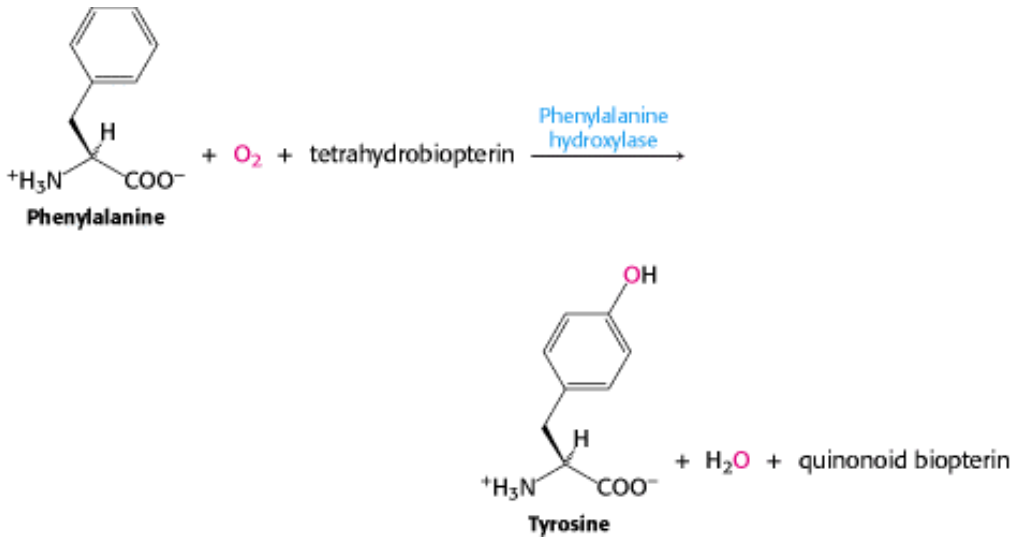
23.5.7. Oxygenases Are Required for the Degradation of Aromatic Amino Acids

The degradation of the aromatic amino acids is not as straightforward as that of the amino acids previously discussed, although the final products—acetoacetate, fumarate, and pyruvate—are common intermediates. For the aromatic amino acids, *molecular oxygen is used to break an aromatic ring.*

The degradation of phenylalanine begins with its hydroxylation to tyrosine, a reaction catalyzed by *phenylalanine hydroxylase.* This enzyme is called a *monooxygenase* (or *mixed-function oxygenase*) because *one atom of* O_2 appears in the product and the other in H_2O.



The reductant here is *tetrahydrobiopterin,* an electron carrier that has not been previously discussed and is derived from the cofactor *biopterin*. Because biopterin is synthesized in the body, it is not a vitamin. Tetrahydrobiopterin is initially formed by the reduction of dihydrobiopterin by NADPH in a reaction catalyzed by *dihydrofolate reductase* (Figure 23.28). NADH reduces the quinonoid form of dihydrobiopterin produced in the hydroxylation of phenylalanine back to tetrahydrobiopterin in a reaction catalyzed by *dihydropteridine reductase.* The sum of the reactions catalyzed by phenylalanine hydroxylase and dihydropteridine reductase is

$$\text{Phenylalanine} + O_2 + \text{NADH} + H^+ \longrightarrow \text{tyrosine} + \text{NAD}^+ + H_2O$$

Note that these reactions can also be used to synthesize tyrosine from phenylalanine.

The next step in the degradation of phenylalanine and tyrosine is the transamination of tyrosine to p-*hydroxyphenylpyruvate* (Figure 23.29). This α-ketoacid then reacts with O_2 to form *homogentisate.* The enzyme catalyzing this complex reaction, p-*hydroxyphenylpyruvate hydroxylase,* is called a *dioxygenase* because *both atoms of* O_2 become incorporated into the product, one on the ring and one in the carboxyl group. The aromatic ring of homogentisate is then cleaved by O_2, which yields 4-maleylacetoacetate. This reaction is catalyzed by *homogentisate oxidase,* another dioxygenase. 4-Maleylacetoacetate is then isomerized to *4-fumarylacetoacetate* by an enzyme that uses glutathione as a cofactor. Finally, 4-fumarylacetoacetate is hydrolyzed to *fumarate* and *acetoacetate.*

Tryptophan degradation requires several oxygenases (Figure 23.30). Tryptophan 2,3-dioxygenase cleaves the pyrrole ring, and kynureinine 3-monooxygenase hydroxylates the remaining benzene ring, a reaction similar to the hydroxylation of phenylalanine to form tyrosine. Alanine is removed and the 3-hydroxyanthranilic acid is cleaved with another dioxygenase and subsequently processed to acetoacetyl CoA (Figure 23.31). *Nearly all cleavages of aromatic rings in*

biological systems are catalyzed by dioxygenases, a class of enzymes discovered by Osamu Hayaishi. The active sites of these enzymes contain iron that is not part of heme or an iron- sulfur cluster.

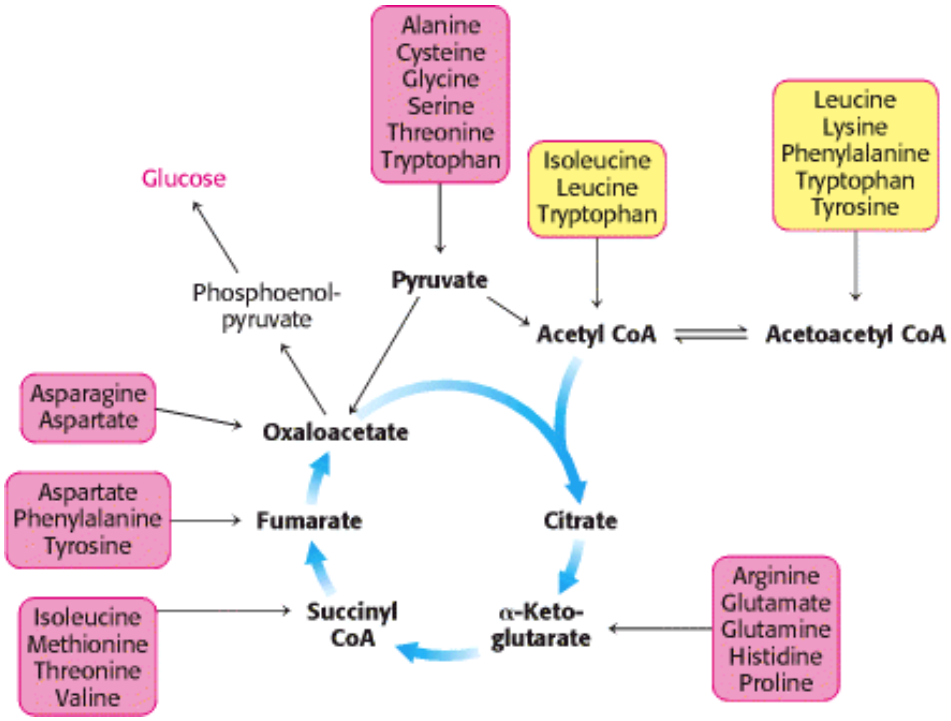


Figure 23.21. Fates of the Carbon Skeletons of Amino Acids. Glucogenic amino acids are shaded red, and ketogenic amino acids are shaded yellow. Most amino acids are both glucogenic and ketogenic.

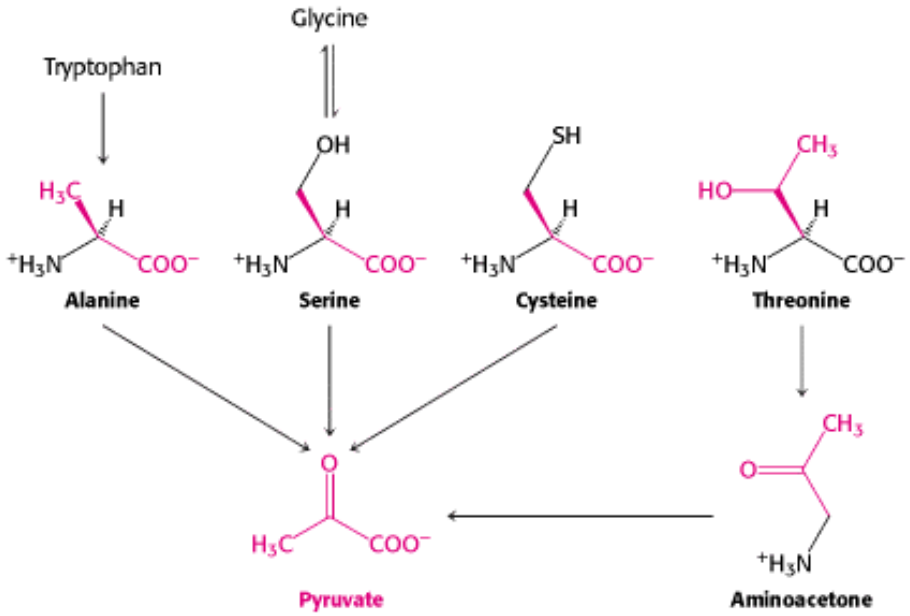


Figure 23.22. Pyruvate Formation from Amino Acids. Pyruvate is the point of entry for alanine, serine, cysteine, glycine, threonine, and tryptophan.

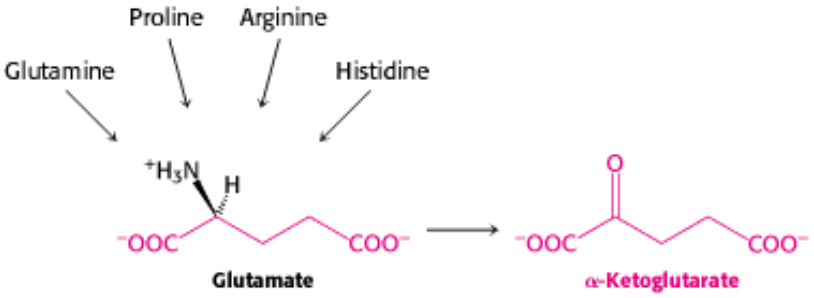


Figure 23.23. α -Ketoglutarate formation from amino acids. α-Ketoglutarate is the point of entry of several five-carbon amino acids that are first converted into glutamate.

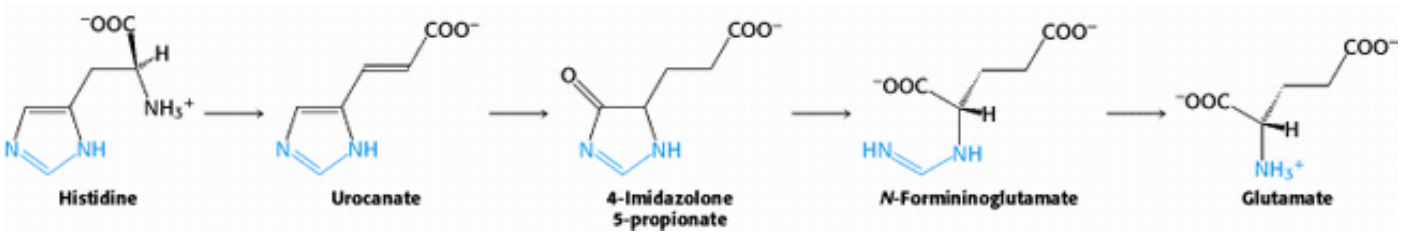


Figure 23.24. Histidine Degradation. Conversion of histidine into glutamate.

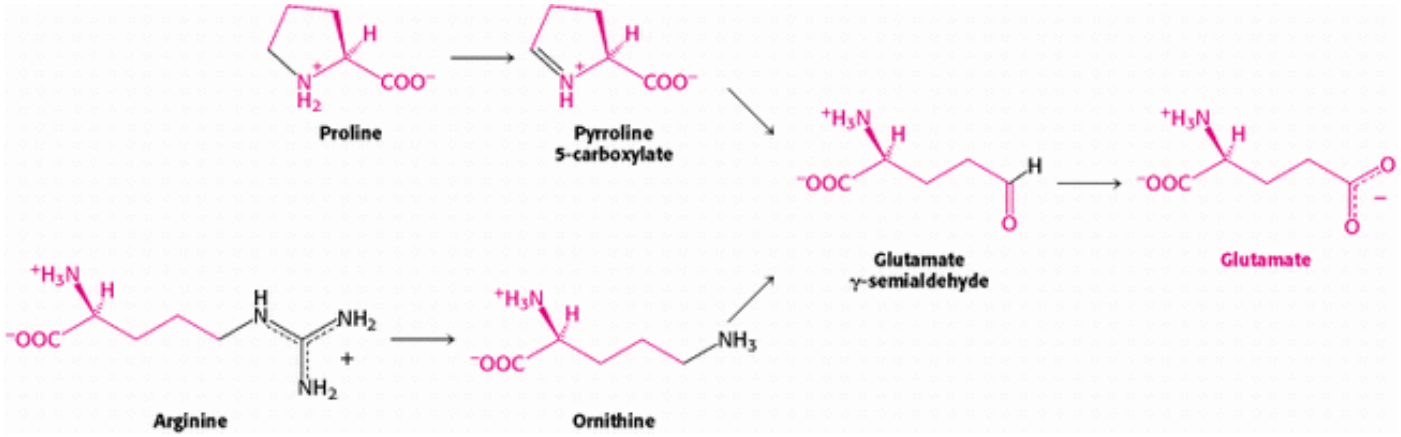


Figure 23.25. Glutamate Formation. Conversion of proline and arginine into glutamate.

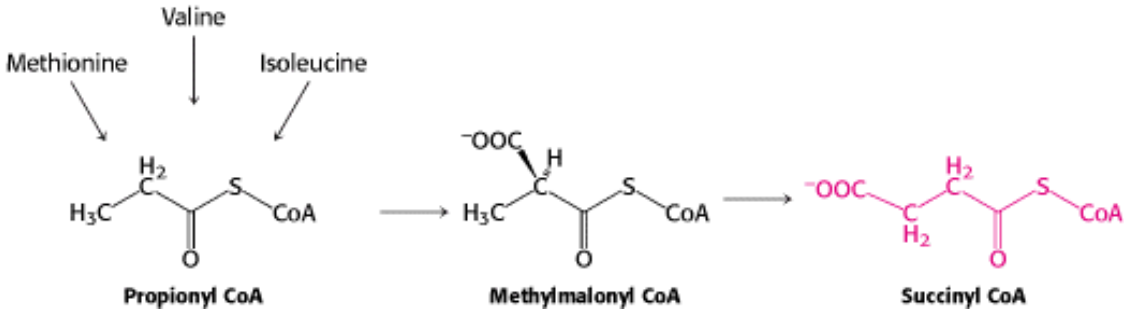


Figure 23.26. Succinyl CoA Formation. Conversion of methionine, isoleucine, and valine into succinyl CoA.

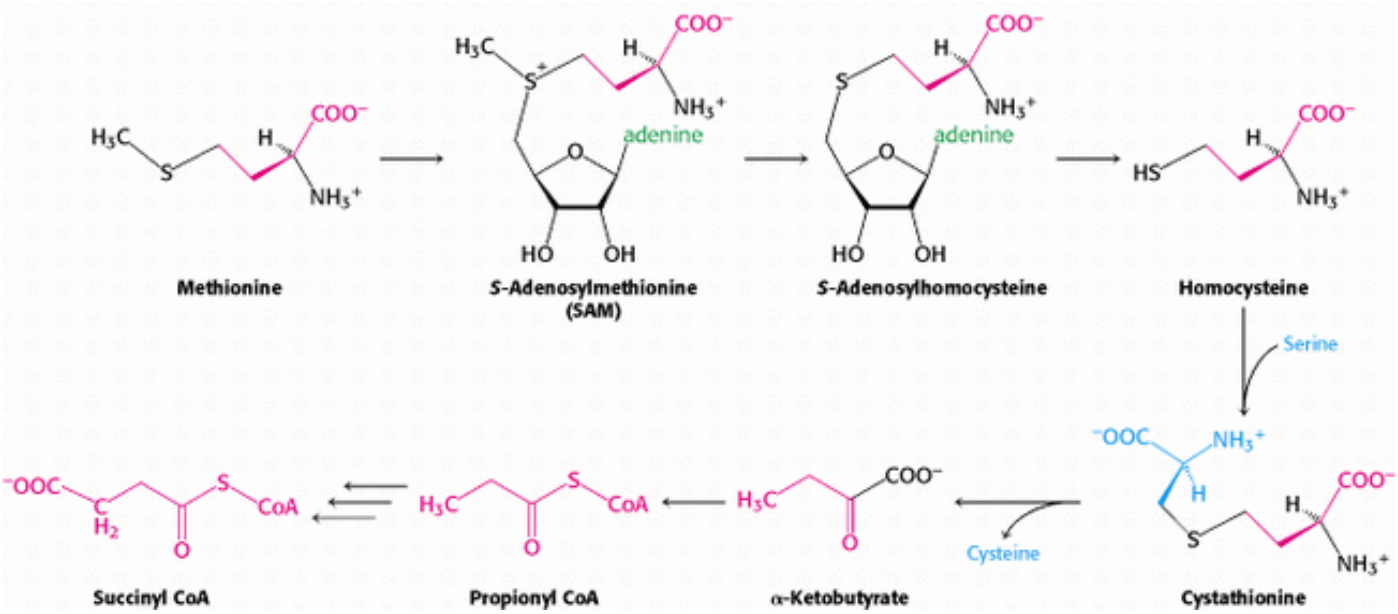


Figure 23.27. Methionine Metabolism. The pathway for the conversion of methionine into succinyl CoA. *S*-Adenosylmethionine, formed along this pathway, is an important molecule for transferring methyl groups.

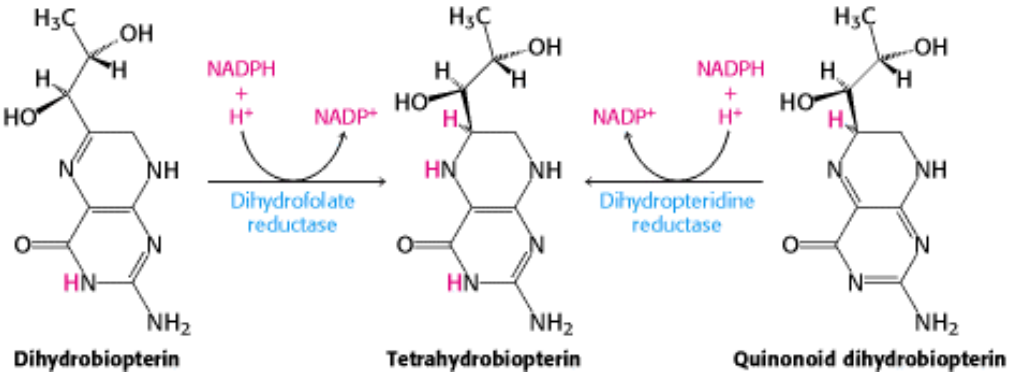


Figure 23.28. Formation of Tetrahydrobiopterin, an Important Electron Carrier. Tetrahydrobiopterin can be formed by the reduction of either of two forms of dihydrobiopterin.

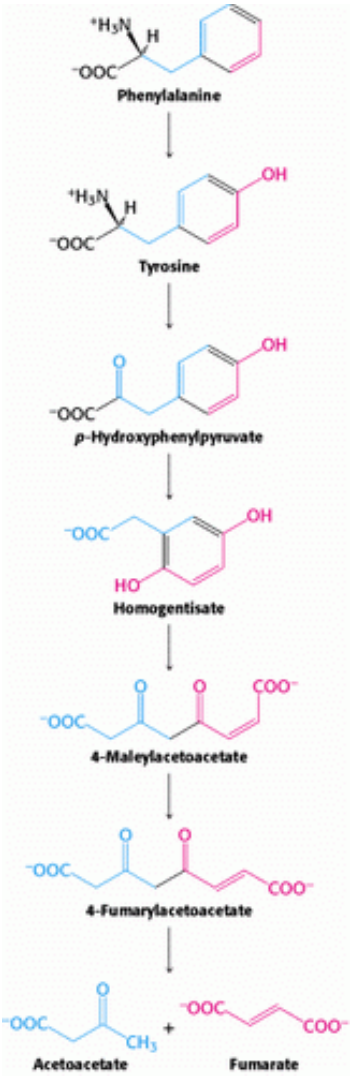


Figure 23.29. Phenylalanine and Tyrosine Degradation. The pathway for the conversion of phenylalanine into acetoacetate and fumarate.

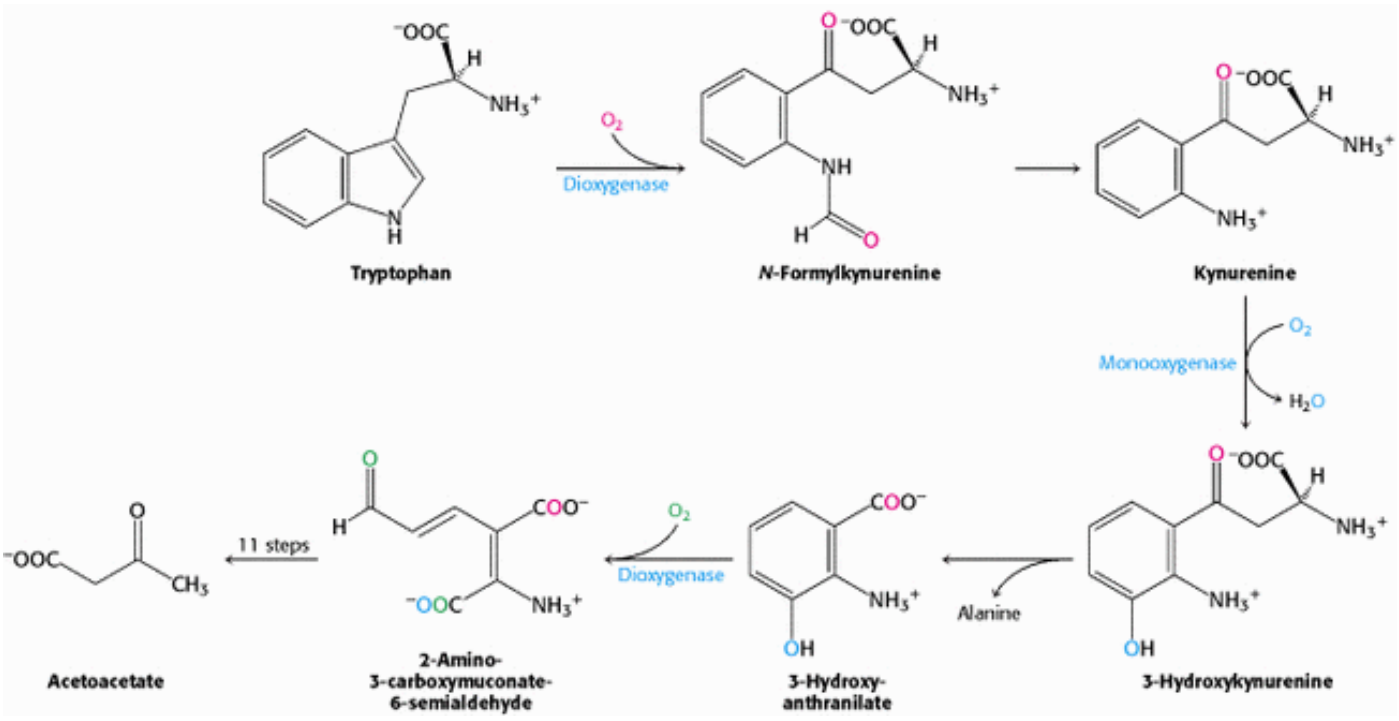


Figure 23.30. Tryptophan Degradation. The pathway for the conversion of tryptophan into alanine and acetoacetate.

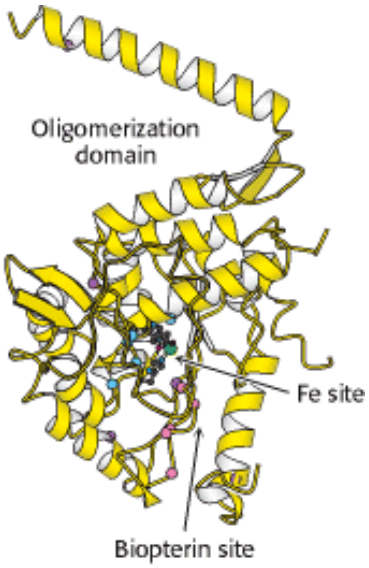
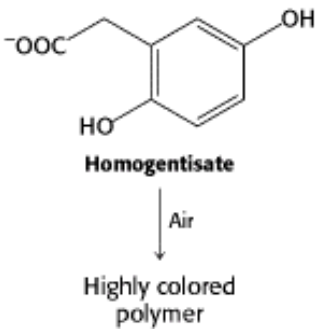


Figure 23.31. Structure of One Subunit of Phenylalanine Hydroxylase. Mutations in the genes encoding this enzyme cause phenylketonuria. More than 200 point mutations have been identified in these genes. The positions of five mutations affecting the active site (blue), the biopterin-binding site (red), and other regions of the protein (purple) are indicated as colored spheres.

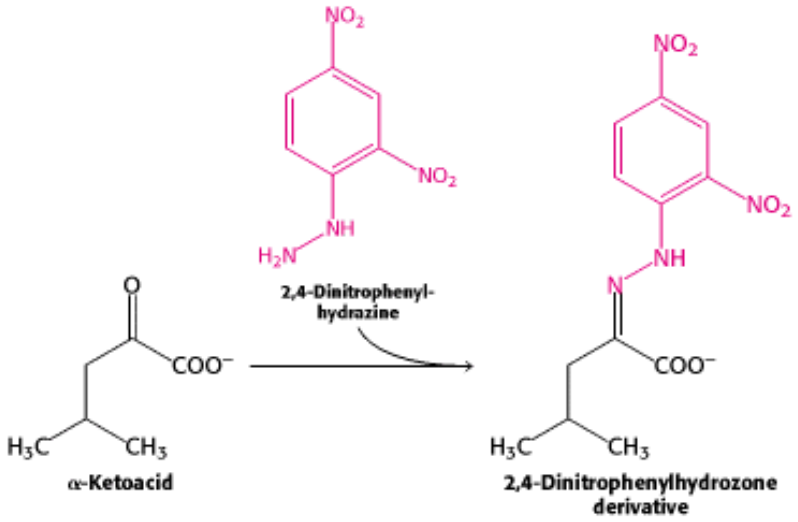
23.6. Inborn Errors of Metabolism Can Disrupt Amino Acid Degradation

Errors in amino acid metabolism provided some of the first correla- tions between biochemical defects and pathological conditions. For instance, *alcaptonuria* is an inherited metabolic disorder caused by the absence of homogentisate oxidase. In 1902, Archibald Garrod showed that alcaptonuria is transmitted as a single recessive Mendelian trait. Furthermore, he recognized that homogentisate is a normal intermediate in the degradation of phenylalanine and tyrosine (see Figure 23.29) and that it accumulates in alcaptonuria because its degradation is blocked. He concluded that "the splitting of the benzene ring in normal metabolism is the work of a special enzyme, that in congenital alcaptonuria this enzyme is wanting." Homogentisate accumulates and is excreted in the urine, which turns dark on standing as homogentisate is oxidized and polymerized to a melanin-like substance.

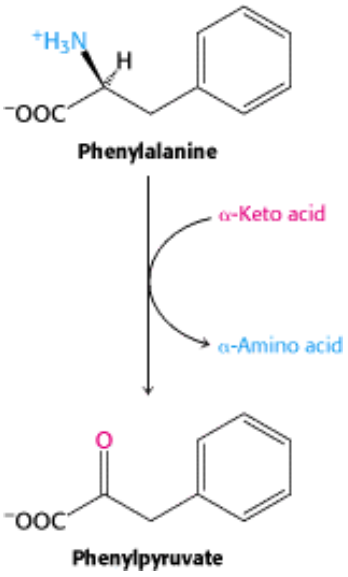


Although alcaptonuria is a relatively harmless condition, such is not the case with other errors in amino acid metabolism. In *maple syrup urine disease*, the oxidative decarboxylation of α-ketoacids derived from valine, isoleucine, and leucine is blocked because the branched-chain dehydrogenase is missing or defective. Hence, the levels of these α-ketoacids and the branched-chain amino acids that give rise to them are markedly elevated in both blood and urine. Indeed, the urine of patients has the odor of maple syrup—hence the name of the disease (also called *branched-chain ketoaciduria*). Maple syrup urine disease usually leads to mental and physical retardation unless the patient is placed on a diet low in valine,

isoleucine, and leucine early in life. The disease can be readily detected in newborns by screening urine samples with 2,4-dinitrophenylhydrazine, which reacts with α-ketoacids to form 2,4-dinitrophenylhydrazone derivatives. A definitive diagnosis can be made by mass spectrometry.



Phenylketonuria is perhaps the best known of the diseases of amino acid metabolism. Phenylketonuria is caused by an *absence or deficiency of phenylalanine hydroxylase* or, more rarely, of its tetrahydrobiopterin cofactor. *Phenylalanine accumulates in all body fluids because it cannot be converted into tyrosine.* Normally, three-quarters of the phenylalanine is converted into tyrosine, and the other quarter becomes incorporated into proteins. Because the major outflow pathway is blocked in phenylketonuria, the blood level of phenylalanine is typically at least 20-fold as high as in normal people. Minor fates of phenylalanine in normal people, such as the formation of phenylpyruvate, become major fates in phenylketonurics.



Indeed, the initial description of phenylketonuria in 1934 was made by observing the reaction of phenylpyruvate with $FeCl_3$, which turns the urine olive green. *Almost all untreated phenylketonurics are severely mentally retarded.* In fact, about 1% of patients in mental institutions have phenylketonuria. The brain weight of these people is below normal, myelination of their nerves is defective, and their reflexes are hyperactive. The life expectancy of untreated phenylketonurics is drastically shortened. Half are dead by age 20 and three-quarters by age 30. *The biochemical basis of their mental retardation is an enigma.*

Phenylketonurics appear normal at birth, but are severely defective by age 1 if untreated. The therapy for phenylketonuria is a *low phenylalanine diet.* The aim is to provide just enough phenylalanine to meet the needs for

growth and replacement. Proteins that have a low content of phenylalanine, such as casein from milk, are hydrolyzed and phenylalanine is removed by adsorption. A low phenylalanine diet must be started very soon after birth to prevent irreversible brain damage. In one study, the average IQ of phenylketonurics treated within a few weeks after birth was 93; a control group treated starting at age 1 had an average IQ of 53.

Early diagnosis of phenylketonuria is essential and has been accomplished by mass screening programs. The phenylalanine level in the blood is the preferred diagnostic criterion because it is more sensitive and reliable than the $FeCl_3$ test. Prenatal diagnosis of phenylketonuria with DNA probes has become feasible because the gene has been cloned and many mutations have been pinpointed to sites in the protein (see Figure 23.31). Interestingly, whereas some mutations affect the activity of the enzyme, others do not affect the activity itself but, instead, decrease the enzyme concentration. These mutations lead to degradation of the enzyme, at least in part by the ubiquitin-proteasome pathway.

The incidence of phenylketonuria is about 1 in 20,000 newborns. The disease is inherited in an *autosomal recessive* manner. Heterozygotes, who make up about 1.5% of a typical population, appear normal. Carriers of the phenylketonuria gene have a reduced level of phenylalanine hydroxylase, as indicated by an increased level of phenylalanine in the blood. However, this criterion is not absolute, because the blood levels of phenylalanine in carriers and normal people overlap to some extent. The measurement of the kinetics of the disappearance of intravenously administered phenylalanine is a more definitive test for the carrier state. It should be noted that a high blood level of phenylalanine in a pregnant woman can result in abnormal development of the fetus. This is a striking example of maternal-fetal relationships at the molecular level. Table 23.3 lists some other diseases of amino acid metabolism.

Table 23.3. Inborn errors of amino acid metabolism

Disease	Enzyme deficiency	Symptoms
Citrullinema	Arginosuccinate lyase	Lethergy, siezures, reduced muscle tension
Tyrosinemia	Various enzymes of tyrosine degradation	Weakness, self-mutilation, liver damage, mental retardation
Albinism	Tyrosinase	Absence of pigmentation
Homocystinuria	Cystathionine β-synthase	Scoliosis, muscle weakness, mental retardation, thin blond hair
Hyperlysinemia	α-Aminoadipic semialdehyde dehydrogenase	Seizures, mental retardation, lack of muscle tone, ataxia

Summary

Proteins Are Degraded to Amino Acids

Dietary protein is digested in the intestine, producing amino acids that are transported throughout the body. Cellular proteins are degraded at widely variable rates, ranging from minutes to the life of the organism.

Protein Turnover Is Tightly Regulated

The turnover of cellular proteins is a regulated process requiring complex enzyme systems. Proteins to be degraded are conjugated with ubi-quitin, a small conserved protein, in a reaction driven by ATP hydrolysis. The ubiquitin conjugating system is composed of three distinct enzymes. A large, barrel-shaped complex called the proteasome digests the