare metabolic disorders involve each of the five urea cycle enzymes.

PROTEIN TURNOVER OCCURS IN ALL FORMS OF LIFE

The continuous degradation and synthesis of cellular proteins occur in all forms of life. Each day humans turn over 1–2% of their total body protein, principally muscle protein. High rates of protein degradation occur in tissues undergoing structural rearrangement—eg, uterine tissue during pregnancy, tadpole tail tissue during metamorphosis, or skeletal muscle in starvation. Of the liberated amino acids, approximately 75% are reutilized. The excess nitrogen forms urea. Since excess amino acids are not stored, those not immediately incorporated into new protein are rapidly degraded.

PROTEASES & PEPTIDASES DEGRADE PROTEINS TO AMINO ACIDS

The susceptibility of a protein to degradation is expressed as its half-life ($t_{1/2}$), the time required to lower its concentration to half the initial value. Half-lives of liver proteins range from under 30 minutes to over 150 hours. Typical "housekeeping" enzymes have $t_{1/2}$ values of over 100 hours. By contrast, many key regulatory enzymes have a $t_{1/2}$ of 0.5–2 hours. PEST sequences, regions rich in proline (P), glutamate (E), serine (S), and threonine (T), target some proteins for rapid degradation. Intracellular proteases hydrolyze internal peptide bonds. The resulting peptides are then degraded to amino acids by endopeptidases that cleave internal bonds and by aminopeptidases and carboxypeptidases that remove amino acids sequentially from the amino and carboxyl terminals, respectively. Degradation of circulating peptides such as hormones follows loss of a sialic acid moiety from the nonreducing ends of their oligosaccharide chains. Asialoglycoproteins are internalized by liver cell asialoglycoprotein receptors and degraded by lysosomal proteases termed cathepsins.

Extracellular, membrane-associated, and long-lived intracellular proteins are degraded in lysosomes by ATP-independent processes. By contrast, degradation of abnormal and other short-lived proteins occurs in the cytosol and requires ATP and ubiquitin. Ubiquitin, so named because it is present in all eukaryotic cells, is a small (8.5 kDa) protein that targets many intracellular proteins for degradation. The primary structure of ubiquitin is highly conserved. Only 3 of 76 residues differ between yeast and human ubiquitin. Several molecules of ubiquitin are attached by non-α-peptide bonds formed between the carboxyl terminal of ubiquitin and the ε-amino groups of lysyl residues in the target protein (Figure 29–1). The residue present at its amino terminal affects whether a protein is ubiquitinated. Amino terminal Met or Ser retards whereas Asp or Arg accelerates ubiquitination. Degradation occurs in a multicatalytic complex of proteases known as the proteasome.

ANIMALS CONVERT α-AMINO NITROGEN TO VARIED END PRODUCTS

Different animals excrete excess nitrogen as ammonia, uric acid, or urea. The aqueous environment of teleostean fish, which are ammonotelic (excrete ammonia), compels them to excrete water continuously, facilitating excretion of highly toxic ammonia. Birds, which must conserve water and maintain low weight, are uricotelic and excrete uric acid as semisolid guano. Many

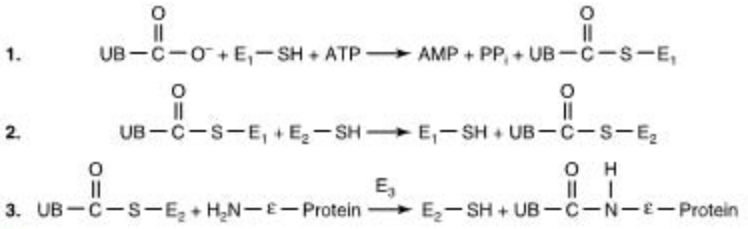

Figure 29–1. Partial reactions in the attachment of ubiquitin (UB) to proteins. (1) The terminal COOH of ubiquitin forms a thioester bond with an -SH of E_1 in a reaction driven by conversion of ATP to AMP and PP_i. Subsequent hydrolysis of PP_i by pyrophosphatase ensures that reaction 1 will proceed readily. (2) A thioester exchange reaction transfers activated ubiquitin to E_2. (3) E_3 catalyzes transfer of ubiquitin to ε-amino groups of lysyl residues of target proteins.

land animals, including humans, are ureotelic and excrete nontoxic, water-soluble urea. High blood urea levels in renal disease are a consequence—not a cause—of impaired renal function.

BIOSYNTHESIS OF UREA

Urea biosynthesis occurs in four stages: (1) transamination, (2) oxidative deamination of glutamate, (3) ammonia transport, and (4) reactions of the urea cycle (Figure 29–2).

Transamination Transfers α-Amino Nitrogen to α-Ketoglutarate, Forming Glutamate

Transamination interconverts pairs of α-amino acids and α-keto acids (Figure 29–3). All the protein amino acids except lysine, threonine, proline, and hydroxyproline participate in transamination. Transamination is readily reversible, and aminotransferases also function in amino acid biosynthesis. The coenzyme pyridoxal phosphate (PLP) is present at the catalytic site of aminotransferases and of many other enzymes that act on amino acids. PLP, a derivative of vitamin B_6, forms an enzyme-bound Schiff base intermediate that can rearrange in various ways. During transamination, bound PLP serves as a carrier of amino groups. Rearrangement forms an α-keto acid and enzyme-bound pyridoxamine phosphate, which forms a Schiff base with a second keto acid. Following removal of α-amino nitrogen by transamination, the remaining carbon "skeleton" is degraded by pathways discussed in Chapter 30.

Alanine-pyruvate aminotransferase (alanine aminotransferase) and glutamate-α-ketoglutarate aminotransferase (glutamate aminotransferase) catalyze the transfer

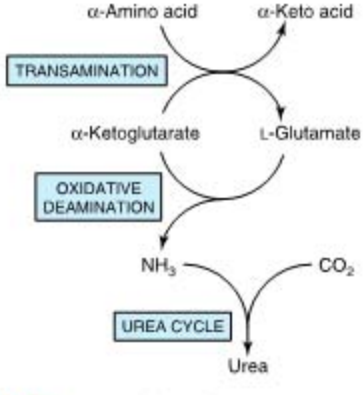

Figure 29–2. Overall flow of nitrogen in amino acid catabolism.

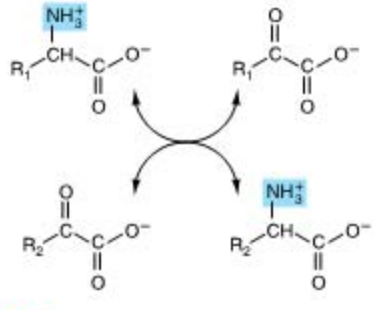

Figure 29–3. Transamination. The reaction is freely reversible with an equilibrium constant close to unity.

of amino groups to pyruvate (forming alanine) or to α-ketoglutarate (forming glutamate) (Figure 29–4). Each aminotransferase is specific for one pair of substrates but nonspecific for the other pair. Since alanine is also a substrate for glutamate aminotransferase, all the amino nitrogen from amino acids that undergo transamination can be concentrated in glutamate. This is important because L-glutamate is the only amino acid that undergoes oxidative deamination at an appreciable rate in mammalian tissues. The formation of ammonia from α-amino groups thus occurs mainly via the α-amino nitrogen of L-glutamate.

Transamination is not restricted to α-amino groups. The δ-amino group of ornithine—but not the ε-amino group of lysine—readily undergoes transamination. Serum levels of aminotransferases are elevated in some disease states (see Figure 7–11).

L-GLUTAMATE DEHYDROGENASE OCCUPIES A CENTRAL POSITION IN NITROGEN METABOLISM

Transfer of amino nitrogen to α-ketoglutarate forms L-glutamate. Release of this nitrogen as ammonia is then catalyzed by hepatic **L-glutamate dehydrogenase (GDH),** which can use either NAD^+ or $NADP^+$ (Figure 29–5). Conversion of α-amino nitrogen to ammonia by the concerted action of glutamate aminotransferase and GDH is often termed "transdeamination." Liver GDH activity is allosterically inhibited by ATP, GTP, and NADH and activated by ADP. The reaction catalyzed by GDH is freely reversible and functions also in amino acid biosynthesis (see Figure 28–1).

Amino Acid Oxidases Also Remove Nitrogen as Ammonia

While their physiologic role is uncertain, L-amino acid oxidases of liver and kidney convert amino acids to an α-imino acid that decomposes to an α-keto acid with release of ammonium ion (Figure 29–6). The reduced flavin is reoxidized by molecular oxygen, forming hydrogen peroxide (H_2O_2), which then is split to O_2 and H_2O by **catalase.**

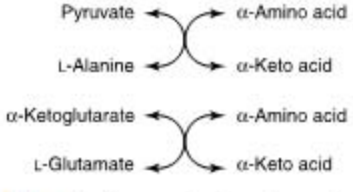

Figure 29–4. Alanine aminotransferase *(top)* and glutamate aminotransferase *(bottom).*

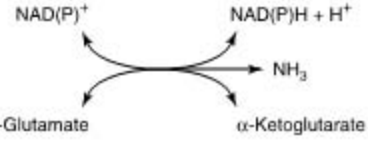

Figure 29–5. The L-glutamate dehydrogenase reaction. $NAD(P)^+$ means that either NAD^+ or $NADP^+$ can serve as co-substrate. The reaction is reversible but favors glutamate formation.

Ammonia Intoxication Is Life-Threatening

The ammonia produced by enteric bacteria and absorbed into portal venous blood and the ammonia produced by tissues are rapidly removed from circulation by the liver and converted to urea. Only traces (10–20 μg/dL) thus normally are present in peripheral blood. This is essential, since ammonia is toxic to the central nervous system. Should portal blood bypass the liver, systemic blood ammonia levels may rise to toxic levels. This occurs in severely impaired hepatic function or the development of collateral links between the portal and systemic veins in cirrhosis. Symptoms of **ammonia intoxication** include tremor, slurred speech, blurred vision, coma, and ultimately death. Ammonia may be toxic to the brain in part because it reacts with α-ketoglutarate to form glutamate. The resulting depleted levels of α-ketoglutarate then impair function of the tricarboxylic acid (TCA) cycle in neurons.

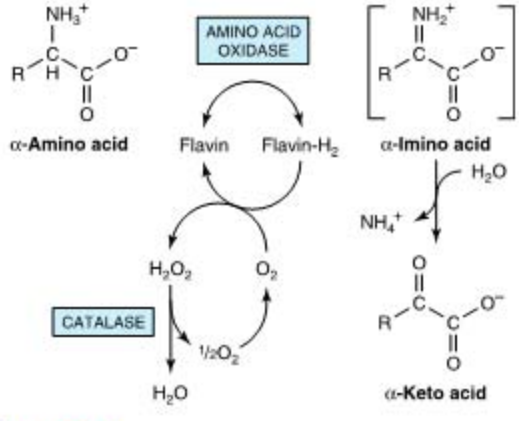

Figure 29–6. Oxidative deamination catalyzed by L-amino acid oxidase (L-α-amino acid:O_2 oxidoreductase). The α-imino acid, shown in brackets, is not a stable intermediate.

Glutamine Synthase Fixes Ammonia as Glutamine

Formation of glutamine is catalyzed by mitochondrial **glutamine synthase** (Figure 29–7). Since amide bond synthesis is coupled to the hydrolysis of ATP to ADP and P_i, the reaction strongly favors glutamine synthesis. One function of glutamine is to sequester ammonia in a nontoxic form.

Glutaminase & Asparaginase Deamidate Glutamine & Asparagine

Hydrolytic release of the amide nitrogen of glutamine as ammonia, catalyzed by **glutaminase** (Figure 29–8), strongly favors glutamate formation. The concerted action of glutamine synthase and glutaminase thus catalyzes the interconversion of free ammonium ion and glutamine. An analogous reaction is catalyzed by L-asparaginase.

Formation & Secretion of Ammonia Maintains Acid-Base Balance

Excretion into urine of ammonia produced by renal tubular cells facilitates cation conservation and regulation of acid-base balance. Ammonia production from intracellular renal amino acids, especially glutamine, increases in **metabolic acidosis** and decreases in **metabolic alkalosis.**

UREA IS THE MAJOR END PRODUCT OF NITROGEN CATABOLISM IN HUMANS

Synthesis of 1 mol of urea requires 3 mol of ATP plus 1 mol each of ammonium ion and of the α-amino nitrogen of aspartate. Five enzymes catalyze the numbered reactions of Figure 29–9. Of the six participating amino acids, *N*-acetylglutamate functions solely as an enzyme activator. The others serve as carriers of the atoms that ultimately become urea. The major metabolic role of **ornithine, citrulline,** and **argininosuccinate** in mammals is urea synthesis. Urea synthesis is a cyclic process. Since the ornithine consumed in reaction 2 is regenerated in reaction 5, there is no net loss or gain of ornithine, citrulline, argininosuccinate, or arginine. Ammonium ion, CO_2, ATP, and aspartate are, however, consumed. Some reactions of urea synthesis occur in the matrix of the mitochondrion, other reactions in the cytosol (Figure 29–9).

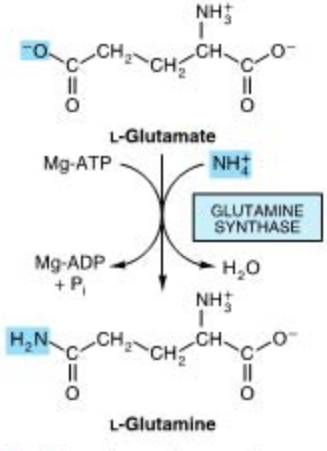

Figure 29–7. The glutamine synthase reaction strongly favors glutamine synthesis.

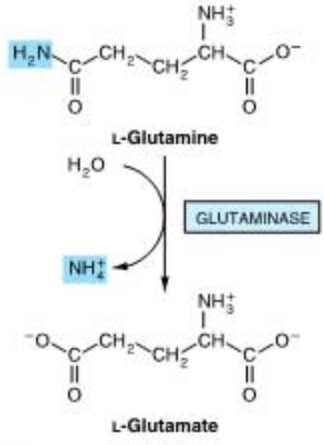

Figure 29–8. The glutaminase reaction proceeds essentially irreversibly in the direction of glutamate and NH_4^+ formation. Note that the *amide* nitrogen, not the α-amino nitrogen, is removed.

Carbamoyl Phosphate Synthase I Initiates Urea Biosynthesis

Condensation of CO_2, ammonia, and ATP to form **carbamoyl phosphate** is catalyzed by mitochondrial **carbamoyl phosphate synthase I** (reaction 1, Figure 29–9). A cytosolic form of this enzyme, carbamoyl phosphate synthase II, uses glutamine rather than ammonia as the nitrogen donor and functions in pyrimidine biosynthesis (see Chapter 34). Carbamoyl phosphate synthase I, the rate-limiting enzyme of the urea cycle, is active only in the presence of its allosteric activator ***N*-acetylglutamate,** which enhances the affinity of the synthase for ATP. Formation of carbamoyl phosphate requires 2 mol of ATP, one of which serves as a phosphate donor. Conversion of the second ATP to AMP and pyrophosphate, coupled to the hydrolysis of pyrophosphate to orthophosphate, provides the driving

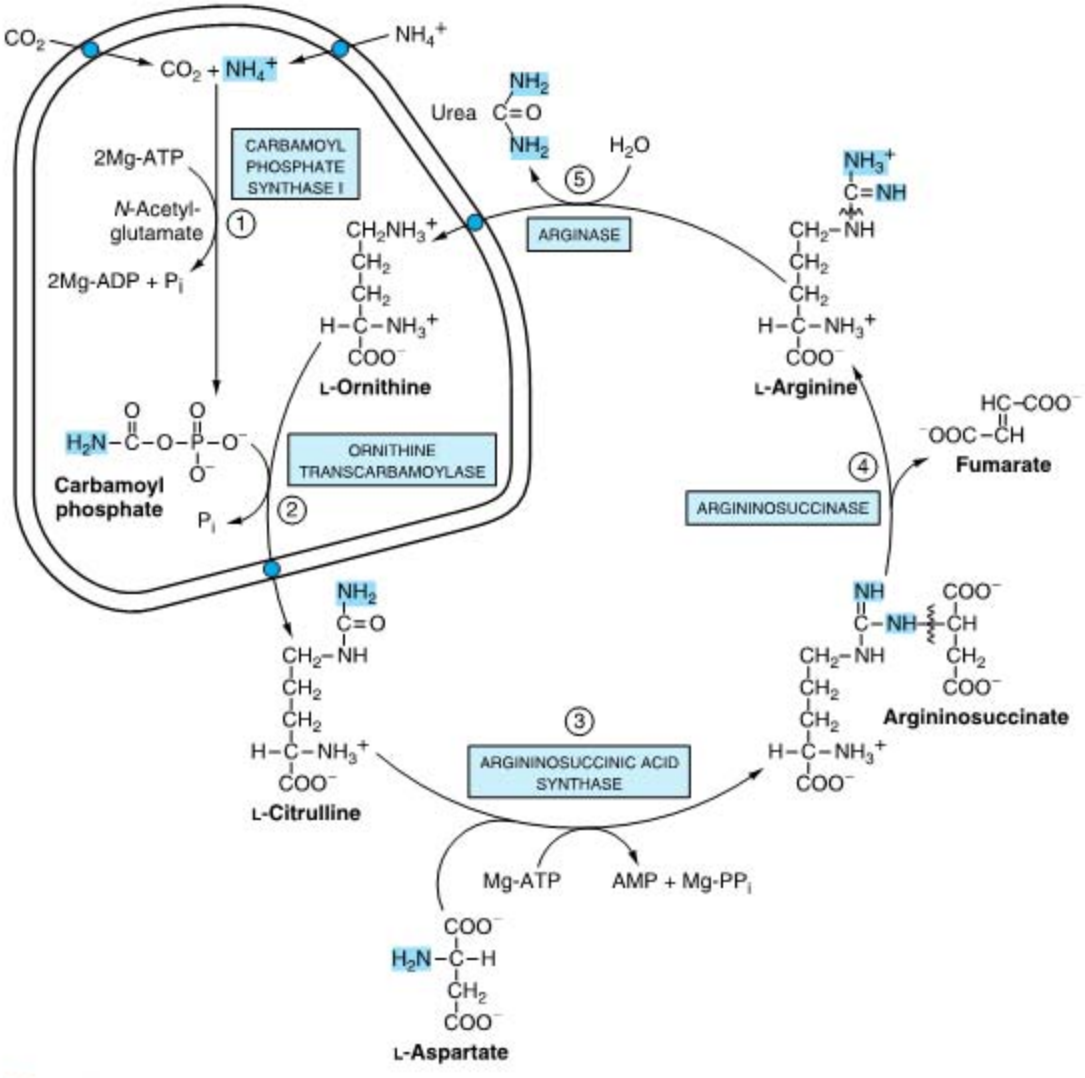

Figure 29–9. Reactions and intermediates of urea biosynthesis. The nitrogen-containing groups that contribute to the formation of urea are shaded. Reactions ① and ② occur in the matrix of liver mitochondria and reactions ③, ④, and ⑤ in liver cytosol. CO_2 (as bicarbonate), ammonium ion, ornithine, and citrulline enter the mitochondrial matrix via specific carriers (see heavy dots) present in the inner membrane of liver mitochondria.

force for synthesis of the amide bond and the mixed acid anhydride bond of carbamoyl phosphate. The concerted action of GDH and carbamoyl phosphate synthase I thus shuttles nitrogen into carbamoyl phosphate, a compound with high group transfer potential. The reaction proceeds stepwise. Reaction of bicarbonate with ATP forms carbonyl phosphate and ADP. Ammonia then displaces ADP, forming carbamate and orthophosphate. Phosphorylation of carbamate by the second ATP then forms carbamoyl phosphate.

Carbamoyl Phosphate Plus Ornithine Forms Citrulline

L-Ornithine transcarbamoylase catalyzes transfer of the carbamoyl group of carbamoyl phosphate to ornithine, forming citrulline and orthophosphate (reaction 2, Figure 29–9). While the reaction occurs in the mitochondrial matrix, both the formation of ornithine and the subsequent metabolism of citrulline take place in the cytosol. Entry of ornithine into mitochondria

and exodus of citrulline from mitochondria therefore involve mitochondrial inner membrane transport systems (Figure 29–9).

Citrulline Plus Aspartate Forms Argininosuccinate

Argininosuccinate synthase links aspartate and citrulline via the amino group of aspartate (reaction 3, Figure 29–9) and provides the second nitrogen of urea. The reaction requires ATP and involves intermediate formation of citrullyl-AMP. Subsequent displacement of AMP by aspartate then forms citrulline.

Cleavage of Argininosuccinate Forms Arginine & Fumarate

Cleavage of argininosuccinate, catalyzed by **argininosuccinase,** proceeds with retention of nitrogen in arginine and release of the aspartate skeleton as fumarate (reaction 4, Figure 29–9). Addition of water to fumarate forms L-malate, and subsequent NAD^+-dependent oxidation of malate forms oxaloacetate. These two reactions are analogous to reactions of the citric acid cycle (see Figure 16–3) but are catalyzed by *cytosolic* fumarase and malate dehydrogenase. Transamination of oxaloacetate by glutamate aminotransferase then re-forms aspartate. The carbon skeleton of aspartate-fumarate thus acts as a carrier of the nitrogen of glutamate into a precursor of urea.

Cleavage of Arginine Releases Urea & Re-forms Ornithine

Hydrolytic cleavage of the guanidino group of arginine, catalyzed by liver **arginase,** releases urea (reaction 5, Figure 29–9). The other product, ornithine, reenters liver mitochondria for additional rounds of urea synthesis. Ornithine and lysine are potent inhibitors of arginase, competitive with arginine. Arginine also serves as the precursor of the potent muscle relaxant nitric oxide (NO) in a Ca^{2+}-dependent reaction catalyzed by NO synthase (see Figure 49–15).

Carbamoyl Phosphate Synthase I Is the Pacemaker Enzyme of the Urea Cycle

The activity of carbamoyl phosphate synthase I is determined by *N*-acetylglutamate, whose steady-state level is dictated by its rate of synthesis from acetyl-CoA and glutamate and its rate of hydrolysis to acetate and glutamate. These reactions are catalyzed by *N*-acetylglutamate synthase and *N*-acetylglutamate hydrolase, respectively. Major changes in diet can increase the concentrations of individual urea cycle enzymes 10-fold to 20-fold. Starvation, for example, elevates enzyme levels, presumably to cope with the increased production of ammonia that accompanies enhanced protein degradation.

METABOLIC DISORDERS ARE ASSOCIATED WITH EACH REACTION OF THE UREA CYCLE

Metabolic disorders of urea biosynthesis, while extremely rare, illustrate four important principles: (1) Defects in any of several enzymes of a metabolic pathway enzyme can result in similar clinical signs and symptoms. (2) The identification of intermediates and of ancillary products that accumulate prior to a metabolic block provides insight into the reaction that is impaired. (3) Precise diagnosis requires quantitative assay of the activity of the enzyme thought to be defective. (4) Rational therapy must be based on an understanding of the underlying biochemical reactions in normal and impaired individuals.

All defects in urea synthesis result in ammonia intoxication. Intoxication is more severe when the metabolic block occurs at reactions 1 or 2 since some covalent linking of ammonia to carbon has already occurred if citrulline can be synthesized. Clinical symptoms common to all urea cycle disorders include vomiting, avoidance of high-protein foods, intermittent ataxia, irritability, lethargy, and mental retardation. The clinical features and treatment of all five disorders discussed below are similar. Significant improvement and minimization of brain damage accompany a low-protein diet ingested as frequent small meals to avoid sudden increases in blood ammonia levels.

Hyperammonemia Type 1. A consequence of **carbamoyl phosphate synthase I** deficiency (reaction 1, Figure 29–9), this relatively infrequent condition (estimated frequency 1:62,000) probably is familial.

Hyperammonemia Type 2. A deficiency of **ornithine transcarbamoylase** (reaction 2, Figure 29–9) produces this X chromosome–linked deficiency. The mothers also exhibit hyperammonemia and an aversion to high-protein foods. Levels of glutamine are elevated in blood, cerebrospinal fluid, and urine, probably due to enhanced glutamine synthesis in response to elevated levels of tissue ammonia.

Citrullinemia. In this rare disorder, plasma and cerebrospinal fluid citrulline levels are elevated and 1–2 g of citrulline are excreted daily. One patient lacked detectable **argininosuccinate synthase** activity (reaction 3, Figure 29–9). In another, the K_m for citrulline was 25 times higher than normal. Citrulline and argininosuccinate, which contain nitrogen destined for urea synthesis, serve as alternative carriers of excess nitrogen. Feeding arginine enhanced excretion of citrulline in these patients. Similarly, feeding benzoate diverts ammonia nitrogen to hippurate via glycine (see Figure 31–1).

Argininosuccinicaciduria. A rare disease characterized by elevated levels of argininosuccinate in blood, cerebrospinal fluid, and urine is associated with friable, tufted hair (trichorrhexis nodosa). Both early-onset and late-onset types are known. The metabolic defect is the absence of **argininosuccinase** (reaction 4, Figure 29–9). Diagnosis by measurement of erythrocyte argininosuccinase activity can be performed on umbilical cord blood or amniotic fluid cells. As for citrullinemia, feeding arginine and benzoate promotes nitrogen excretion.

Hyperargininemia. This defect is characterized by elevated blood and cerebrospinal fluid arginine levels, low erythrocyte levels of arginase (reaction 5, Figure 29–9), and a urinary amino acid pattern resembling that of lysine-cystinuria. This pattern may reflect competition by arginine with lysine and cystine for reabsorption in the renal tubule. A low-protein diet lowers plasma ammonia levels and abolishes lysine-cystinuria.

Gene Therapy Offers Promise for Correcting Defects in Urea Biosynthesis

Gene therapy for rectification of defects in the enzymes of the urea cycle is an area of active investigation. Encouraging preliminary results have been obtained, for example, in animal models using an adenoviral vector to treat citrullinemia.

SUMMARY

- Human subjects degrade 1–2% of their body protein daily at rates that vary widely between proteins and with physiologic state. Key regulatory enzymes often have short half-lives.
- Proteins are degraded by both ATP-dependent and ATP-independent pathways. Ubiquitin targets many intracellular proteins for degradation. Liver cell surface receptors bind and internalize circulating asialoglycoproteins destined for lysosomal degradation.
- Ammonia is highly toxic. Fish excrete NH_3 directly; birds convert NH_3 to uric acid. Higher vertebrates convert NH_3 to urea.
- Transamination channels α-amino acid nitrogen into glutamate. L-Glutamate dehydrogenase (GDH) occupies a central position in nitrogen metabolism.
- Glutamine synthase converts NH_3 to nontoxic glutamine. Glutaminase releases NH_3 for use in urea synthesis.
- NH_3, CO_2, and the amide nitrogen of aspartate provide the atoms of urea.
- Hepatic urea synthesis takes place in part in the mitochondrial matrix and in part in the cytosol. Inborn errors of metabolism are associated with each reaction of the urea cycle.
- Changes in enzyme levels and allosteric regulation of carbamoyl phosphate synthase by *N*-acetylglutamate regulate urea biosynthesis.

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Catabolism of the Carbon Skeletons of Amino Acids

30

Victor W. Rodwell, PhD

BIOMEDICAL IMPORTANCE

This chapter considers conversion of the carbon skeletons of the common L-amino acids to amphibolic intermediates and the metabolic diseases or "inborn errors of metabolism" associated with these processes. Left untreated, they can result in irreversible brain damage and early mortality. Prenatal or early postnatal detection and timely initiation of treatment thus are essential. Many of the enzymes concerned can be detected in cultured amniotic fluid cells, which facilitates early diagnosis by amniocentesis. Treatment consists primarily of feeding diets low in the amino acids whose catabolism is impaired. While many changes in the primary structure of enzymes have no adverse effects, others modify the three-dimensional structure of catalytic or regulatory sites, lower catalytic efficiency (lower V_{max} or elevate K_m), or alter the affinity for an allosteric regulator of activity. A variety of mutations thus may give rise to the same clinical signs and symptoms.

TRANSAMINATION TYPICALLY INITIATES AMINO ACID CATABOLISM

Removal of α-amino nitrogen by transamination (see Figure 28–3) is the first catabolic reaction of amino acids except in the case of proline, hydroxyproline, threonine, and lysine. The residual hydrocarbon skeleton is then degraded to amphibolic intermediates as outlined in Figure 30–1.

Asparagine, Aspartate, Glutamine, and Glutamate. All four carbons of asparagine and aspartate form **oxaloacetate** (Figure 30–2, top). Analogous reactions convert glutamine and glutamate to **α-ketoglutarate** (Figure 30–2, bottom). Since the enzymes also fulfill anabolic functions, no metabolic defects are associated with the catabolism of these four amino acids.

Proline. Proline forms dehydroproline, glutamate-γ-semialdehyde, glutamate, and, ultimately, **α-ketoglutarate** (Figure 30–3, top). The metabolic block in **type I hyperprolinemia** is at **proline dehydrogenase.**

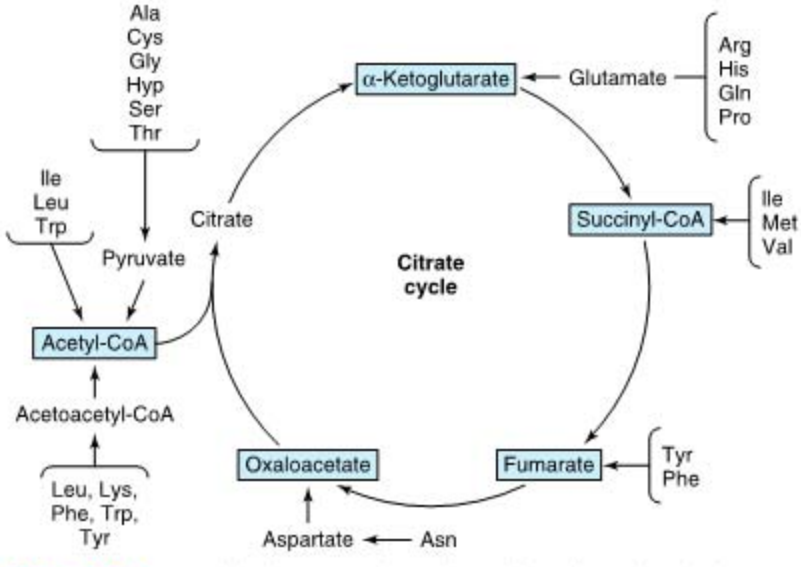

Figure 30–1. Amphibolic intermediates formed from the carbon skeletons of amino acids.

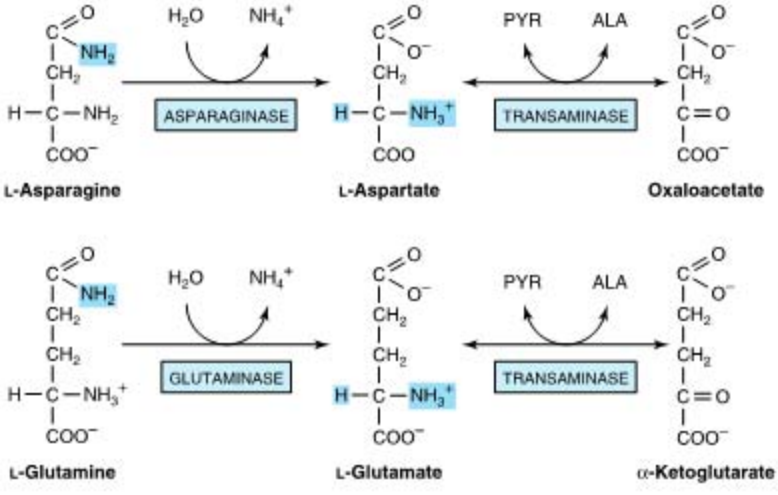

Figure 30–2. Catabolism of L-asparagine (*top*) and of L-glutamine (*bottom*) to amphibolic intermediates. (PYR, pyruvate; ALA, L-alanine.) In this and subsequent figures, color highlights portions of the molecules undergoing chemical change.

There is no associated impairment of hydroxyproline catabolism. The metabolic block in **type II hyperprolinemia** is at **glutamate-γ-semialdehyde dehydrogenase,** which also functions in hydroxyproline catabolism. Both proline and hydroxyproline catabolism thus are affected and Δ^1-pyrroline-3-hydroxy-5-carboxylate (see Figure 30–10) is excreted.

Arginine and Ornithine. Arginine is converted to ornithine, glutamate γ-semialdehyde, and then **α-ketoglutarate** (Figure 30–3, bottom). Mutations in **ornithine δ-aminotransferase** elevate plasma and urinary ornithine and cause **gyrate atrophy of the retina.** Treatment involves restricting dietary arginine. In **hyperornithinemia-hyperammonemia syndrome,** a defective mitochondrial **ornithine-citrulline antiporter** (see Figure 29–9) impairs transport of ornithine into mitochondria for use in urea synthesis.

Histidine. Catabolism of histidine proceeds via urocanate, 4-imidazolone-5-propionate, and *N*-formiminoglutamate (Figlu). Formimino group transfer to tetrahydrofolate forms glutamate, then **α-ketoglutarate** (Figure 30–4). In folic acid deficiency, group transfer is impaired and Figlu is excreted. Excretion of Figlu following a dose of histidine thus has been used to detect folic acid deficiency. Benign disorders of histidine catabolism include **histidinemia** and **urocanic aciduria** associated with impaired **histidase.**

SIX AMINO ACIDS FORM PYRUVATE

All of the carbons of glycine, serine, alanine, and cysteine and two carbons of threonine form pyruvate and subsequently acetyl-CoA.

Glycine. The **glycine synthase complex** of liver mitochondria splits glycine to CO_2 and NH_4^+ and forms N^5,N^{10}-methylene tetrahydrofolate (Figure 30–5).

Glycinuria results from a defect in renal tubular reabsorption. The defect in **primary hyperoxaluria** is the failure to catabolize glyoxylate formed by deamination of glycine. Subsequent oxidation of glyoxylate to oxalate results in urolithiasis, nephrocalcinosis, and early mortality from renal failure or hypertension.

Serine. Following conversion to glycine, catalyzed by **serine hydroxymethyltransferase** (Figure 30–5), serine catabolism merges with that of glycine (Figure 30–6).

Alanine. Transamination of alanine forms pyruvate. Perhaps for the reason advanced under glutamate and aspartate catabolism, there is no known metabolic defect of alanine catabolism. **Cysteine.** Cystine is first reduced to cysteine by **cystine reductase** (Figure 30–7). Two different pathways then convert cysteine to pyruvate (Figure 30–8).

There are numerous abnormalities of cysteine metabolism. Cystine, lysine, arginine, and ornithine are excreted in **cystine-lysinuria (cystinuria),** a defect in renal reabsorption. Apart from cystine calculi, cystinuria is benign. The mixed disulfide of L-cysteine and L-homocysteine (Figure 30–9) excreted by cystinuric patients is more soluble than cystine and reduces formation of cystine calculi. Several metabolic defects result in vitamin B_6-responsive or -unresponsive **homocystinurias.** Defective carrier-mediated transport of cystine results in **cystinosis (cystine storage disease)** with deposition of cystine crystals in tissues and early mortality from acute renal failure. Despite

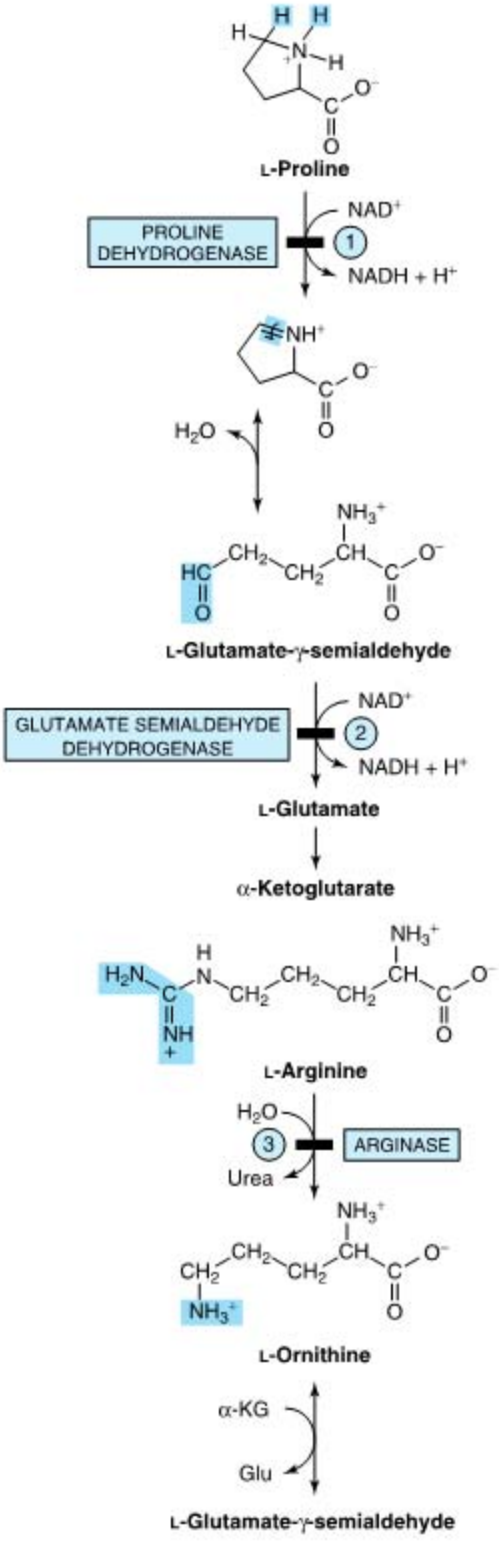

Figure 30–3. **Top:** Catabolism of proline. Numerals indicate sites of the metabolic defects in ① type I and ② type II hyperprolinemias. **Bottom:** Catabolism of arginine. Glutamate-γ-semialdehyde forms α-ketoglutarate as shown above. ③, site of the metabolic defect in hyperargininemia.

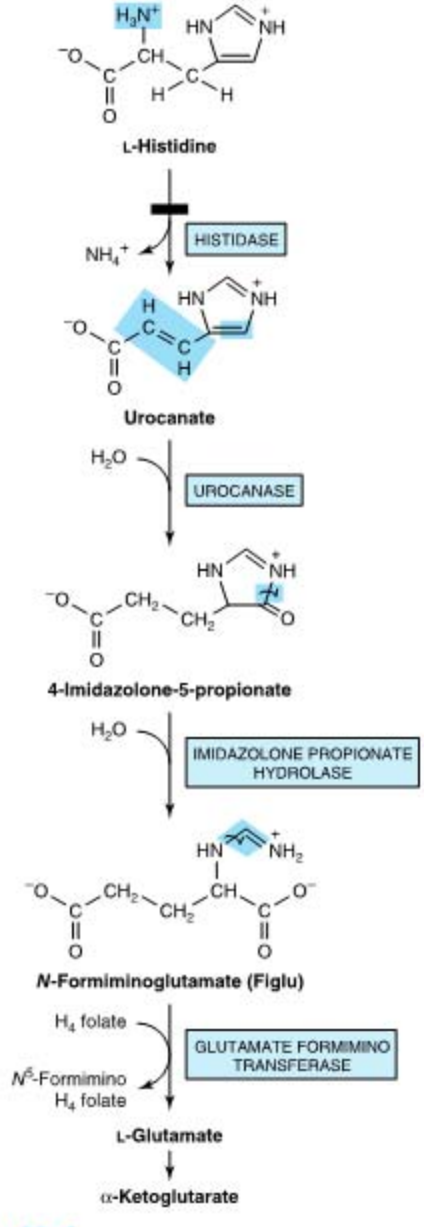

Figure 30–4. Catabolism of L-histidine to α-ketoglutarate. (H_4 folate, tetrahydrofolate.) Histidase is the probable site of the metabolic defect in histidinemia.

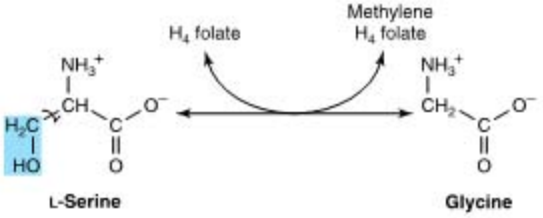

Figure 30–5. Interconversion of serine and glycine catalyzed by serine hydroxymethyltransferase. (H_4 folate, tetrahydrofolate.)

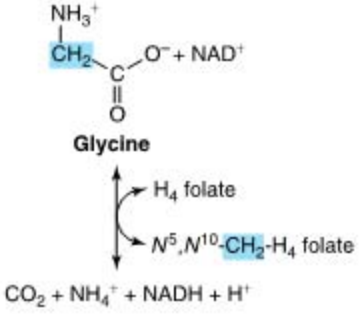

Figure 30–6. Reversible cleavage of glycine by the mitochondrial glycine synthase complex. (PLP, pyridoxal phosphate.)

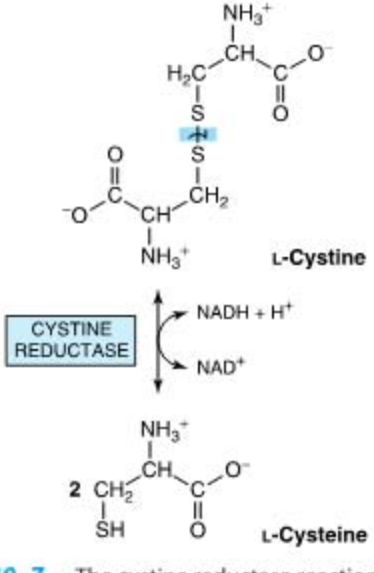

Figure 30–7. The cystine reductase reaction.

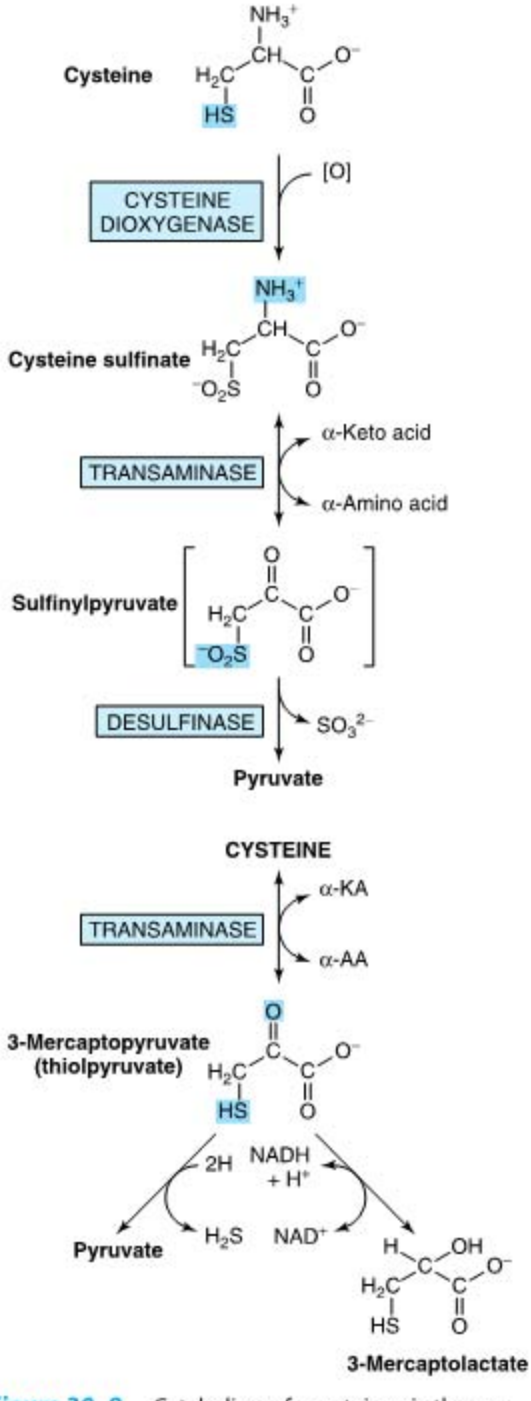

Figure 30–8. Catabolism of L-cysteine via the cysteine sulfinate pathway *(top)* and by the 3-mercaptopyruvate pathway *(bottom)*.

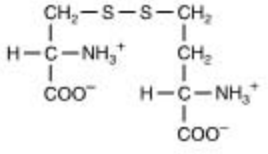

Figure 30–9. Mixed disulfide of cysteine and homocysteine.

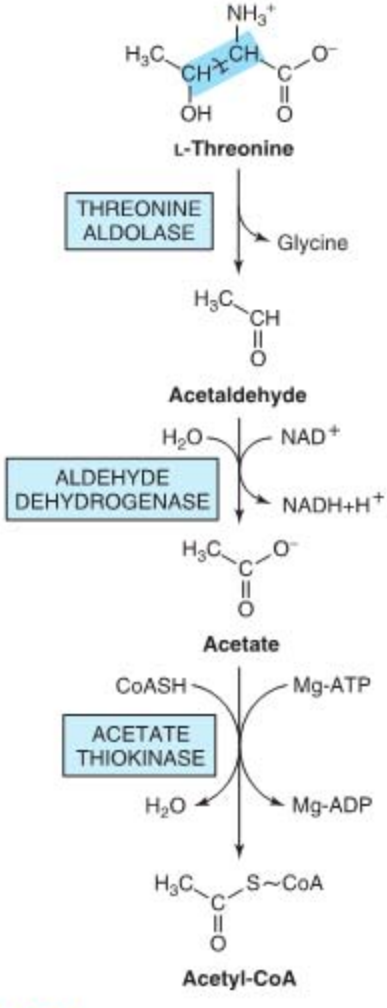

Figure 30–10. Conversion of threonine to glycine (see Figure 30–6) and acetyl-CoA.

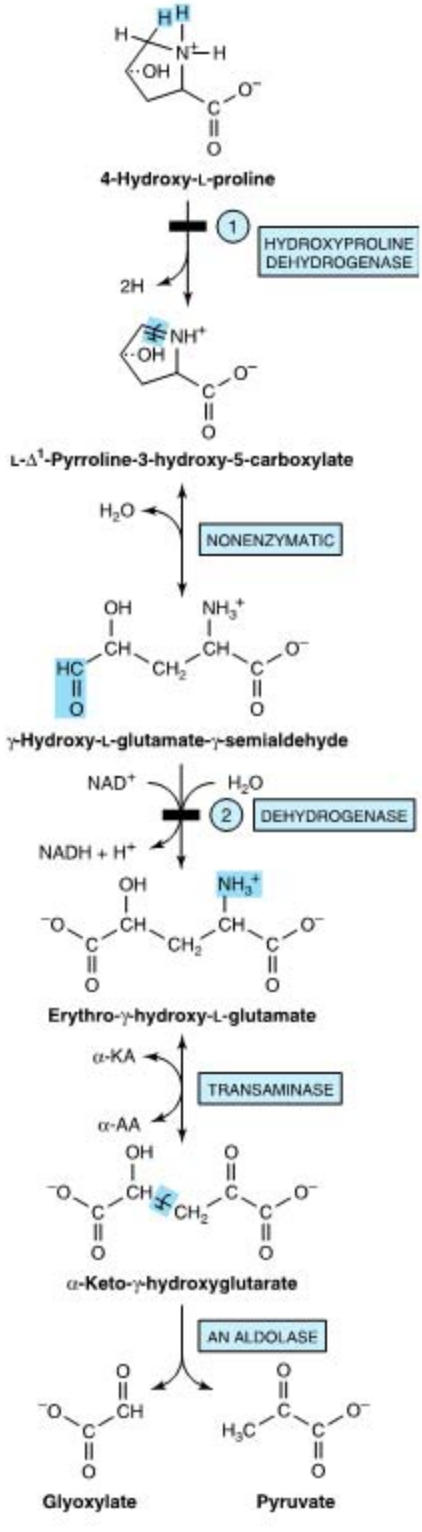

Figure 30–11. Intermediates in L-hydroxyproline catabolism. (α-KA, α-keto acid; α-AA, α-amino acid.) Numerals identify sites of metabolic defects in ① hyperhydroxyprolinemia and ② type II hyperprolinemia.

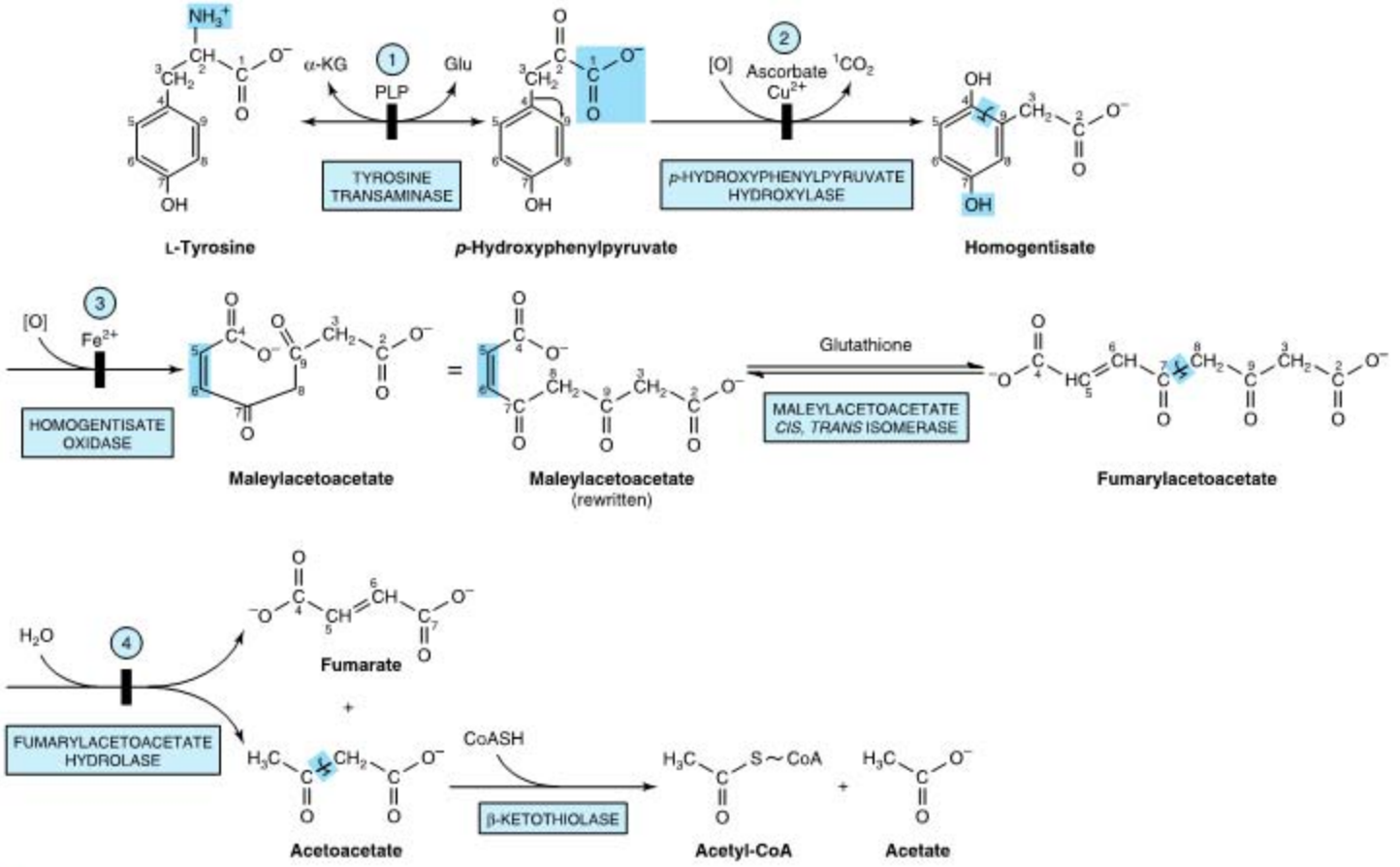

Figure 30–12. Intermediates in tyrosine catabolism. Carbons are numbered to emphasize their ultimate fate. (α-KG, α-ketoglutarate; Glu, glutamate; PLP, pyridoxal phosphate.) Circled numerals represent the probable sites of the metabolic defects in ① type II tyrosinemia; ② neonatal tyrosinemia; ③ alkaptonuria; and ④ type I tyrosinemia, or tyrosinosis.

epidemiologic data suggesting a relationship between plasma homocysteine and cardiovascular disease, whether homocysteine represents a causal cardiovascular risk factor remains controversial.

Threonine. Threonine is cleaved to acetaldehyde and glycine. Oxidation of acetaldehyde to acetate is followed by formation of acetyl-CoA (Figure 30–10). Catabolism of glycine is discussed above.

4-Hydroxyproline. Catabolism of 4-hydroxy-L-proline forms, successively, L-Δ^1-pyrroline-3-hydroxy-5-carboxylate, γ-hydroxy-L-glutamate-γ-semialdehyde, erythro-γ-hydroxy-L-glutamate, and α-keto-γ-hydroxyglutarate. An aldol-type cleavage then forms glyoxylate plus pyruvate (Figure 30–11). A defect in **4-hydroxyproline dehydrogenase** results in **hyperhydroxyprolinemia,** which is benign. There is no associated impairment of proline catabolism.

TWELVE AMINO ACIDS FORM ACETYL-CoA

Tyrosine. Figure 30–12 diagrams the conversion of tyrosine to amphibolic intermediates. Since ascorbate is the reductant for conversion of *p*-hydroxyphenylpyruvate to homogentisate, scorbutic patients excrete incompletely oxidized products of tyrosine catabolism. Subsequent catabolism forms maleylacetoacetate, fumarylacetoacetate, fumarate, acetoacetate, and ultimately **acetyl-CoA.**

The probable metabolic defect in **type I tyrosinemia (tyrosinosis)** is at **fumarylacetoacetate hydrolase** (reaction 4, Figure 30–12). Therapy employs a diet low in tyrosine and phenylalanine. Untreated acute and chronic tyrosinosis leads to death from liver failure. Alternate metabolites of tyrosine are also excreted in **type II tyrosinemia (Richner-Hanhart syndrome),** a defect in **tyrosine aminotransferase** (reaction 1, Figure 30–12), and in **neonatal tyrosinemia,** due to lowered *p*-hydroxyphenylpyruvate hydroxylase activity (reaction 2, Figure 30–12). Therapy employs a diet low in protein.

Alkaptonuria was first described in the 16th century. Characterized in 1859, it provided the basis for Garrod's classic ideas concerning heritable metabolic disorders. The defect is lack of **homogentisate oxidase** (reaction 3, Figure 30–12). The urine darkens on exposure to air due to oxidation of excreted homogentisate. Late in the disease, there is arthritis and connective tissue pigmentation (ochronosis) due to oxidation of homogentisate to benzoquinone acetate, which polymerizes and binds to connective tissue.

Phenylalanine. Phenylalanine is first converted to tyrosine (see Figure 28–10). Subsequent reactions are those of tyrosine (Figure 30–12). **Hyperphenylalaninemias** arise from defects in phenylalanine hydroxylase itself (**type I, classic phenylketonuria** or **PKU**), in dihydrobiopterin reductase (**types II and III**), or in dihydrobiopterin biosynthesis (**types IV and V**) (Figure 28–10). Alternative catabolites are excreted (Figure 30–13). DNA probes facilitate prenatal diagnosis of defects in phenylalanine hydroxylase or dihydrobiopterin reductase. A diet low in phenylalanine can prevent the mental retardation of PKU (frequency 1:10,000

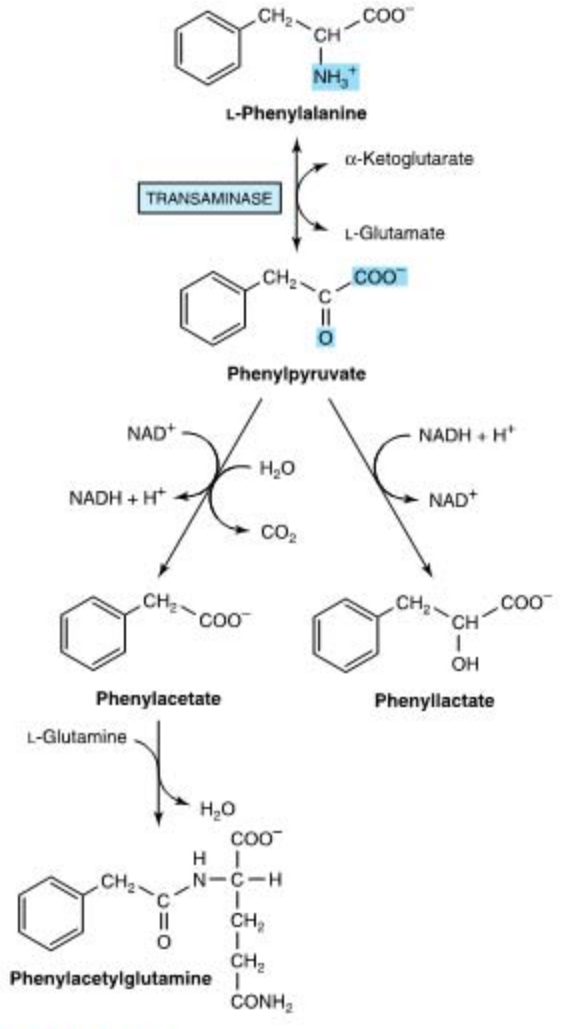

Figure 30–13. Alternative pathways of phenylalanine catabolism in phenylketonuria. The reactions also occur in normal liver tissue but are of minor significance.

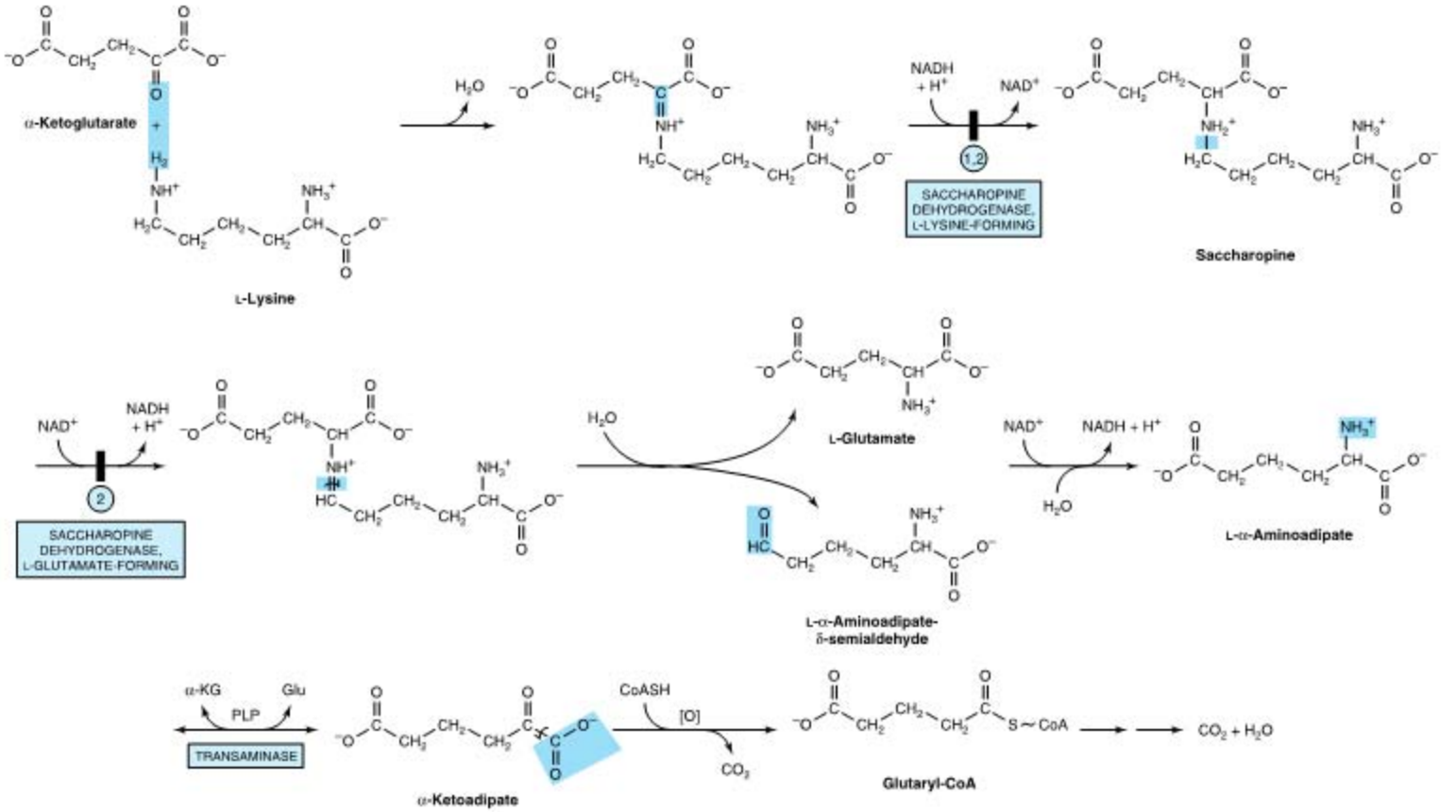

Figure 30–14. Catabolism of L-lysine. (α-KG, α-ketoglutarate; Glu, glutamate; PLP, pyridoxal phosphate.) Circled numerals indicate the probable sites of the metabolic defects in ① periodic hyperlysinemia with associated hyperammonemia; and ② persistent hyperlysinemia without associated hyperammonemia.

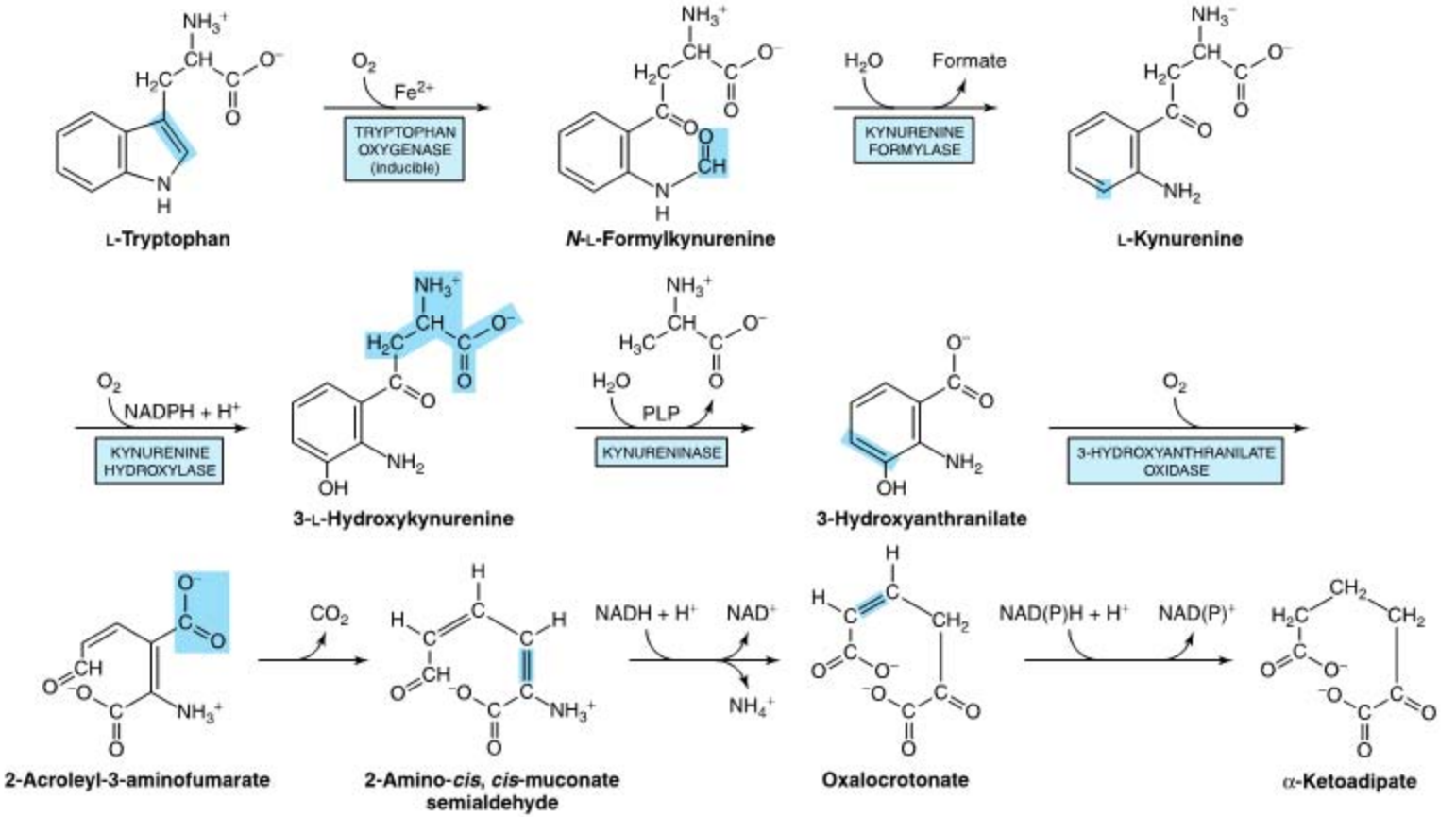

Figure 30–15. Catabolism of L-tryptophan. (PLP, pyridoxal phosphate.)

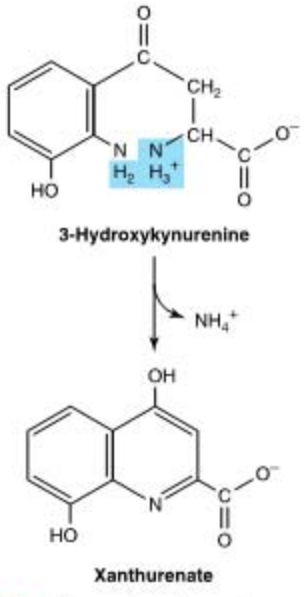

Figure 30–16. Formation of xanthurenate in vitamin B_6 deficiency. Conversion of the tryptophan metabolite 3-hydroxykynurenine to 3-hydroxyanthranilate is impaired (see Figure 30–15). A large portion is therefore converted to xanthurenate.

births). Elevated blood phenylalanine may not be detectable until 3–4 days postpartum. False-positives in premature infants may reflect delayed maturation of enzymes of phenylalanine catabolism. A less reliable screening test employs $FeCl_3$ to detect urinary phenylpyruvate. $FeCl_3$ screening for PKU of the urine of newborn infants is compulsory in the United States and many other countries.

Lysine. Figure 30–14 summarizes the catabolism of lysine. Lysine first forms a Schiff base with α-ketoglutarate, which is reduced to **saccharopine.** In one form of **periodic hyperlysinemia**, elevated lysine competitively inhibits liver **arginase** (see Figure 29–9), causing hyperammonemia. Restricting dietary lysine relieves the ammonemia, whereas ingestion of a lysine load precipitates severe crises and coma. In a different **periodic hyperlysinemia,** lysine catabolites accumulate, but even a lysine load does not trigger hyperammonemia. In addition to impaired synthesis of saccharopine, some patients cannot cleave saccharopine.

Tryptophan. Tryptophan is degraded to amphibolic intermediates via the kynurenine-anthranilate pathway (Figure 30–15). **Tryptophan oxygenase (tryptophan pyrrolase)** opens the indole ring, incorporates molecular oxygen, and forms *N*-formylkynurenine. An iron porphyrin metalloprotein that is inducible in liver by adrenal corticosteroids and by tryptophan, tryptophan oxygenase is feedback-inhibited by nicotinic acid derivatives, including NADPH. Hydrolytic removal of the formyl group of *N*-formylkynurenine, catalyzed by **kynurenine formylase,** produces kynurenine. Since **kynureninase** requires pyridoxal phosphate, excretion of xanthurenate (Figure 30–16) in response to a tryptophan load is diagnostic of vitamin B_6 deficiency. **Hartnup disease** reflects impaired intestinal and renal transport of tryptophan and other neutral amino acids. Indole derivatives of unabsorbed tryptophan formed by intestinal bacteria are excreted. The defect limits tryptophan availability for niacin biosynthesis and accounts for the pellagra-like signs and symptoms.

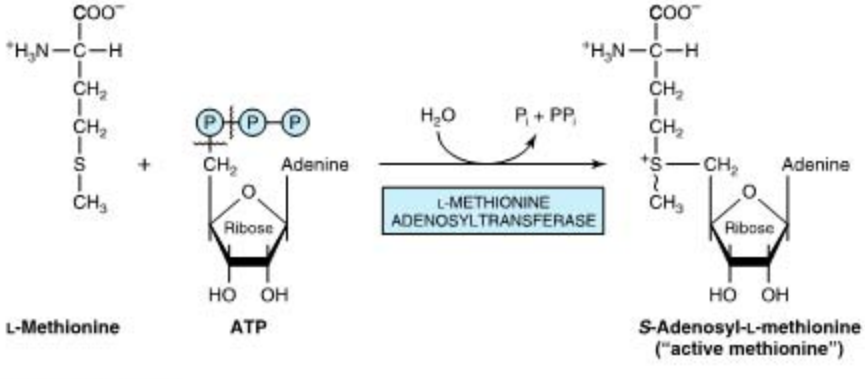

Figure 30–17. Formation of *S*-adenosylmethionine. ~CH_3 represents the high group transfer potential of "active methionine."

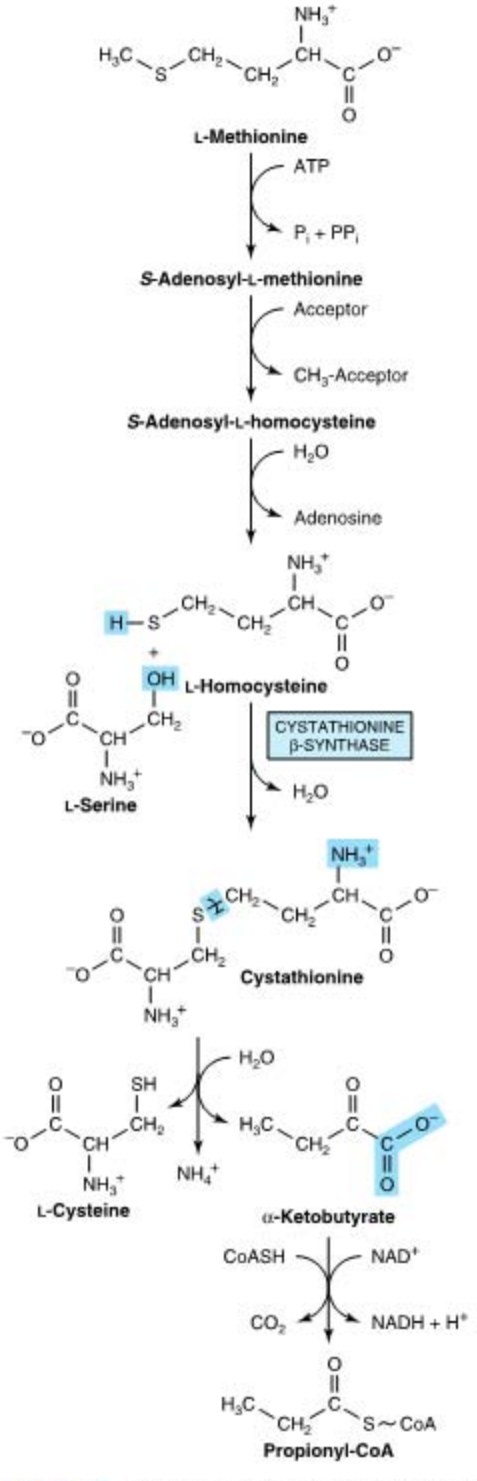

Figure 30–18. Conversion of methionine to propionyl-CoA.

Methionine. Methionine reacts with ATP forming *S*-adenosylmethionine, "active methionine" (Figure 30–17). Subsequent reactions form propionyl-CoA (Figure 30–18) and ultimately succinyl-CoA (see Figure 19–2).

THE INITIAL REACTIONS ARE COMMON TO ALL THREE BRANCHED-CHAIN AMINO ACIDS

Reactions 1–3 of Figure 30–19 are analogous to those of fatty acid catabolism. Following transamination, all three α-keto acids undergo oxidative decarboxylation catalyzed by mitochondrial **branched-chain α-keto acid dehydrogenase.** This multimeric enzyme complex of a decarboxylase, a transacylase, and a dihydrolipoyl dehydrogenase closely resembles pyruvate dehydrogenase (see Figure 17–5). Its regulation also parallels that of pyruvate dehydrogenase, being inactivated by phosphorylation and reactivated by dephosphorylation (see Figure 17–6).

Reaction 3 is analogous to the dehydrogenation of fatty acyl-CoA thioesters (see Figure 22–3). In **isovaleric acidemia,** ingestion of protein-rich foods elevates isovalerate, the deacylation product of isovaleryl-CoA. Figures 30–20, 30–21, and 30–22 illustrate the subsequent reactions unique to each amino acid skeleton.

METABOLIC DISORDERS OF BRANCHED-CHAIN AMINO ACID CATABOLISM

As the name implies, the odor of urine in **maple syrup urine disease (branched-chain ketonuria)** suggests maple syrup or burnt sugar. The biochemical defect involves the **α-keto acid decarboxylase complex** (reaction 2, Figure 30–19). Plasma and urinary levels of leucine, isoleucine, valine, α-keto acids, and α-hydroxy acids (reduced α-keto acids) are elevated. The mechanism of toxicity is unknown. Early diagnosis, especially prior to 1 week of age, employs enzymatic analysis. Prompt replacement of dietary protein by an amino acid mixture that lacks leucine, isoleucine, and valine averts brain damage and early mortality.

Mutation of the dihydrolipoate reductase component impairs decarboxylation of branched-chain α-keto acids, of pyruvate, and of α-ketoglutarate. In **intermittent branched-chain ketonuria,** the α-keto acid decarboxylase retains some activity, and symptoms occur later in life. The impaired enzyme in **isovaleric acidemia** is **isovaleryl-CoA dehydrogenase** (reaction 3, Figure 30–19). Vomiting, acidosis, and coma follow ingestion of excess protein. Accumulated

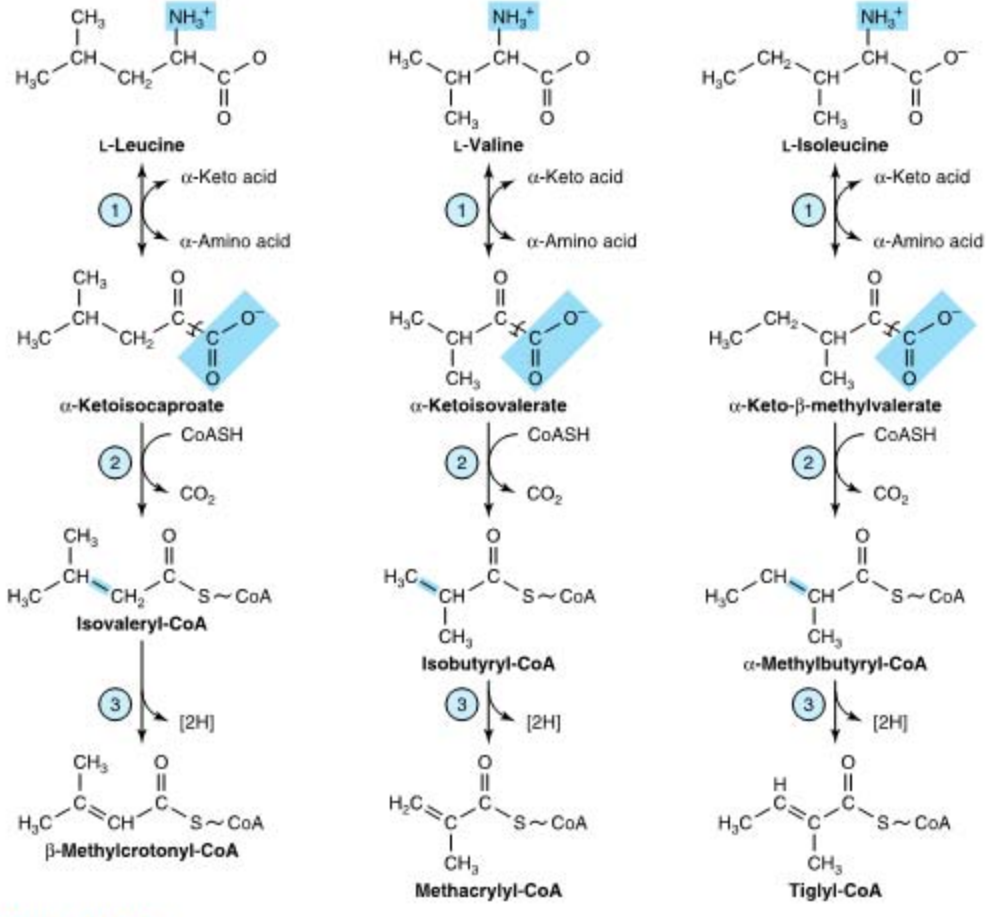

Figure 30–19. The analogous first three reactions in the catabolism of leucine, valine, and isoleucine. Note also the analogy of reactions ② and ③ to reactions of the catabolism of fatty acids (see Figure 22–3). The analogy to fatty acid catabolism continues, as shown in subsequent figures.

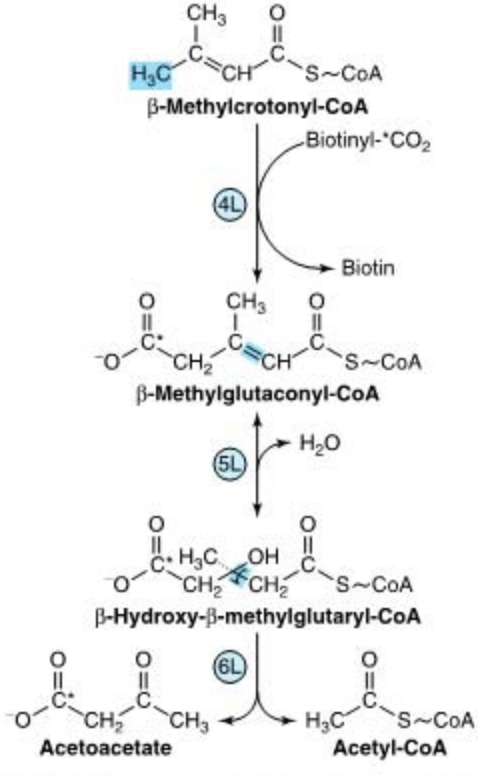

Figure 30–20. Catabolism of the β-methylcrotonyl-CoA formed from L-leucine. Asterisks indicate carbon atoms derived from CO_2.

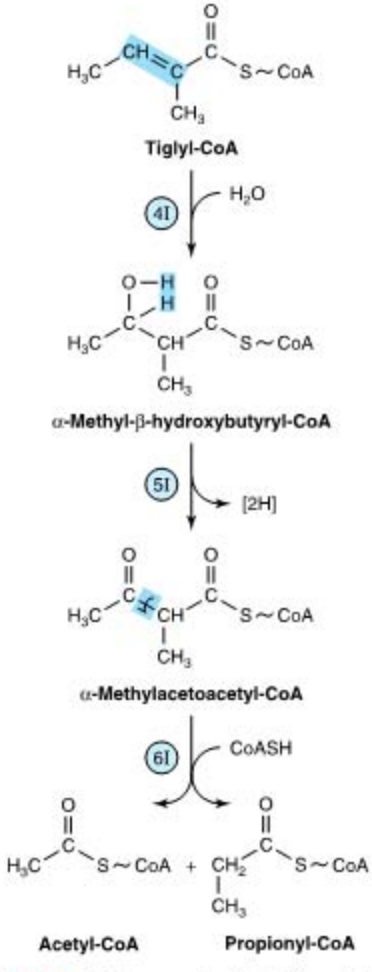

Figure 30–21. Subsequent catabolism of the tiglyl-CoA formed from L-isoleucine.

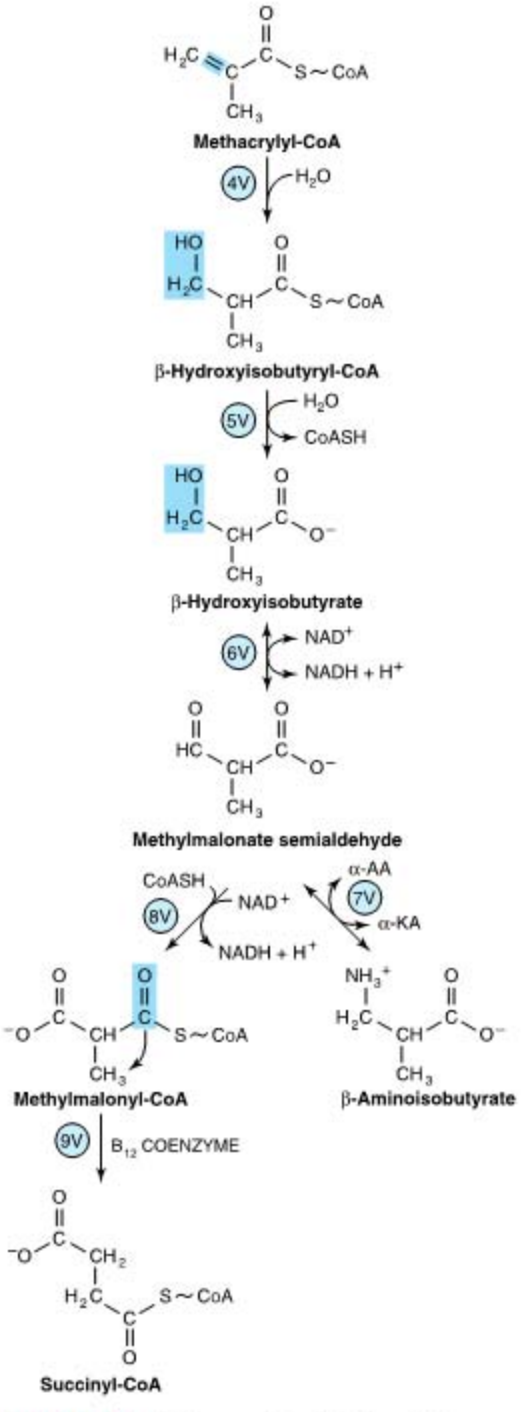

Figure 30–22. Subsequent catabolism of the methacrylyl-CoA formed from L-valine (see Figure 30–19). (α-KA, α-keto acid; α-AA, α-amino acid.)

isovaleryl-CoA is hydrolyzed to isovalerate and excreted.

SUMMARY

- Excess amino acids are catabolized to amphibolic intermediates used as sources of energy or for carbohydrate and lipid biosynthesis.
- Transamination is the most common initial reaction of amino acid catabolism. Subsequent reactions remove any additional nitrogen and restructure the hydrocarbon skeleton for conversion to oxaloacetate, α-ketoglutarate, pyruvate, and acetyl-CoA.
- Metabolic diseases associated with glycine catabolism include glycinuria and primary hyperoxaluria.
- Two distinct pathways convert cysteine to pyruvate. Metabolic disorders of cysteine catabolism include cystine-lysinuria, cystine storage disease, and the homocystinurias.
- Threonine catabolism merges with that of glycine after threonine aldolase cleaves threonine to glycine and acetaldehyde.
- Following transamination, the carbon skeleton of tyrosine is degraded to fumarate and acetoacetate. Metabolic diseases of tyrosine catabolism include tyrosinosis, Richner-Hanhart syndrome, neonatal tyrosinemia, and alkaptonuria.
- Metabolic disorders of phenylalanine catabolism include phenylketonuria (PKU) and several hyperphenylalaninemias.
- Neither nitrogen of lysine undergoes transamination. Metabolic diseases of lysine catabolism include periodic and persistent forms of hyperlysinemia-ammonemia.
- The catabolism of leucine, valine, and isoleucine presents many analogies to fatty acid catabolism. Metabolic disorders of branched-chain amino acid catabolism include hypervalinemia, maple syrup urine disease, intermittent branched-chain ketonuria, isovaleric acidemia, and methylmalonic aciduria.

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Conversion of Amino Acids to Specialized Products

31

Victor W. Rodwell, PhD

BIOMEDICAL IMPORTANCE

Important products derived from amino acids include heme, purines, pyrimidines, hormones, neurotransmitters, and biologically active peptides. In addition, many proteins contain amino acids that have been modified for a specific function such as binding calcium or as intermediates that serve to stabilize proteins—generally structural proteins—by subsequent covalent cross-linking. The amino acid residues in those proteins serve as precursors for these modified residues. Small peptides or peptide-like molecules not synthesized on ribosomes fulfill specific functions in cells. Histamine plays a central role in many allergic reactions. Neurotransmitters derived from amino acids include γ-aminobutyrate, 5-hydroxytryptamine (serotonin), dopamine, norepinephrine, and epinephrine. Many drugs used to treat neurologic and psychiatric conditions affect the metabolism of these neurotransmitters.

Glycine

Metabolites and pharmaceuticals excreted as water-soluble glycine conjugates include glycocholic acid (Chapter 24) and hippuric acid formed from the food additive benzoate (Figure 31–1). Many drugs, drug metabolites, and other compounds with carboxyl groups are excreted in the urine as glycine conjugates. Glycine is incorporated into creatine (see Figure 31–6), the nitrogen and α-carbon of glycine are incorporated into the pyrrole rings and the methylene bridge carbons of heme (Chapter 32), and the entire glycine molecule becomes atoms 4, 5, and 7 of purines (Figure 34–1).

β-Alanine

β-Alanine, a metabolite of cysteine (Figure 34–9), is present in coenzyme A and as β-alanyl dipeptides, principally carnosine (see below). Mammalian tissues form β-alanine from cytosine (Figure 34–9), carnosine, and anserine (Figure 31–2). Mammalian tissues transaminate β-alanine, forming malonate semialdehyde. Body fluid and tissue levels of β-alanine, taurine, and β-aminoisobutyrate are elevated in the rare metabolic disorder hyperbeta-alaninemia.

β-Alanyl Dipeptides

The β-alanyl dipeptides carnosine and anserine (*N*-methylcarnosine) (Figure 31–2) activate myosin ATPase, chelate copper, and enhance copper uptake. β-Alanyl-imidazole buffers the pH of anaerobically contracting skeletal muscle. Biosynthesis of carnosine is catalyzed by carnosine synthetase in a two-stage reaction that involves initial formation of an enzyme-bound acyl-adenylate of β-alanine and subsequent transfer of the β-alanyl moiety to L-histidine.

$$\text{ATP} + \beta\text{-Alanine} \rightarrow \beta\text{-Alanyl} - \text{AMP} \rightarrow + PP_i$$

$$\beta\text{-Alanyl} - \text{AMP} + \text{L-Histidine} \rightarrow \text{Carnosine} + \text{AMP}$$

Hydrolysis of carnosine to β-alanine and L-histidine is catalyzed by carnosinase. The heritable disorder carnosinase deficiency is characterized by carnosinuria.

Homocarnosine (Figure 31–2), present in human brain at higher levels than carnosine, is synthesized in brain tissue by carnosine synthetase. Serum carnosinase does not hydrolyze homocarnosine. Homocarnosinosis, a rare genetic disorder, is associated with progressive spastic paraplegia and mental retardation.

Phosphorylated Serine, Threonine, & Tyrosine

The phosphorylation and dephosphorylation of seryl, threonyl, and tyrosyl residues regulate the activity of certain enzymes of lipid and carbohydrate metabolism and the properties of proteins that participate in signal transduction cascades.

Methionine

S-Adenosylmethionine, the principal source of methyl groups in the body, also contributes its carbon skeleton for the biosynthesis of the 3-diaminopropane portions of the polyamines spermine and spermidine (Figure 31–4).

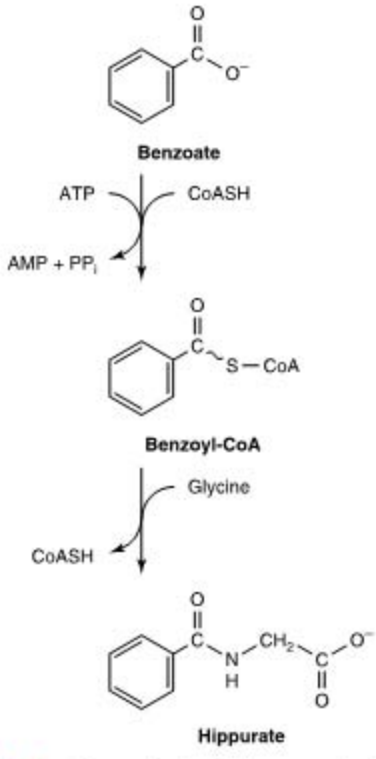

Figure 31–1. Biosynthesis of hippurate. Analogous reactions occur with many acidic drugs and catabolites.

Cysteine

L-Cysteine is a precursor of the thioethanolamine portion of coenzyme A and of the taurine that conjugates with bile acids such as taurocholic acid (Chapter 26).

Histidine

Decarboxylation of histidine to histamine is catalyzed by a broad-specificity aromatic L-amino acid decarboxylase that also catalyzes the decarboxylation of dopa, 5-hydroxytryptophan, phenylalanine, tyrosine, and tryptophan. α-Methyl amino acids, which inhibit decarboxylase activity, find application as antihypertensive agents. Histidine compounds present in the human body include ergothioneine, carnosine, and dietary anserine (Figure 31–2). Urinary levels of 3-methylhistidine are unusually low in patients with Wilson's disease.

Ornithine & Arginine

Arginine is the formamidine donor for creatine synthesis (Figure 31–6) and via ornithine to putrescine, spermine, and spermidine (Figure 31–3) Arginine is also the precursor of the intercellular signaling molecule ni-

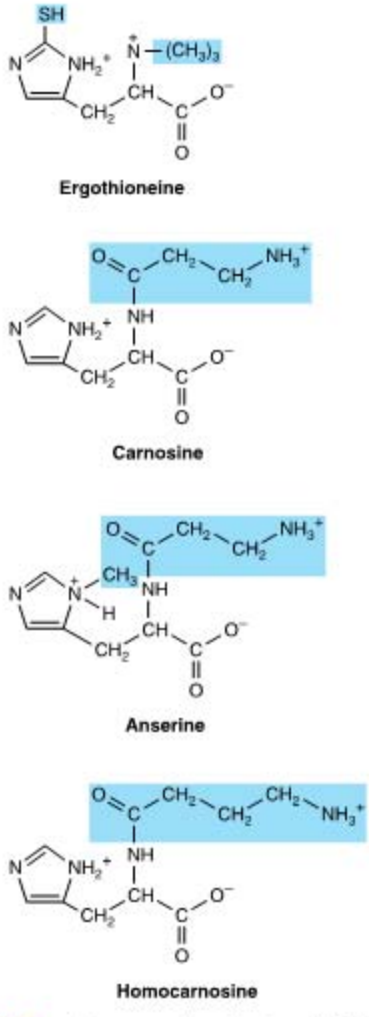

Figure 31–2. Compounds related to histidine. The boxes surround the components not derived from histidine. The SH group of ergothioneine derives from cysteine.

tric oxide (NO) that serves as a neurotransmitter, smooth muscle relaxant, and vasodilator. Synthesis of NO, catalyzed by NO synthase, involves the NADPH-dependent reaction of L-arginine with O_2 to yield L-citrulline and NO.

Polyamines

The polyamines spermidine and spermine (Figure 31–4) function in cell proliferation and growth, are growth factors for cultured mammalian cells, and stabilize intact cells, subcellular organelles, and membranes. Pharmacologic doses of polyamines are hypothermic

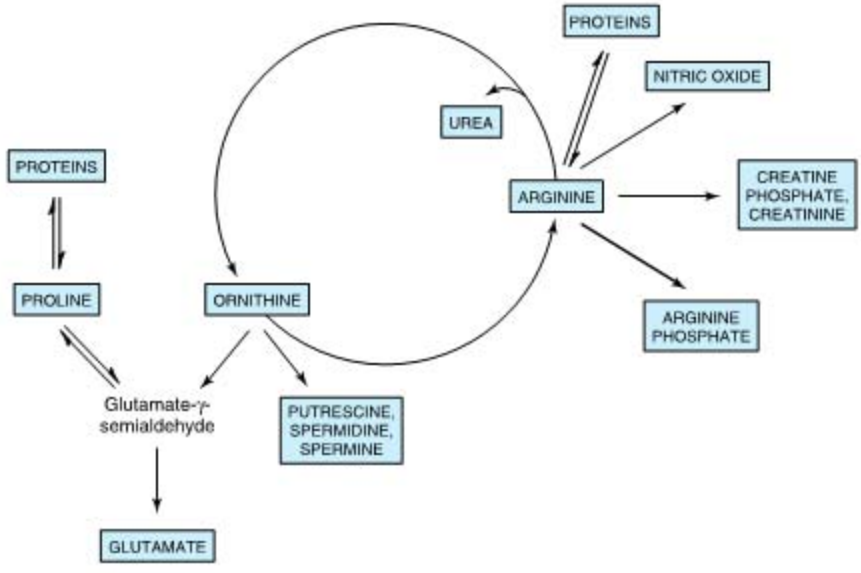

Figure 31–3. Arginine, ornithine, and proline metabolism. Reactions with solid arrows all occur in mammalian tissues. Putrescine and spermine synthesis occurs in both mammals and bacteria. Arginine phosphate of invertebrate muscle functions as a phosphagen analogous to creatine phosphate of mammalian muscle (see Figure 31–6).

and hypotensive. Since they bear multiple positive charges, polyamines associate readily with DNA and RNA. Figure 31–4 summarizes polyamine biosynthesis.

Tryptophan

Following hydroxylation of tryptophan to 5-hydroxytryptophan by liver tyrosine hydroxylase, subsequent decarboxylation forms serotonin (5-hydroxytryptamine), a potent vasoconstrictor and stimulator of smooth muscle contraction. Catabolism of serotonin is initiated by monoamine oxidase-catalyzed oxidative deamination to 5-hydroxyindoleacetate. The psychic stimulation that follows administration of iproniazid results from its ability to prolong the action of serotonin by inhibiting monoamine oxidase. In carcinoid (argentaffinoma), tumor cells overproduce serotonin. Urinary metabolites of serotonin in patients with carci-

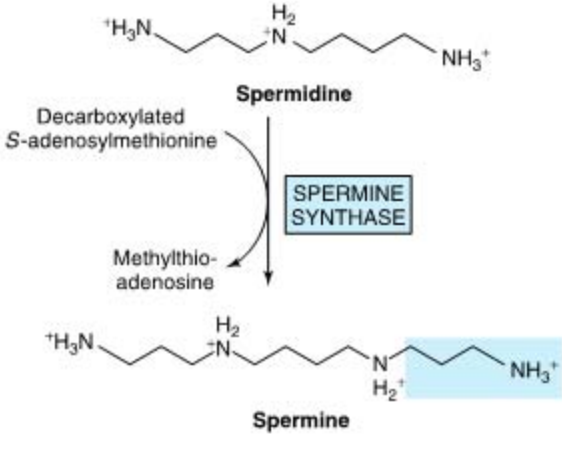

Figure 31–4. Conversion of spermidine to spermine. Spermidine formed from putrescine (decarboxylated L-ornithine) by transfer of a propylamine moiety from decarboxylated *S*-adenosylmethionine accepts a second propylamine moiety to form spermidine.

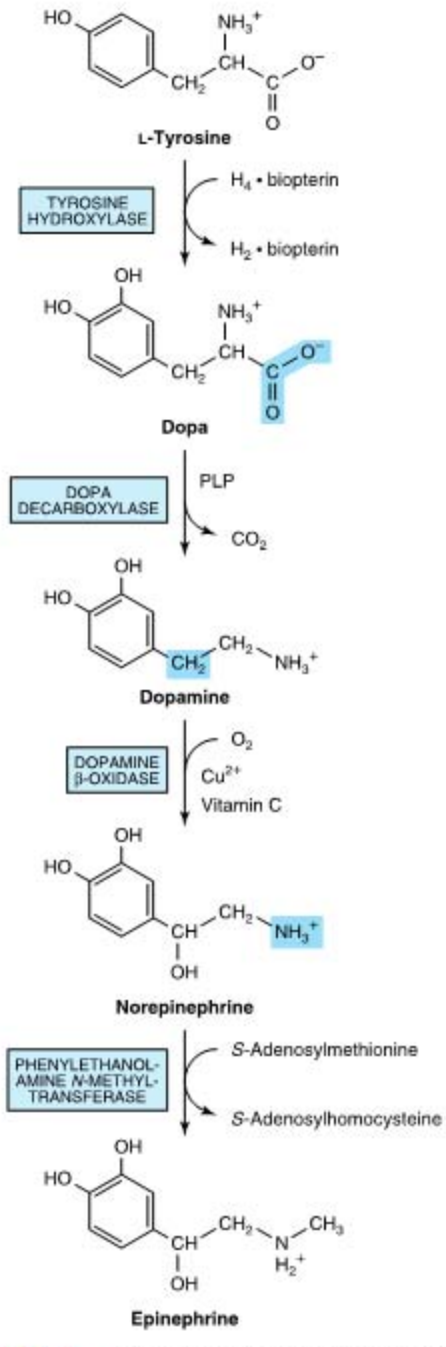

Figure 31–5. Conversion of tyrosine to epinephrine and norepinephrine in neuronal and adrenal cells. (PLP, pyridoxal phosphate.)

noid include *N*-acetylserotonin glucuronide and the glycine conjugate of 5-hydroxyindoleacetate. Serotonin and 5-methoxytryptamine are metabolized to the corresponding acids by monoamine oxidase. *N*-Acetylation of serotonin, followed by *O*-methylation in the pineal body, forms melatonin. Circulating melatonin is taken up by all tissues, including brain, but is rapidly metabolized by hydroxylation followed by conjugation with sulfate or with glucuronic acid.

Kidney tissue, liver tissue, and fecal bacteria all convert tryptophan to tryptamine, then to indole 3-acetate. The principal normal urinary catabolites of tryptophan are 5-hydroxyindoleacetate and indole 3-acetate.

Tyrosine

Neural cells convert tyrosine to epinephrine and norepinephrine (Figure 31–5). While dopa is also an intermediate in the formation of melanin, different enzymes hydroxylate tyrosine in melanocytes. Dopa decarboxylase, a pyridoxal phosphate-dependent enzyme, forms dopamine. Subsequent hydroxylation by dopamine β-oxidase then forms norepinephrine. In the adrenal medulla, phenylethanolamine-*N*-methyltransferase utilizes *S*-adenosylmethionine to methylate the primary amine of norepinephrine, forming epinephrine (Figure 31–5). Tyrosine is also a precursor of triiodothyronine and thyroxine (Chapter 42).

Creatinine

Creatinine is formed in muscle from creatine phosphate by irreversible, nonenzymatic dehydration and loss of phosphate (Figure 31–6). The 24-hour urinary excretion of creatinine is proportionate to muscle mass. Glycine, arginine, and methionine all participate in creatine biosynthesis. Synthesis of creatine is completed by methylation of guanidoacetate by *S*-adenosylmethionine (Figure 31–6).

γ-Aminobutyrate

γ-Aminobutyrate (GABA) functions in brain tissue as an inhibitory neurotransmitter by altering transmembrane potential differences. It is formed by decarboxylation of L-glutamate, a reaction catalyzed by L-glutamate decarboxylase (Figure 31–7). Transamination of γ-aminobutyrate forms succinate semialdehyde (Figure 31–7), which may then undergo reduction to γ-hydroxybutyrate, a reaction catalyzed by L-lactate dehydrogenase, or oxidation to succinate and thence via the citric acid cycle to CO_2 and H_2O. A rare genetic disorder of GABA metabolism involves a defective GABA aminotransferase, an enzyme that participates in the catabolism of GABA subsequent to its postsynaptic release in brain tissue.

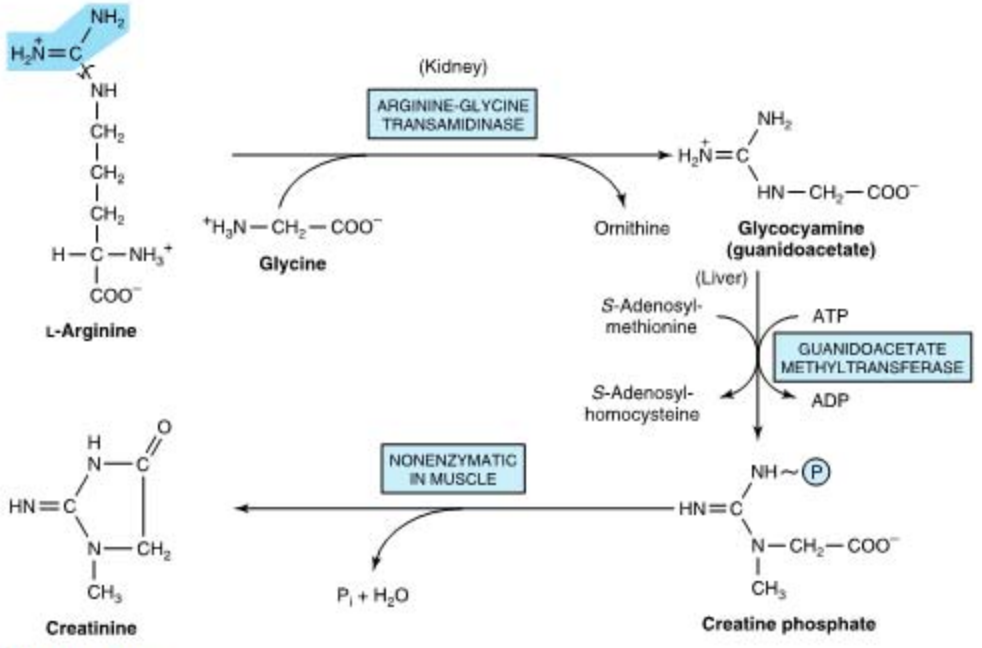

Figure 31–6. Biosynthesis and metabolism of creatine and creatinine.

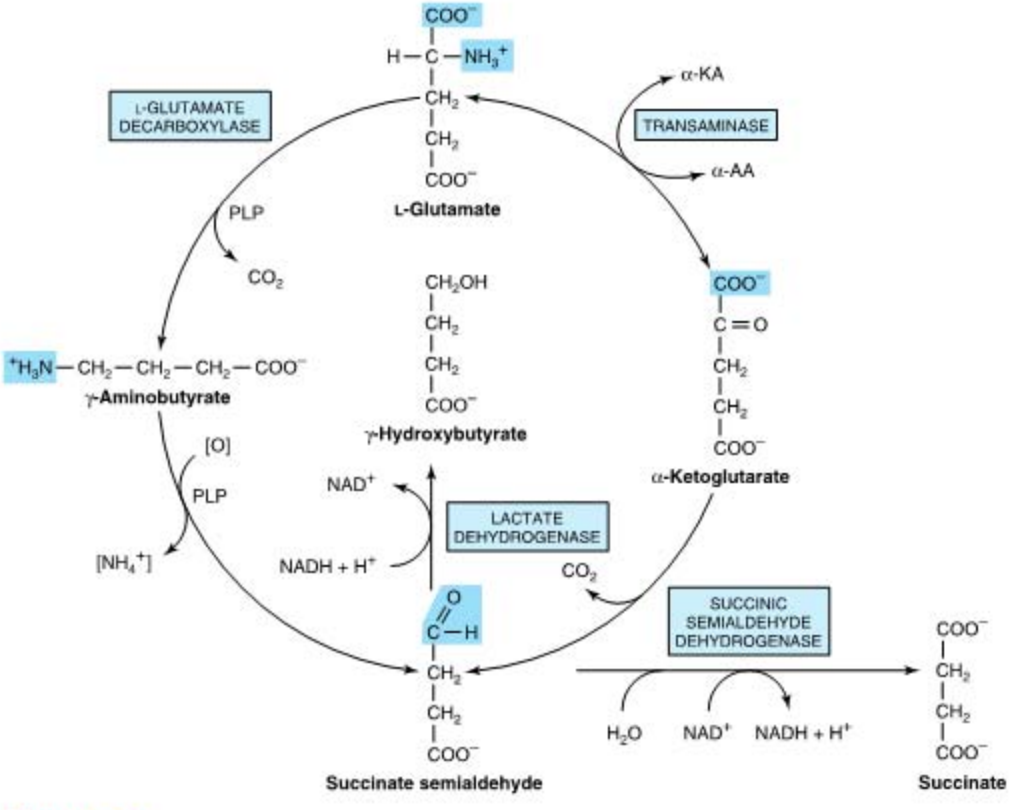

Figure 31–7. Metabolism of γ-aminobutyrate. (α-KA, α-keto acids; α-AA, α-amino acids; PLP, pyridoxal phosphate.)

SUMMARY

- In addition to their roles in proteins and polypeptides, amino acids participate in a wide variety of additional biosynthetic processes.
- Glycine participates in the biosynthesis of heme, purines, and creatine and is conjugated to bile acids and to the urinary metabolites of many drugs.
- In addition to its roles in phospholipid and sphingosine biosynthesis, serine provides carbons 2 and 8 of purines and the methyl group of thymine.
- *S*-Adenosylmethionine, the methyl group donor for many biosynthetic processes, also participates directly in spermine and spermidine biosynthesis.
- Glutamate and ornithine form the neurotransmitter γ-aminobutyrate (GABA).
- The thioethanolamine of coenzyme A and the taurine of taurocholic acid arise from cysteine.
- Decarboxylation of histidine forms histamine, and several dipeptides are derived from histidine and β-alanine.
- Arginine serves as the formamidine donor for creatine biosynthesis, participates in polyamine biosynthesis, and provides the nitrogen of nitric oxide (NO).
- Important tryptophan metabolites include serotonin, melanin, and melatonin.
- Tyrosine forms both epinephrine and norepinephrine, and its iodination forms thyroid hormone.

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Porphyrins & Bile Pigments

32

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BIOMEDICAL IMPORTANCE

The biochemistry of the porphyrins and of the bile pigments is presented in this chapter. These topics are closely related, because heme is synthesized from porphyrins and iron, and the products of degradation of heme are the bile pigments and iron.

Knowledge of the biochemistry of the porphyrins and of heme is basic to understanding the varied functions of hemoproteins (see below) in the body. The **porphyrias** are a group of diseases caused by abnormalities in the pathway of biosynthesis of the various porphyrins. Although porphyrias are not very prevalent, physicians must be aware of them. A much more prevalent clinical condition is **jaundice,** due to elevation of bilirubin in the plasma. This elevation is due to overproduction of bilirubin or to failure of its excretion and is seen in numerous diseases ranging from hemolytic anemias to viral hepatitis and to cancer of the pancreas.

METALLOPORPHYRINS & HEMOPROTEINS ARE IMPORTANT IN NATURE

Porphyrins are cyclic compounds formed by the linkage of four pyrrole rings through —HC═ methenyl bridges (Figure 32–1). A characteristic property of the porphyrins is the formation of complexes with metal ions bound to the nitrogen atom of the pyrrole rings. Examples are the **iron porphyrins** such as **heme** of hemoglobin and the **magnesium**-containing porphyrin **chlorophyll,** the photosynthetic pigment of plants.

Proteins that contain heme (hemoproteins) are widely distributed in nature. Examples of their importance in humans and animals are listed in Table 32–1.

Natural Porphyrins Have Substituent Side Chains on the Porphin Nucleus

The porphyrins found in nature are compounds in which various **side chains** are substituted for the eight hydrogen atoms numbered in the porphin nucleus shown in Figure 32–1. As a simple means of showing these substitutions, Fischer proposed a shorthand formula in which the methenyl bridges are omitted and each pyrrole ring is shown as indicated with the eight substituent positions numbered as shown in Figure 32–2. Various porphyrins are represented in Figures 32–2, 32–3, and 32–4.

The arrangement of the acetate (A) and propionate (P) substituents in the uroporphyrin shown in Figure 32–2 is asymmetric (in ring IV, the expected order of the A and P substituents is reversed). A porphyrin with this type of **asymmetric substitution** is classified as a type III porphyrin. A porphyrin with a completely symmetric arrangement of the substituents is classified as a type I porphyrin. Only types I and III are found in nature, and the type III series is far more abundant (Figure 32–3)—and more important because it includes heme.

Heme and its immediate precursor, protoporphyrin IX (Figure 32–4), are both type III porphyrins (ie, the methyl groups are asymmetrically distributed, as in type III coproporphyrin). However, they are sometimes identified as belonging to series IX, because they were designated ninth in a series of isomers postulated by Hans Fischer, the pioneer worker in the field of porphyrin chemistry.

HEME IS SYNTHESIZED FROM SUCCINYL-COA & GLYCINE

Heme is synthesized in living cells by a pathway that has been much studied. The two starting materials are **succinyl-CoA,** derived from the citric acid cycle in mitochondria, and the amino acid **glycine.** Pyridoxal phosphate is also necessary in this reaction to "activate" glycine. The product of the condensation reaction between succinyl-CoA and glycine is α-amino-β-ketoadipic acid, which is rapidly decarboxylated to form α-aminolevulinate (ALA) (Figure 32–5). This reaction sequence is catalyzed by **ALA synthase,** the rate-controlling enzyme in porphyrin biosynthesis in mammalian liver. Synthesis of ALA occurs in **mitochondria.** In the cytosol, two molecules of ALA are condensed by the enzyme **ALA dehydratase** to form two molecules of water and one of **porphobilinogen** (PBG) (Figure 32–5). ALA dehydratase is a zinc-containing enzyme and is sensitive to inhibition by **lead,** as can occur in lead poisoning.

The formation of a cyclic tetrapyrrole—ie, a porphyrin—occurs by condensation of four molecules of PBG (Figure 32–6). These four molecules condense in a head-to-tail manner to form a linear tetrapyrrole, hy-

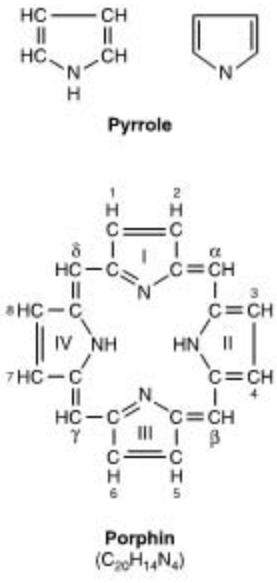

Figure 32–1. The porphin molecule. Rings are labeled I, II, III, and IV. Substituent positions on the rings are labeled 1, 2, 3, 4, 5, 6, 7, and 8. The methenyl bridges (—HC═) are labeled α, β, γ, and δ.

droxymethylbilane (HMB). The reaction is catalyzed by uroporphyrinogen I synthase, also named PBG deaminase or HMB synthase. HMB cyclizes spontaneously to form **uroporphyrinogen I** (left-hand side of Figure 32–6) or is converted to **uroporphyrinogen III** by the action of uroporphyrinogen III synthase (right-hand side of Figure 32–6). Under normal conditions, the uroporphyrinogen formed is almost exclusively the III isomer, but in certain of the porphyrias (discussed below), the type I isomers of porphyrinogens are formed in excess.

Note that both of these uroporphyrinogens have the pyrrole rings connected by methylene bridges

Table 32–1. Examples of some important human and animal hemoproteins.[1]

Protein	Function
Hemoglobin	Transport of oxygen in blood
Myoglobin	Storage of oxygen in muscle
Cytochrome *c*	Involvement in electron transport chain
Cytochrome P450	Hydroxylation of xenobiotics
Catalase	Degradation of hydrogen peroxide
Tryptophan pyrrolase	Oxidation of trypotophan

[1]The functions of the above proteins are described in various chapters of this text.

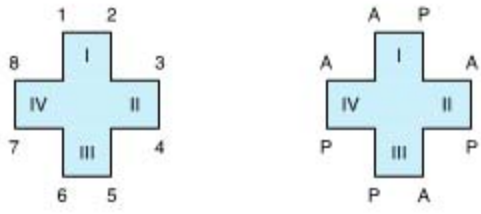

Figure 32–2. Uroporphyrin III. A (acetate) = —CH_2COOH; P (propionate) = —CH_2CH_2COOH.

(—CH_2—), which do not form a conjugated ring system. Thus, these compounds are colorless (as are all porphyrinogens). However, the porphyrinogens are readily auto-oxidized to their respective colored porphyrins. These oxidations are catalyzed by light and by the porphyrins that are formed.

Uroporphyrinogen III is converted to coproporphyrinogen III by decarboxylation of all of the acetate (A) groups, which changes them to methyl (M) substituents. The reaction is catalyzed by **uroporphyrinogen decarboxylase,** which is also capable of converting uroporphyrinogen I to coproporphyrinogen I (Figure 32–7). Coproporphyrinogen III then enters the mitochondria, where it is converted to **protoporphyrinogen III** and then to **protoporphyrin III.** Several steps are involved in this conversion. The mitochondrial enzyme **coproporphyrinogen oxidase** catalyzes the decarboxylation and oxidation of two propionic side chains to form protoporphyrinogen. This enzyme is able to act only on type III coproporphyrinogen, which would explain why type I protoporphyrins do not generally occur in nature. The oxidation of protoporphyrinogen to protoporphyrin is catalyzed by another mitochondrial enzyme, **protoporphyrinogen oxidase.** In mammalian liver, the conversion of coproporphyrinogen to protoporphyrin requires molecular oxygen.

Formation of Heme Involves Incorporation of Iron Into Protoporphyrin

The final step in heme synthesis involves the incorporation of ferrous iron into protoporphyrin in a reaction catalyzed by **ferrochelatase (heme synthase),** another mitochondrial enzyme (Figure 32–4).

A summary of the steps in the biosynthesis of the porphyrin derivatives from PBG is given in Figure 32–8. The last three enzymes in the pathway and ALA synthase are located in the mitochondrion, whereas the other enzymes are cytosolic. Both erythroid and nonerythroid ("housekeeping") forms of the first four enzymes are found. Heme biosynthesis occurs in most mammalian cells with the exception of mature erythrocytes, which do not contain mitochondria. However,

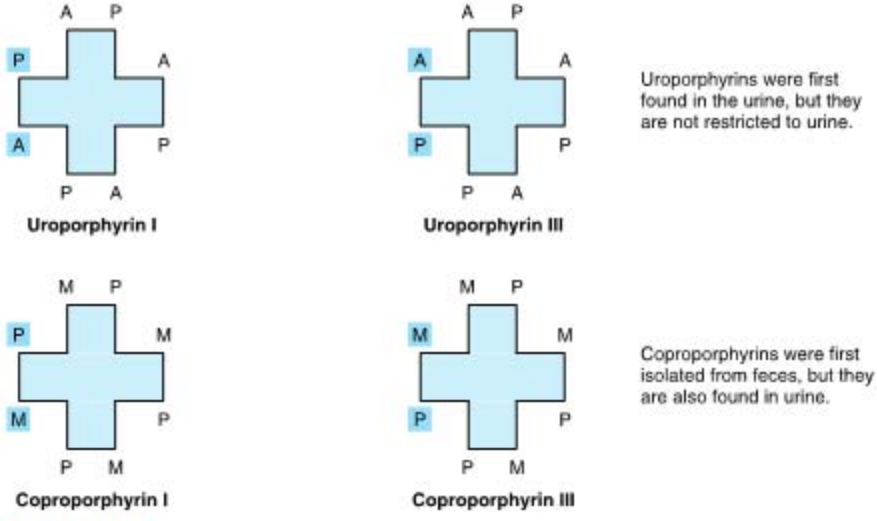

Figure 32–3. Uroporphyrins and coproporphyrins. A (acetate); P (propionate); M (methyl) = —CH_3; V (vinyl) = —CH—CH_2.

approximately 85% of heme synthesis occurs in erythroid precursor cells in the **bone marrow** and the majority of the remainder in **hepatocytes.**

The porphyrinogens described above are colorless, containing six extra hydrogen atoms as compared with the corresponding colored porphyrins. These **reduced porphyrins** (the porphyrinogens) and not the corresponding porphyrins are the actual intermediates in the biosynthesis of protoporphyrin and of heme.

ALA Synthase Is the Key Regulatory Enzyme in Hepatic Biosynthesis of Heme

ALA synthase occurs in both hepatic (ALAS1) and erythroid (ALAS2) forms. The rate-limiting reaction in the synthesis of heme in liver is that catalyzed by ALAS1 (Figure 32–5), a regulatory enzyme. It appears that heme, probably acting through an aporepressor molecule, acts as a negative regulator of the synthesis of ALAS1. This repression-derepression mechanism is depicted diagrammatically in Figure 32–9. Thus, the rate of synthesis of ALAS1 increases greatly in the absence of heme and is diminished in its presence. The turnover rate of ALAS1 in rat liver is normally rapid (half-life about 1 hour), a common feature of an enzyme catalyzing a rate-limiting reaction. Heme also affects translation of the enzyme and its transfer from the cytosol to the mitochondrion.

Many drugs when administered to humans can result in a marked increase in ALAS1. Most of these drugs are metabolized by a system in the liver that utilizes a specific hemoprotein, **cytochrome P450** (see Chapter 53). During their metabolism, the utilization of heme by cytochrome P450 is greatly increased, which in turn diminishes the intracellular heme concentration. This latter event effects a derepression of ALAS1 with a corresponding increased rate of heme synthesis to meet the needs of the cells.

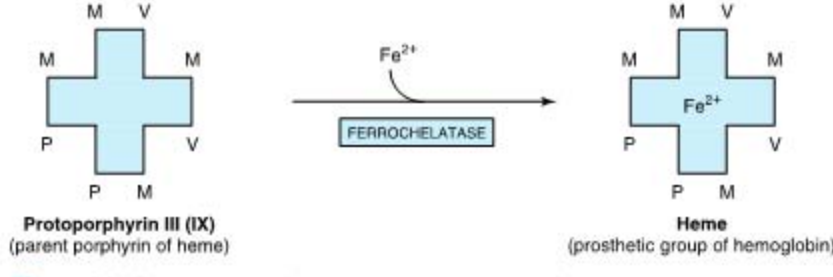

Figure 32–4. Addition of iron to protoporphyrin to form heme.

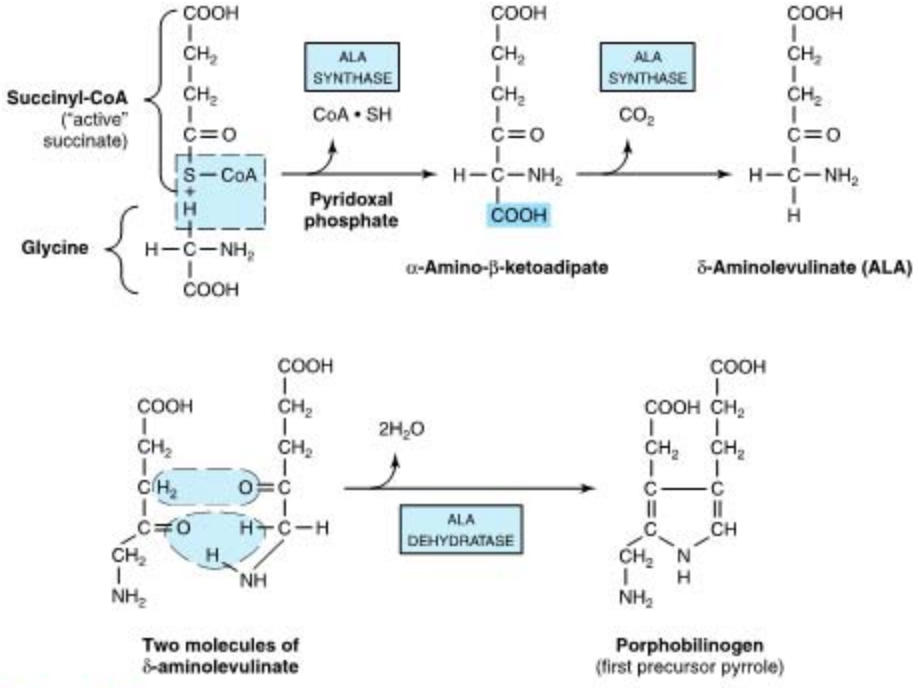

Figure 32–5. Biosynthesis of porphobilinogen. ALA synthase occurs in the mitochondria, whereas ALA dehydratase is present in the cytosol.

Several factors affect drug-mediated derepression of ALAS1 in liver—eg, the administration of glucose can prevent it, as can the administration of hematin (an oxidized form of heme).

The importance of some of these regulatory mechanisms is further discussed below when the porphyrias are described.

Regulation of the **erythroid** form of ALAS (ALAS2) differs from that of ALAS1. For instance, it is not induced by the drugs that affect ALAS1, and it does not undergo feedback regulation by heme.

PORPHYRINS ARE COLORED & FLUORESCE

The various porphyrinogens are colorless, whereas the various porphyrins are all colored. In the study of porphyrins or porphyrin derivatives, the characteristic absorption spectrum that each exhibits—in both the visible and the ultraviolet regions of the spectrum—is of great value. An example is the absorption curve for a solution of porphyrin in 5% hydrochloric acid (Figure 32–10). Note particularly the sharp absorption band near 400 nm. This is a distinguishing feature of the porphin ring and is characteristic of all porphyrins regardless of the side chains present. This band is termed the **Soret band** after its discoverer, the French physicist Charles Soret.

When porphyrins dissolved in strong mineral acids or in organic solvents are illuminated by ultraviolet light, they emit a strong red **fluorescence.** This fluorescence is so characteristic that it is often used to detect small amounts of free porphyrins. The double bonds joining the pyrrole rings in the porphyrins are responsible for the characteristic absorption and fluorescence of these compounds; these double bonds are absent in the porphyrinogens.

An interesting application of the photodynamic properties of porphyrins is their possible use in the treatment of certain types of cancer, a procedure called **cancer phototherapy.** Tumors often take up more porphyrins than do normal tissues. Thus, hematoporphyrin or other related compounds are administered to a patient with an appropriate tumor. The tumor is then exposed to an argon laser, which excites the porphyrins, producing cytotoxic effects.

Spectrophotometry Is Used to Test for Porphyrins & Their Precursors

Coproporphyrins and uroporphyrins are of clinical interest because they are excreted in increased amounts in

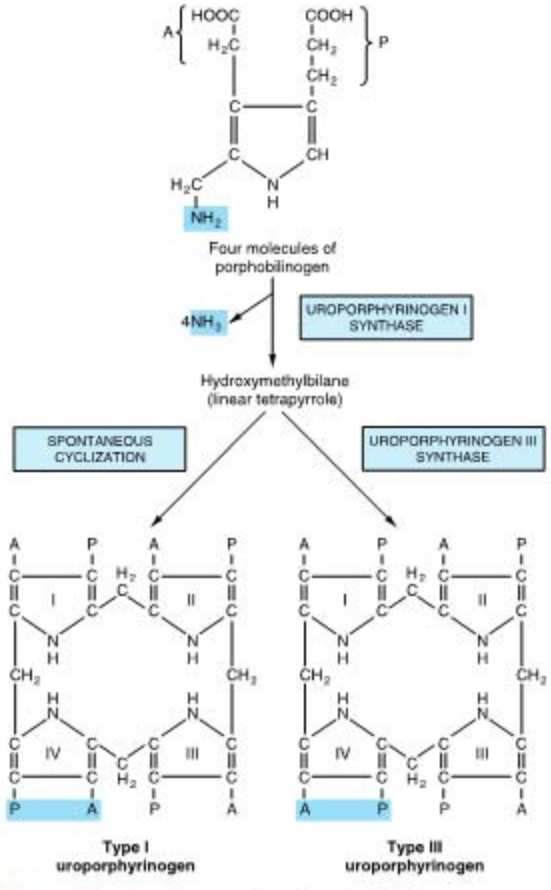

Figure 32–6. Conversion of porphobilinogen to uroporphyrinogens. Uroporphyrinogen synthase I is also called porphobilinogen (PBG) deaminase or hydroxymethylbilane (HMB) synthase.

the porphyrias. These compounds, when present in urine or feces, can be separated from each other by extraction with appropriate solvent mixtures. They can then be identified and quantified using spectrophotometric methods.

ALA and PBG can also be measured in urine by appropriate colorimetric tests.

THE PORPHYRIAS ARE GENETIC DISORDERS OF HEME METABOLISM

The **porphyrias** are a group of disorders due to abnormalities in the pathway of biosynthesis of heme; they can be genetic or acquired. They are not prevalent, but it is important to consider them in certain circumstances (eg, in the differential diagnosis of abdominal pain and of a variety of neuropsychiatric findings); otherwise, patients will be subjected to inappropriate treatments. It has been speculated that King George III had a type of porphyria, which may account for his periodic confinements in Windsor Castle and perhaps for some of his views regarding American colonists. Also, the **photosensitivity** (favoring nocturnal activities) and severe **disfigurement** exhibited by some victims of congenital erythropoietic porphyria have led to the suggestion that these individuals may have been the prototypes of so-called werewolves. No evidence to support this notion has been adduced.

Biochemistry Underlies the Causes, Diagnoses, & Treatments of the Porphyrias

Six major types of porphyria have been described, resulting from depressions in the activities of enzymes 3 through 8 shown in Figure 32–9 (see also Table 32–2). Assay of the activity of one or more of these enzymes using an appropriate source (eg, red blood cells) is thus important in making a definitive diagnosis in a suspected case of porphyria. Individuals with low activities of enzyme 1 (ALAS2) develop anemia, not porphyria (see Table 32–2). Patients with low activities of enzyme 2 (ALA dehydratase) have been reported, but very rarely; the resulting condition is called ALA dehydratase-deficient porphyria.

In general, the porphyrias described are inherited in an autosomal dominant manner, with the exception of congenital erythropoietic porphyria, which is inherited in a recessive mode. The precise abnormalities in the genes directing synthesis of the enzymes involved in heme biosynthesis have been determined in some instances. Thus, the use of appropriate gene probes has made possible the prenatal diagnosis of some of the porphyrias.

As is true of most inborn errors, the signs and symptoms of porphyria result from either a deficiency of metabolic products beyond the enzymatic block or from an accumulation of metabolites behind the block.

If the enzyme lesion occurs early in the pathway prior to the formation of porphyrinogens (eg, enzyme 3 of Figure 32–9, which is affected in acute intermittent porphyria), ALA and PBG will accumulate in body tissues and fluids (Figure 32–11). Clinically, patients complain of abdominal pain and neuropsychiatric symptoms. The precise biochemical cause of these symptoms has not been determined but may relate to elevated levels of ALA or PBG or to a deficiency of heme.

On the other hand, enzyme blocks later in the pathway result in the accumulation of the porphyrinogens

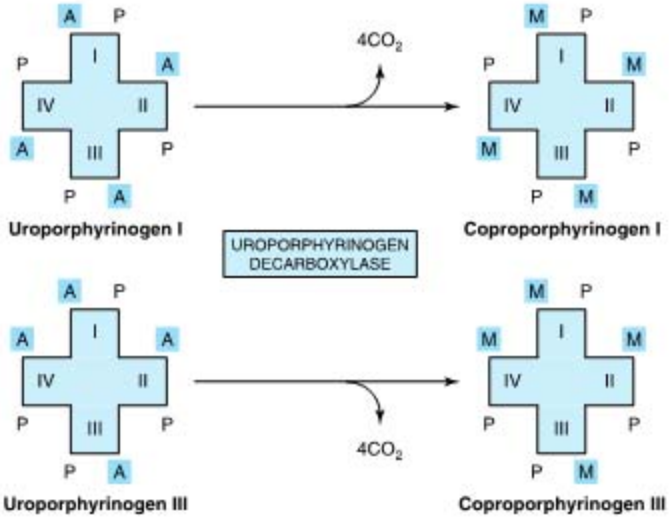

Figure 32–7. Decarboxylation of uroporphyrinogens to coproporphyrinogens in cytosol. (A, acetyl; M, methyl; P, propionyl.)

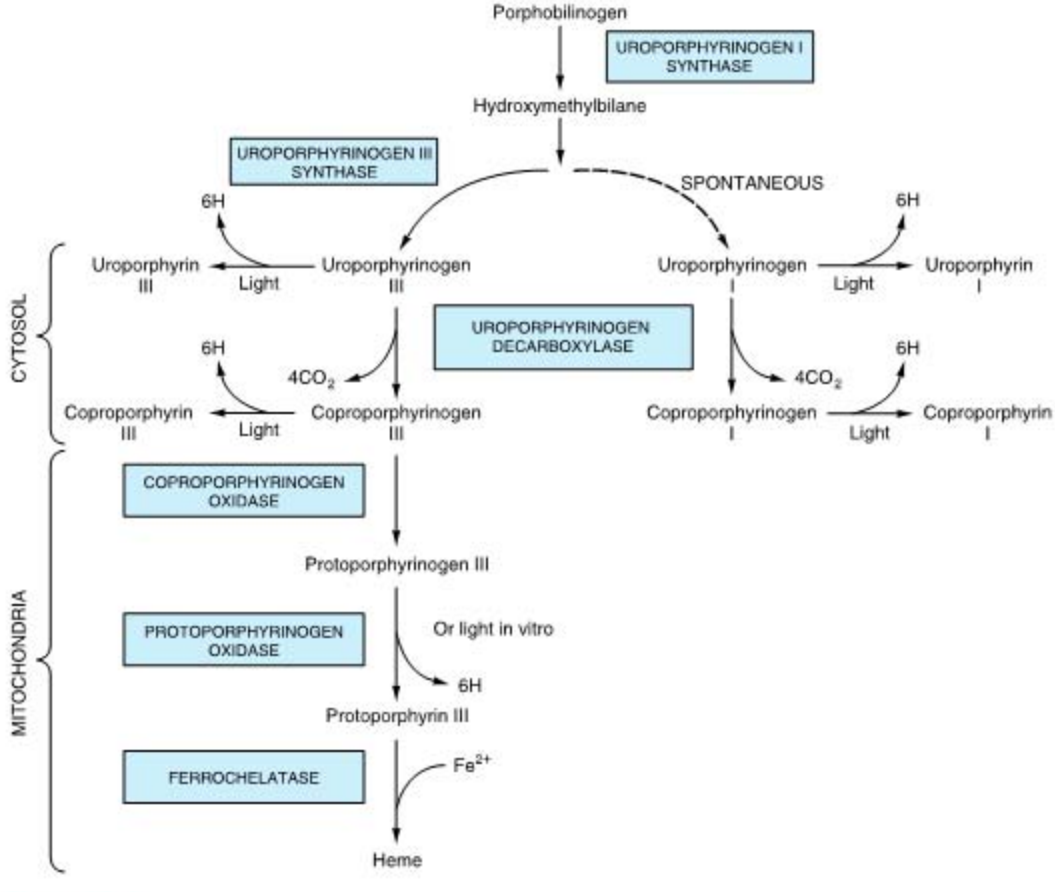

Figure 32–8. Steps in the biosynthesis of the porphyrin derivatives from porphobilinogen. Uroporphyrinogen I synthase is also called porphobilinogen deaminase or hydroxymethylbilane synthase.

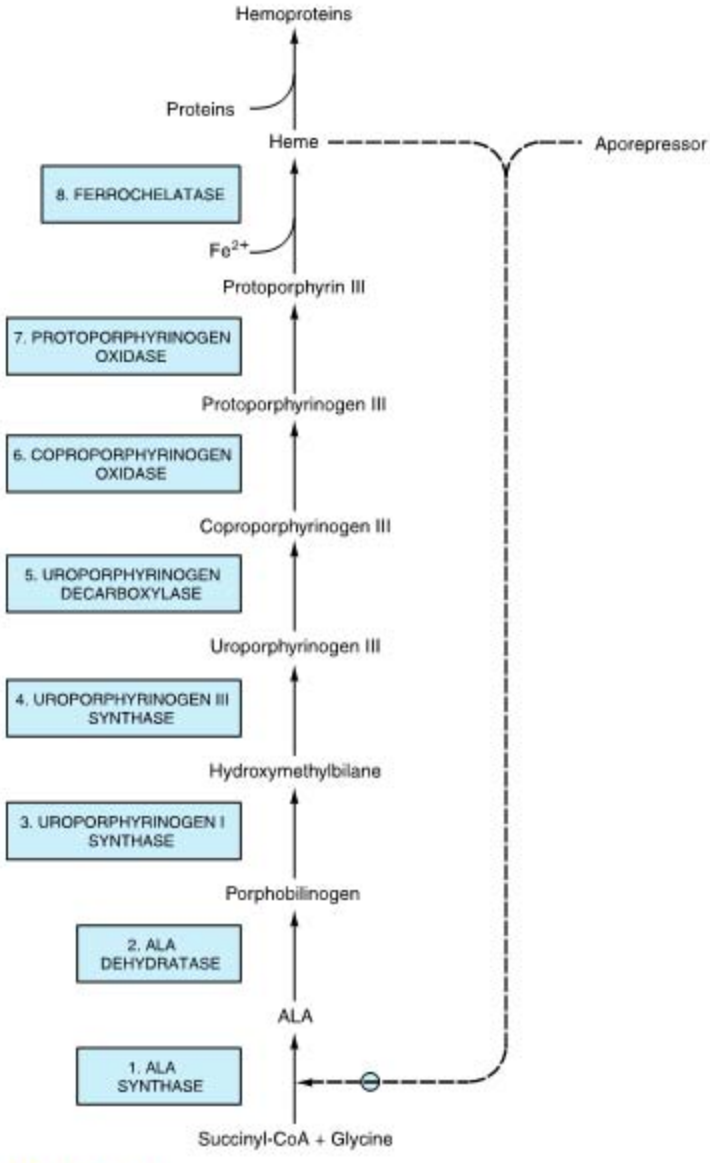

Figure 32–9. Intermediates, enzymes, and regulation of heme synthesis. The enzyme numbers are those referred to in column 1 of Table 32–2. Enzymes 1, 6, 7, and 8 are located in mitochondria, the others in the cytosol. Mutations in the gene encoding enzyme 1 causes X-linked sideroblastic anemia. Mutations in the genes encoding enzymes 2–8 cause the porphyrias, though only a few cases due to deficiency of enzyme 2 have been reported. Regulation of hepatic heme synthesis occurs at ALA synthase (ALAS1) by a repression-derepression mechanism mediated by heme and its hypothetical aporepressor. The dotted lines indicate the negative (⊖) regulation by repression. Enzyme 3 is also called porphobilinogen deaminase or hydroxymethylbilane synthase.

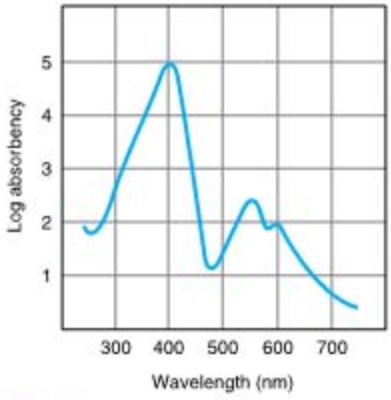

Figure 32–10. Absorption spectrum of hematoporphyrin (0.01% solution in 5% HCl).

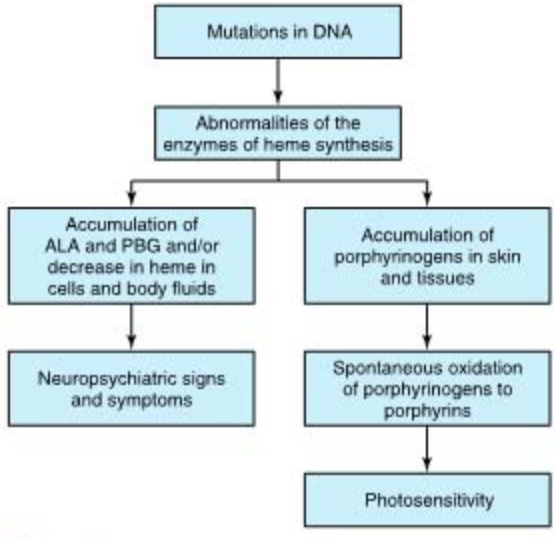

Figure 32–11. Biochemical causes of the major signs and symptoms of the porphyrias.

Table 32–2. Summary of major findings in the porphyrias.[1]

Enzyme Involved[2]	Type, Class, and MIM Number	Major Signs and Symptoms	Results of Laboratory Tests
1. ALA synthase (erythroid form)	X-linked sideroblastic anemia[3] (erythropoietic) (MIM 201300)	Anemia	Red cell counts and hemoglobin decreased
2. ALA dehydratase	ALA dehydratase deficiency (hepatic) (MIM 125270)	Abdominal pain, neuropsychiatric symptoms	Urinary δ-aminolevulinic acid
3. Uroporphyrinogen I synthase[4]	Acute intermittent porphyria (hepatic) (MIM 176000)	Abdominal pain, neuropsychiatric symptoms	Urinary porphobilinogen positive, uroporphyrin positive
4. Uroporphyrinogen III synthase	Congenital erythropoietic (erythropoietic) (MIM 263700)	No photosensitivity	Uroporphyrin positive, porphobilinogen negative
5. Uroporphyrinogen decarboxylase	Porphyria cutanea tarda (hepatic) (MIM 176100)	Photosensitivity	Uroporphyrin positive, porphobilinogen negative
6. Coproporphyrinogen oxidase	Hereditary coproporphyria (hepatic) (MIM 121300)	Photosensitivity, abdominal pain, neuropsychiatric symptoms	Urinary porphobilinogen positive, urinary uroporphyrin positive, fecal protoporphyrin positive
7. Protoporphyrinogen oxidase	Variegate porphyria (hepatic) (MIM 176200)	Photosensitivity, abdominal pain, neuropsychiatric symptoms	Urinary porphobilinogen positive, fecal protoporphyrin positive
8. Ferrochelatase	Protoporphyria (erythropoietic) (MIM 177000)	Photosensitivity	Fecal protoporphyrin positive, red cell protoporphyrin positive

[1]Only the biochemical findings in the active stages of these diseases are listed. Certain biochemical abnormalities are detectable in the latent stages of some of the above conditions. Conditions 3, 5, and 8 are generally the most prevalent porphyrias.
[2]The numbering of the enzymes in this table corresponds to that used in Figure 32-9.
[3]X-linked sideroblastic anemia is not a porphyria but is included here because δ–aminolevulinic acid synthase is involved.
[4]This enzyme is also called porphobilinogen deaminase or hydroxymethylbilane synthase.

indicated in Figures 32–9 and 32–11. Their oxidation products, the corresponding porphyrin derivatives, cause photosensitivity, a reaction to visible light of about 400 nm. The porphyrins, when exposed to light of this wavelength, are thought to become "excited" and then react with molecular oxygen to form oxygen radicals. These latter species injure lysosomes and other organelles. Damaged lysosomes release their degradative enzymes, causing variable degrees of skin damage, including scarring.

The porphyrias can be **classified** on the basis of the organs or cells that are most affected. These are generally organs or cells in which synthesis of heme is particularly active. The bone marrow synthesizes considerable hemoglobin, and the liver is active in the synthesis of another hemoprotein, cytochrome P450. Thus, one classification of the porphyrias is to designate them as predominantly either **erythropoietic** or **hepatic;** the types of porphyrias that fall into these two classes are so characterized in Table 32–2. Porphyrias can also be classified as acute or cutaneous on the basis of their clinical features. Why do specific types of porphyria affect certain organs more markedly than others? A partial answer is that the levels of metabolites that cause damage (eg, ALA, PBG, specific porphyrins, or lack of heme) can vary markedly in different organs or cells depending upon the differing activities of their heme-forming enzymes.

As described above, **ALAS1** is the key regulatory enzyme of the heme biosynthetic pathway in liver. A large number of **drugs** (eg, barbiturates, griseofulvin) induce the enzyme. Most of these drugs do so by inducing cytochrome P450 (see Chapter 53), which uses up heme and thus derepresses (induces) ALAS1. In patients with porphyria, increased activities of ALAS1 result in increased levels of potentially harmful heme precursors prior to the metabolic block. Thus, taking drugs that cause induction of cytochrome P450 (so-called microsomal inducers) can precipitate attacks of porphyria.

The **diagnosis** of a specific type of porphyria can generally be established by consideration of the clinical and family history, the physical examination, and appropriate laboratory tests. The major findings in the six principal types of porphyria are listed in Table 32–2.

High levels of **lead** can affect heme metabolism by combining with SH groups in enzymes such as ferrochelatase and ALA dehydratase. This affects porphyrin metabolism. Elevated levels of protoporphyrin are found in red blood cells, and elevated levels of ALA and of coproporphyrin are found in urine.

It is hoped that **treatment** of the porphyrias at the gene level will become possible. In the meantime, treatment is essentially symptomatic. It is important for patients to avoid drugs that cause induction of cytochrome P450. Ingestion of large amounts of carbohydrates (glucose loading) or administration of hematin (a hydroxide of heme) may repress ALAS1, resulting in diminished production of harmful heme precursors. Patients exhibiting photosensitivity may benefit from administration of β-carotene; this compound appears to lessen production of free radicals, thus diminishing photosensitivity. Sunscreens that filter out visible light can also be helpful to such patients.

CATABOLISM OF HEME PRODUCES BILIRUBIN

Under physiologic conditions in the human adult, 1–2 $\times 10^8$ erythrocytes are destroyed per hour. Thus, in 1 day, a 70-kg human turns over approximately 6 g of hemoglobin. When hemoglobin is destroyed in the body, **globin** is degraded to its constituent amino acids, which are reused, and the **iron** of heme enters the iron pool, also for reuse. The iron-free **porphyrin** portion of heme is also degraded, mainly in the reticuloendothelial cells of the liver, spleen, and bone marrow.

The catabolism of heme from all of the heme proteins appears to be carried out in the microsomal fractions of cells by a complex enzyme system called **heme oxygenase.** By the time the heme derived from heme proteins reaches the oxygenase system, the iron has usually been oxidized to the ferric form, constituting **hemin.** The heme oxygenase system is substrate-inducible. As depicted in Figure 32–12, the hemin is reduced to heme with NADPH, and, with the aid of more NADPH, oxygen is added to the α-methenyl bridge between pyrroles I and II of the porphyrin. The ferrous iron is again oxidized to the ferric form. With the further addition of oxygen, **ferric ion** is released, **carbon monoxide** is produced, and an equimolar quantity of **biliverdin** results from the splitting of the tetrapyrrole ring.

In birds and amphibia, the green biliverdin IX is excreted; in mammals, a soluble enzyme called **biliverdin reductase** reduces the methenyl bridge between pyrrole III and pyrrole IV to a methylene group to produce **bilirubin,** a yellow pigment (Figure 32–12).

It is estimated that 1 g of hemoglobin yields 35 mg of bilirubin. The daily bilirubin formation in human adults is approximately 250–350 mg, deriving mainly from hemoglobin but also from ineffective erythropoiesis and from various other heme proteins such as cytochrome P450.

The chemical conversion of heme to bilirubin by reticuloendothelial cells can be observed in vivo as the purple color of the heme in a hematoma is slowly converted to the yellow pigment of bilirubin.

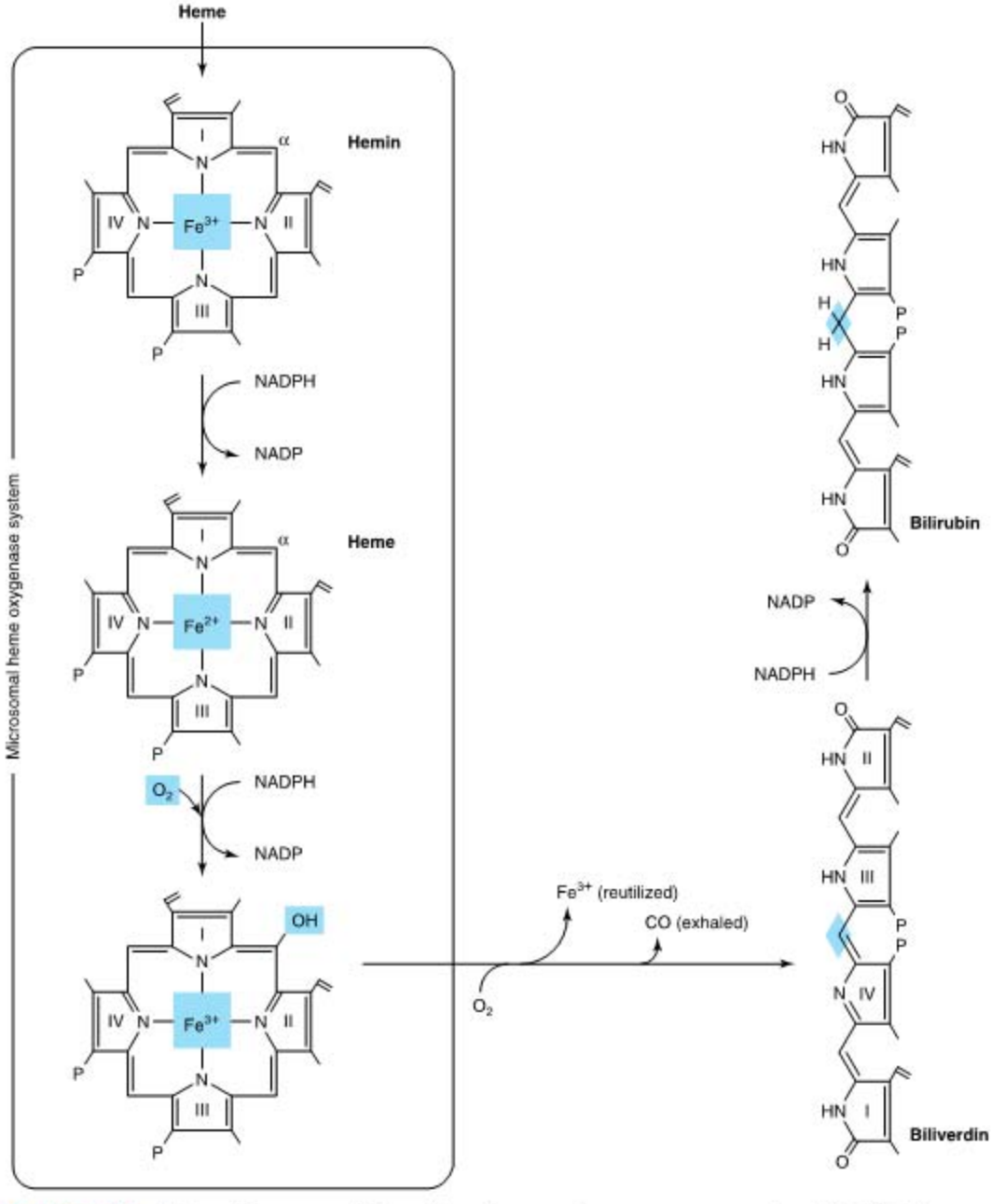

Figure 32–12. Schematic representation of the microsomal heme oxygenase system. (Modified from Schmid R, McDonough AF in: *The Porphyrins*. Dolphin D [editor]. Academic Press, 1978.)

Bilirubin formed in peripheral tissues is transported to the liver by plasma albumin. The further metabolism of bilirubin occurs primarily in the liver. It can be divided into three processes: (1) uptake of bilirubin by liver parenchymal cells, (2) conjugation of bilirubin with glucuronate in the endoplasmic reticulum, and (3) secretion of conjugated bilirubin into the bile. Each of these processes will be considered separately.

THE LIVER TAKES UP BILIRUBIN

Bilirubin is only sparingly soluble in water, but its solubility in plasma is increased by noncovalent binding to albumin. Each molecule of albumin appears to have one high-affinity site and one low-affinity site for bilirubin. In 100 mL of plasma, approximately 25 mg of bilirubin can be tightly bound to albumin at its high-affinity site. Bilirubin in excess of this quantity can be bound only loosely and thus can easily be detached and diffuse into tissues. A number of compounds such as antibiotics and other drugs compete with bilirubin for the high-affinity binding site on albumin. Thus, these compounds can displace bilirubin from albumin and have significant clinical effects.

In the liver, the bilirubin is removed from albumin and taken up at the sinusoidal surface of the hepatocytes by a carrier-mediated saturable system. This **facilitated transport system** has a very large capacity, so that even under pathologic conditions the system does not appear to be rate-limiting in the metabolism of bilirubin.

Since this facilitated transport system allows the equilibrium of bilirubin across the sinusoidal membrane of the hepatocyte, the net uptake of bilirubin will be dependent upon the removal of bilirubin via subsequent metabolic pathways.

Once bilirubin enters the hepatocytes, it can bind to certain cytosolic proteins, which help to keep it solubilized prior to conjugation. Ligandin (a family of glutathione S-transferases) and protein Y are the involved proteins. They may also help to prevent efflux of bilirubin back into the blood stream.

Conjugation of Bilirubin With Glucuronic Acid Occurs in the Liver

Bilirubin is nonpolar and would persist in cells (eg, bound to lipids) if not rendered water-soluble. Hepatocytes convert bilirubin to a polar form, which is readily excreted in the bile, by adding glucuronic acid molecules to it. This process is called conjugation and can employ polar molecules other than glucuronic acid (eg, sulfate). Many steroid hormones and drugs are also converted to water-soluble derivatives by conjugation in preparation for excretion (see Chapter 53).

The conjugation of bilirubin is catalyzed by a specific glucuronosyltransferase. The enzyme is mainly located in the endoplasmic reticulum, uses UDP-glucuronic acid as the glucuronosyl donor, and is referred to as bilirubin-UGT. Bilirubin monoglucuronide is an intermediate and is subsequently converted to the diglucuronide (Figures 32–13 and 32–14). Most of the bilirubin excreted in the bile of mammals is in the form of bilirubin diglucuronide. However, when bilirubin conjugates exist abnormally in human plasma (eg, in obstructive jaundice), they are predominantly monoglucuronides. Bilirubin-UGT activity can be **induced** by a number of clinically useful drugs, including phenobarbital. More information about glucuronosylation is presented below in the discussion of inherited disorders of bilirubin conjugation.

Bilirubin Is Secreted Into Bile

Secretion of conjugated bilirubin into the bile occurs by an active transport mechanism, which is probably rate-limiting for the entire process of hepatic bilirubin metabolism. The protein involved is MRP-2 (multidrug resistance-like protein 2), also called multispecific organic anion transporter (MOAT). It is located in the plasma membrane of the bile canalicular membrane and handles a number of organic anions. It is a member of the family of ATP-binding cassette (ABC) transporters. The hepatic transport of conjugated bilirubin into the bile is inducible by those same drugs that are capable of inducing the conjugation of bilirubin. Thus, the conjugation and excretion systems for bilirubin behave as a coordinated functional unit.

Figure 32–15 summarizes the three major processes involved in the transfer of bilirubin from blood to bile. Sites that are affected in a number of conditions causing jaundice (see below) are also indicated.

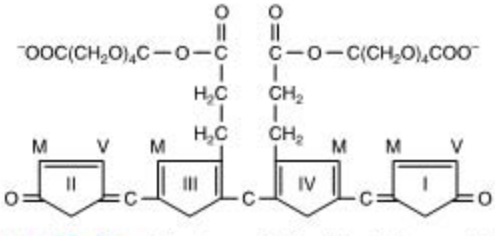

Figure 32–13. Structure of bilirubin diglucuronide (conjugated, "direct-reacting" bilirubin). Glucuronic acid is attached via ester linkage to the two propionic acid groups of bilirubin to form an acylglucuronide.

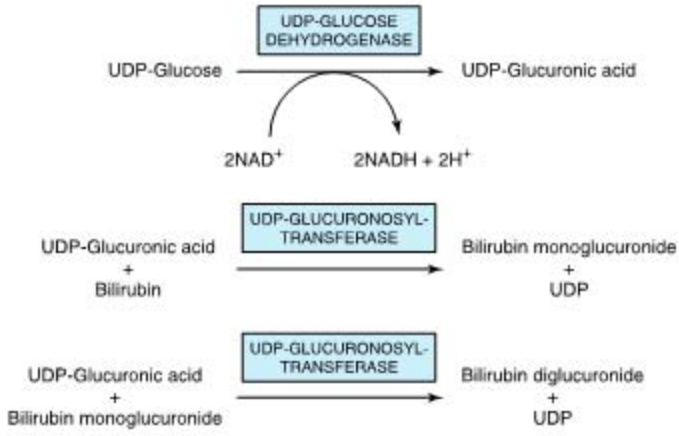

Figure 32–14. Conjugation of bilirubin with glucuronic acid. The glucuronate donor, UDP-glucuronic acid, is formed from UDP-glucose as depicted. The UDP-glucuronosyl-transferase is also called bilirubin-UGT.

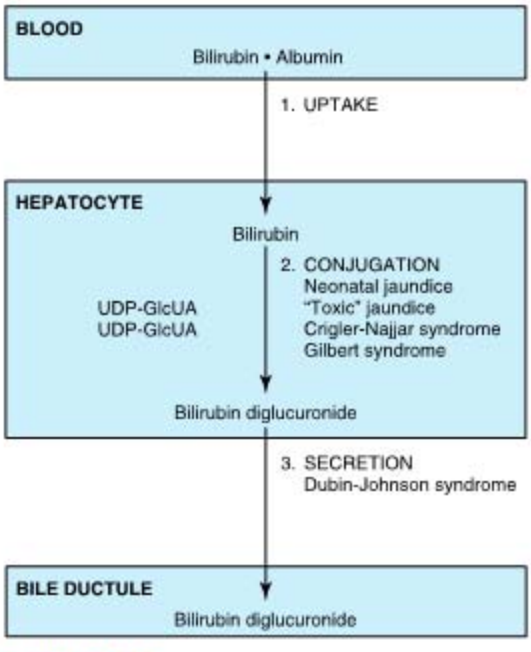

Figure 32–15. Diagrammatic representation of the three major processes (uptake, conjugation, and secretion) involved in the transfer of bilirubin from blood to bile. Certain proteins of hepatocytes, such as ligandin (a family of glutathione *S*-transferase) and Y protein, bind intracellular bilirubin and may prevent its efflux into the blood stream. The process affected in a number of conditions causing jaundice is also shown.

Conjugated Bilirubin Is Reduced to Urobilinogen by Intestinal Bacteria

As the conjugated bilirubin reaches the terminal ileum and the large intestine, the glucuronides are removed by specific bacterial enzymes **(β-glucuronidases),** and the pigment is subsequently reduced by the fecal flora to a group of colorless tetrapyrrolic compounds called **urobilinogens** (Figure 32–16). In the terminal ileum and large intestine, a small fraction of the urobilinogens is reabsorbed and reexcreted through the liver to constitute the **enterohepatic urobilinogen cycle.** Under abnormal conditions, particularly when excessive bile pigment is formed or liver disease interferes with this intrahepatic cycle, urobilinogen may also be excreted in the urine.

Normally, most of the colorless urobilinogens formed in the colon by the fecal flora are oxidized there to urobilins (colored compounds) and are excreted in the feces (Figure 32–16). Darkening of feces upon standing in air is due to the oxidation of residual urobilinogens to urobilins.

HYPERBILIRUBINEMIA CAUSES JAUNDICE

When bilirubin in the blood exceeds 1 mg/dL (17.1 µmol/L), hyperbilirubinemia exists. Hyperbilirubinemia may be due to the production of more bilirubin than the normal liver can excrete, or it may result from the failure of a damaged liver to excrete bilirubin produced in normal amounts. In the absence of hepatic damage, obstruction of the excretory ducts of the liver—by preventing the excretion of bilirubin—will also cause hyperbilirubinemia. In all these situations, bilirubin accumulates in the blood, and when it reaches a certain concentration (approximately 2–2.5 mg/dL),

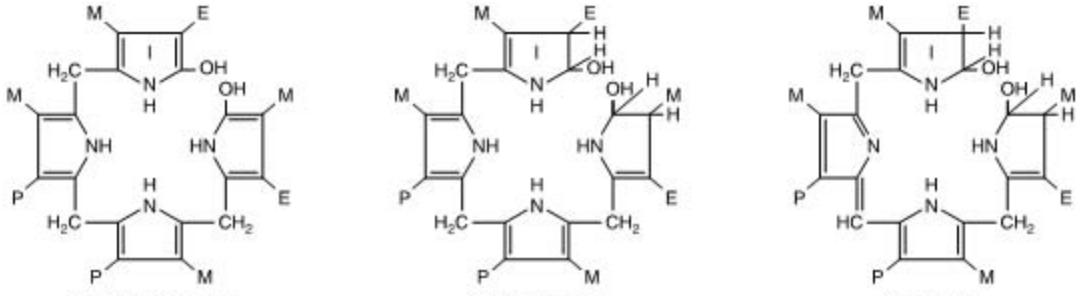

Figure 32–16. Structure of some bile pigments.

it diffuses into the tissues, which then become yellow. That condition is called **jaundice** or **icterus.**

In clinical studies of jaundice, measurement of bilirubin in the serum is of great value. A method for quantitatively assaying the bilirubin content of the serum was first devised by van den Bergh by application of Ehrlich's test for bilirubin in urine. The Ehrlich reaction is based on the coupling of diazotized sulfanilic acid (Ehrlich's diazo reagent) and bilirubin to produce a reddish-purple azo compound. In the original procedure as described by Ehrlich, methanol was used to provide a solution in which both bilirubin and the diazo regent were soluble. Van den Bergh inadvertently omitted the methanol on an occasion when assay of bile pigment in human bile was being attempted. To his surprise, normal development of the color occurred "directly." This form of bilirubin that would react without the addition of methanol was thus termed **"direct-reacting."** It was then found that this same direct reaction would also occur in serum from cases of jaundice due to biliary obstruction. However, it was still necessary to add methanol to detect bilirubin in normal serum or that which was present in excess in serum from cases of hemolytic jaundice where no evidence of obstruction was to be found. To that form of bilirubin which could be measured only after the addition of methanol, the term **"indirect-reacting"** was applied.

It was subsequently discovered that the indirect bilirubin is "free" (unconjugated) bilirubin en route to the liver from the reticuloendothelial tissues, where the bilirubin was originally produced by the breakdown of heme porphyrins. Since this bilirubin is not water-soluble, it requires methanol to initiate coupling with the diazo reagent. In the liver, the free bilirubin becomes conjugated with glucuronic acid, and the conjugate, bilirubin glucuronide, can then be excreted into the bile. Furthermore, conjugated bilirubin, being water-soluble, can react directly with the diazo reagent, so that the "direct bilirubin" of van den Bergh is actually a bilirubin conjugate (bilirubin glucuronide).

Depending on the type of bilirubin present in plasma—ie, unconjugated or conjugated—hyperbilirubinemia may be classified as **retention hyperbilirubinemia,** due to overproduction, or **regurgitation hyperbilirubinemia,** due to reflux into the bloodstream because of biliary obstruction.

Because of its hydrophobicity, only unconjugated bilirubin can cross the blood-brain barrier into the central nervous system; thus, encephalopathy due to hyperbilirubinemia **(kernicterus)** can occur only in connection with unconjugated bilirubin, as found in retention hyperbilirubinemia. On the other hand, because of its water-solubility, only conjugated bilirubin can appear in urine. Accordingly, **choluric jaundice** (choluria is the presence of bile pigments in the urine) occurs only in regurgitation hyperbilirubinemia, and **acholuric jaundice** occurs only in the presence of an excess of unconjugated bilirubin.

Elevated Amounts of Unconjugated Bilirubin in Blood Occur in a Number of Conditions

A. Hemolytic Anemias

Hemolytic anemias are important causes of unconjugated hyperbilirubinemia, though unconjugated hyperbilirubinemia is usually only slight (< 4 mg/dL; < 68.4 µmol/L) even in the event of extensive hemolysis because of the healthy liver's large capacity for handling bilirubin.

B. Neonatal "Physiologic Jaundice"

This transient condition is the most common cause of unconjugated hyperbilirubinemia. It results from an ac-

celerated hemolysis around the time of birth and an immature hepatic system for the uptake, conjugation, and secretion of bilirubin. Not only is the bilirubin-UGT activity reduced, but there probably is reduced synthesis of the substrate for that enzyme, UDP-glucuronic acid. Since the increased amount of bilirubin is unconjugated, it is capable of penetrating the blood-brain barrier when its concentration in plasma exceeds that which can be tightly bound by albumin (20–25 mg/dL). This can result in a hyperbilirubinemic toxic encephalopathy, or **kernicterus,** which can cause mental retardation. Because of the recognized inducibility of this bilirubin-metabolizing system, phenobarbital has been administered to jaundiced neonates and is effective in this disorder. In addition, exposure to blue light (phototherapy) promotes the hepatic excretion of unconjugated bilirubin by converting some of the bilirubin to other derivatives such as maleimide fragments and geometric isomers that are excreted in the bile.

C. Crigler-Najjar Syndrome, Type I; Congenital Nonhemolytic Jaundice

Type I Crigler-Najjar syndrome is a rare autosomal recessive disorder. It is characterized by severe congenital jaundice (serum bilirubin usually exceeds 20 mg/dL) due to mutations in the gene encoding bilirubin-UGT activity in hepatic tissues. The disease is often fatal within the first 15 months of life. Children with this condition have been treated with phototherapy, resulting in some reduction in plasma bilirubin levels. Phenobarbital has no effect on the formation of bilirubin glucuronides in patients with type I Crigler-Najjar syndrome. A liver transplant may be curative.

D. Crigler-Najjar Syndrome, Type II

This rare inherited disorder also results from mutations in the gene encoding bilirubin-UGT, but some activity of the enzyme is retained and the condition has a more benign course than type I. Serum bilirubin concentrations usually do not exceed 20 mg/dL. Patients with this condition can respond to treatment with large doses of phenobarbital.

E. Gilbert Syndrome

Again, this is caused by mutations in the gene encoding bilirubin-UGT, but approximately 30% of the enzyme's activity is preserved and the condition is entirely harmless.

F. Toxic Hyperbilirubinemia

Unconjugated hyperbilirubinemia can result from toxin-induced liver dysfunction such as that caused by chloroform, arsphenamines, carbon tetrachloride, acetaminophen, hepatitis virus, cirrhosis, and *Amanita* mushroom poisoning. These acquired disorders are due to hepatic parenchymal cell damage, which impairs conjugation.

Obstruction in the Biliary Tree Is the Commonest Cause of Conjugated Hyperbilirubinemia

A. Obstruction of the Biliary Tree

Conjugated hyperbilirubinemia commonly results from blockage of the hepatic or common bile ducts, most often due to a gallstone or to cancer of the head of the pancreas. Because of the obstruction, bilirubin diglucuronide cannot be excreted. It thus regurgitates into the hepatic veins and lymphatics, and conjugated bilirubin appears in the blood and urine (choluric jaundice).

The term **cholestatic jaundice** is used to include all cases of extrahepatic obstructive jaundice. It also covers those cases of jaundice that exhibit conjugated hyperbilirubinemia due to micro-obstruction of intrahepatic biliary ductules by swollen, damaged hepatocytes (eg, as may occur in infectious hepatitis).

B. Dubin-Johnson Syndrome

This benign autosomal recessive disorder consists of conjugated hyperbilirubinemia in childhood or during adult life. The hyperbilirubinemia is caused by mutations in the gene encoding MRP-2 (see above), the protein involved in the secretion of conjugated bilirubin into bile. The centrilobular hepatocytes contain an abnormal black pigment that may be derived from epinephrine.

C. Rotor Syndrome

This is a rare benign condition characterized by chronic conjugated hyperbilirubinemia and normal liver histology. Its precise cause has not been identified, but it is thought to be due to an abnormality in hepatic storage.

Some Conjugated Bilirubin Can Bind Covalently to Albumin

When levels of conjugated bilirubin remain high in plasma, a fraction can bind covalently to albumin (delta bilirubin). Because it is bound covalently to albumin, this fraction has a longer half-life in plasma than does conventional conjugated bilirubin. Thus, it remains elevated during the recovery phase of obstructive jaundice after the remainder of the conjugated bilirubin has declined to normal levels; this explains why some patients continue to appear jaundiced after conjugated bilirubin levels have returned to normal.

Table 32–3. Laboratory results in normal patients and patients with three different causes of jaundice.

Condition	Serum Bilirubin	Urine Urobilinogen	Urine Bilirubin	Fecal Urobilinogen
Normal	Direct: 0.1–0.4 mg/dL Indirect: 0.2–0.7 mg/dL	0–4 mg/24 h	Absent	40–280 mg/24 h
Hemolytic anemia	↑ Indirect	Increased	Absent	Increased
Hepatitis	↑ Direct and indirect	Decreased if micro-obstruction is present	Present if micro-obstruction occurs	Decreased
Obstructive jaundice[1]	↑ Direct	Absent	Present	Trace to absent

[1]The commonest causes of obstructive (posthepatic) jaundice are cancer of the head of the pancreas and a gallstone lodged in the common bile duct. The presence of bilirubin in the urine is sometimes referred to as choluria—therefore, hepatitis and obstruction of the common bile duct cause choluric jaundice, whereas the jaundice of hemolytic anemia is referred to as acholuric. The laboratory results in patients with hepatitis are variable, depending on the extent of damage to parenchymal cells and the extent of micro-obstruction to bile ductules. Serum levels of ALT and AST are usually markedly elevated in hepatitis, whereas serum levels of alkaline phosphatase are elevated in obstructive liver disease.

Urobilinogen & Bilirubin in Urine Are Clinical Indicators

Normally, there are mere traces of urobilinogen in the urine. In **complete obstruction of the bile duct,** no urobilinogen is found in the urine, since bilirubin has no access to the intestine, where it can be converted to urobilinogen. In this case, the presence of bilirubin (conjugated) in the urine without urobilinogen suggests obstructive jaundice, either intrahepatic or posthepatic.

In **jaundice secondary to hemolysis,** the increased production of bilirubin leads to increased production of urobilinogen, which appears in the urine in large amounts. Bilirubin is not usually found in the urine in hemolytic jaundice (because unconjugated bilirubin does not pass into the urine), so that the combination of increased urobilinogen and absence of bilirubin is suggestive of hemolytic jaundice. Increased blood destruction from any cause brings about an increase in urine urobilinogen.

Table 32–3 summarizes laboratory results obtained on patients with three different causes of jaundice—hemolytic anemia (a prehepatic cause), hepatitis (a hepatic cause), and obstruction of the common bile duct (a posthepatic cause). Laboratory tests on blood (evaluation of the possibility of a hemolytic anemia and measurement of prothrombin time) and on serum (eg, electrophoresis of proteins; activities of the enzymes ALT, AST, and alkaline phosphatase) are also important in helping to distinguish between prehepatic, hepatic, and posthepatic causes of jaundice.

SUMMARY

- Hemoproteins, such as hemoglobin and the cytochromes, contain heme. Heme is an iron-porphyrin compound (Fe^{2+}-protoporphyrin IX) in which four pyrrole rings are joined by methenyl bridges. The eight side groups (methyl, vinyl, and propionyl substituents) on the four pyrrole rings of heme are arranged in a specific sequence.
- Biosynthesis of the heme ring occurs in mitochondria and cytosol via eight enzymatic steps. It commences with formation of δ-aminolevulinate (ALA) from succinyl-CoA and glycine in a reaction catalyzed by ALA synthase, the regulatory enzyme of the pathway.
- Genetically determined abnormalities of seven of the eight enzymes involved in heme biosynthesis result in the inherited porphyrias. Red blood cells and liver are the major sites of metabolic expression of the porphyrias. Photosensitivity and neurologic problems are common complaints. Intake of certain compounds (such as lead) can cause acquired porphyrias. Increased amounts of porphyrins or their precursors can be detected in blood and urine, facilitating diagnosis.
- Catabolism of the heme ring is initiated by the enzyme heme oxygenase, producing a linear tetrapyrrole.
- Biliverdin is an early product of catabolism and on reduction yields bilirubin. The latter is transported by albumin from peripheral tissues to the liver, where it is taken up by hepatocytes. The iron of heme and the amino acids of globin are conserved and reutilized.
- In the liver, bilirubin is made water-soluble by conjugation with two molecules of glucuronic acid and is secreted into the bile. The action of bacterial enzymes in the gut produces urobilinogen and urobilin, which are excreted in the feces and urine.
- Jaundice is due to elevation of the level of bilirubin in the blood. The causes of jaundice can be classified

as prehepatic (eg, hemolytic anemias), hepatic (eg, hepatitis), and posthepatic (eg, obstruction of the common bile duct). Measurements of plasma total and nonconjugated bilirubin, of urinary urobilinogen and bilirubin, and of certain serum enzymes as well as inspection of stool samples help distinguish between these causes.

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