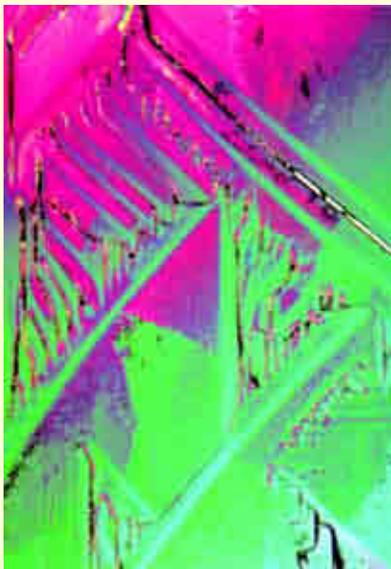


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Most of the ureotelic animals including man and shark secrete the excess ammonia as urea.

CHAPTER

26

Oxidation of Amino Acids

INTRODUCTION

Amino acids are the final class of biomolecules whose oxidation makes a significant contribution towards generation of metabolic energy. The fraction of metabolic energy derived from amino acids varies greatly with the type of organism and with the metabolic situation in which an organism finds itself. **Carnivores** may derive up to 90% of their energy requirements from amino acid oxidation. **Herbivores**, on the other hand, may obtain only a small fraction of their energy needs from this source. Most **microorganisms** can scavenge amino acids from their environment if they are available; these can be oxidized as fuel when the metabolic conditions so demand. **Photosynthetic plants**, on the contrary, rarely oxidize amino acids to provide energy. Instead they convert CO₂ and H₂O into the carbohydrate glucose that is used almost exclusively as an energy source. *Amino acid metabolism does occur in plants, but it is generally concerned with the production of metabolites for other biosynthetic pathways.*

In animals, the amino acids can be oxidatively degraded in 3 different metabolic conditions:

- (a) *During normal protein synthesis:* Some of the amino acids released during protein breakdown will undergo oxidative degradation.
- (b) *During protein-rich diet:* The surplus may be catabolized and amino acids cannot be stored.
- (c) *During starvation or in diabetes mellitus:* Body proteins are used as fuel.

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Under these different circumstances, amino acids lose their amino groups, and the resulting α -keto acids may undergo oxidation to produce CO_2 and H_2O .

Pathways leading to amino acid degradation are quite alike in most organisms. *As is the case for sugar and fatty acid catabolic pathways, the processes of amino acid degradation converge on the central catabolic pathways for carbon metabolism.* However, one major factor distinguishes amino acid degradation from the catabolic processes described till now, *i.e.*, every amino acid contains an amino group. As such every degradative pathway passes through a key step in which α -amino group is separated from the carbon skeleton and shunted into the specialized pathways for amino group metabolism (Fig. 26–1). This biochemical fork in the road is the point around which this chapter is centered.

Amino acids are needed for the synthesis of proteins and other biomolecules. The excess amount of amino acids, in contrast with glucose and fatty acids, cannot be stored; nor are they excreted. Rather surplus amino acids are used as metabolic fuel. *The α -amino group of the amino acids is removed and the resulting carbon skeleton is converted into a major metabolic intermediate.* Most of the amino groups of surplus amino acids are converted into urea whereas their carbon skeletons are transferred to acetyl-CoA, acetoacetyl-CoA, pyruvate, or one of the intermediates of the citric acid cycle. It follows that *amino acids can form glucose, fatty acids and ketone bodies.* The major site of amino acid degradation in mammals is the liver. The fate of the α -amino groups will be dealt with first, followed by that of the carbon skeleton.

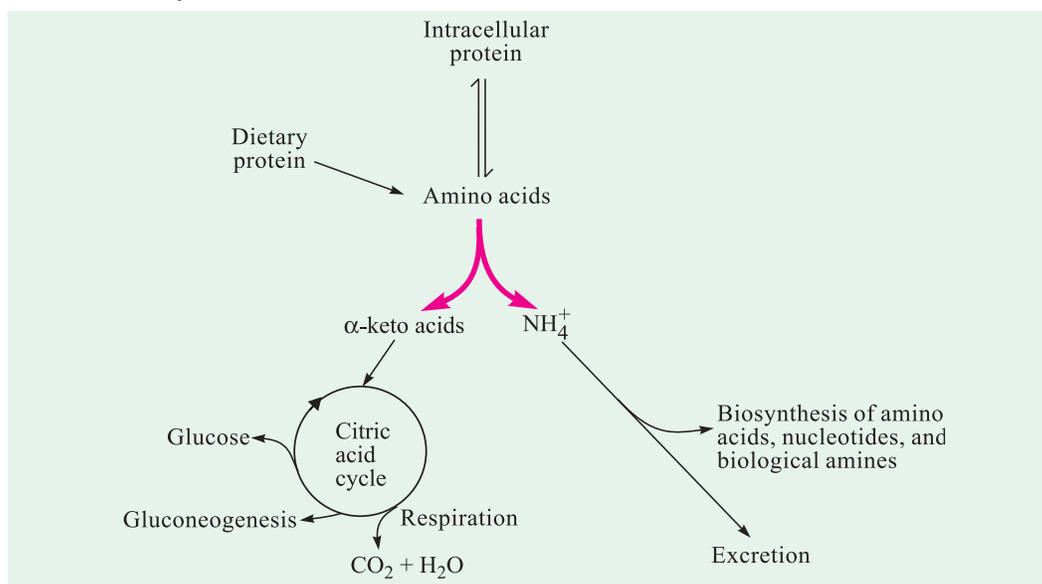


Fig. 26–1. An overview of the catabolism of amino acids

The thick bifurcated arrow indicates the separate paths taken by the carbon skeleton and the amino groups.

AMINO GROUP METABOLISM (= METABOLIC FATES OF AMINO GROUPS)

Nitrogen is the fourth most important contributor (after carbon, hydrogen and oxygen) to the mass of living cells. Atmospheric nitrogen is abundant but is too inert for use in most biochemical processes. Only a few microorganisms have the capacity to convert into biologically useful forms (such as NH_3) and as such amino groups are used with great economy in biological systems.

The catabolism of ammonia and amino groups in vertebrates is presented in Fig. 26–2. Amino acids derived from dietary proteins are the source of most amino groups. *Most of the amino groups*

are metabolized in the liver. Some of the ammonia that is generated is recycled and used in a variety of biosynthetic processes; the excess is either excreted directly or converted to uric acid or urea for excretion. Excess ammonia generated in extrahepatic (*i.e.*, other than liver) tissues is transported to the liver in the form of amino groups, as described below, for conversion to the appropriate excreted form. The coenzyme pyridoxal phosphate (PLP or PALP) participates in these reactions.

Two amino acids, glutamate and its amide form glutamine, play crucial roles in these pathways. Amino groups from amino acids are generally first transferred to a α -ketoglutarate in the cytosol of liver cells (= hepatocytes) to form glutamate. Glutamate is then transported into the mitochondria. In muscle, excess amino groups are generally transferred to pyruvate to form alanine. Alanine is another important molecule in the transport of amino groups, transporting them from muscle to the liver.

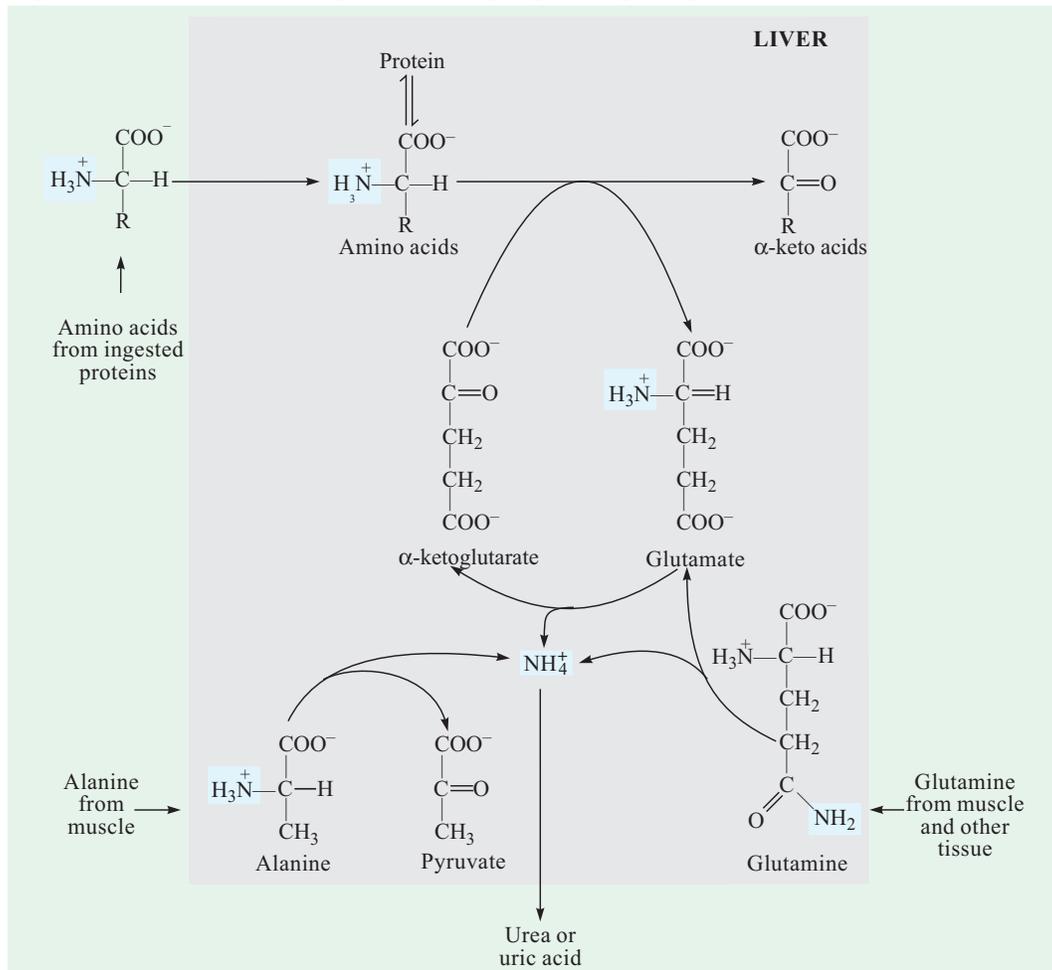


Fig. 26-2. An overview of amino group catabolism in the vertebrate liver

Note that the excess NH_4^+ is excreted as urea or uric acid

A. Transfer of Amino Groups to Glutamate

The α -amino groups of the 20 l-amino acids, commonly found in proteins, are removed during the oxidative degradation of the amino acids. If not reused for the synthesis of new amino acids, these amino groups are channelled into a single excretory product (Fig. 26-3). Many aquatic organisms simply release ammonia as NH_4^+ into the surrounding medium. Most terrestrial vertebrates first convert ammonia into either urea (*e.g.*, humans, other mammals and adult amphibians) or uric acid (*e.g.*, reptiles, birds).

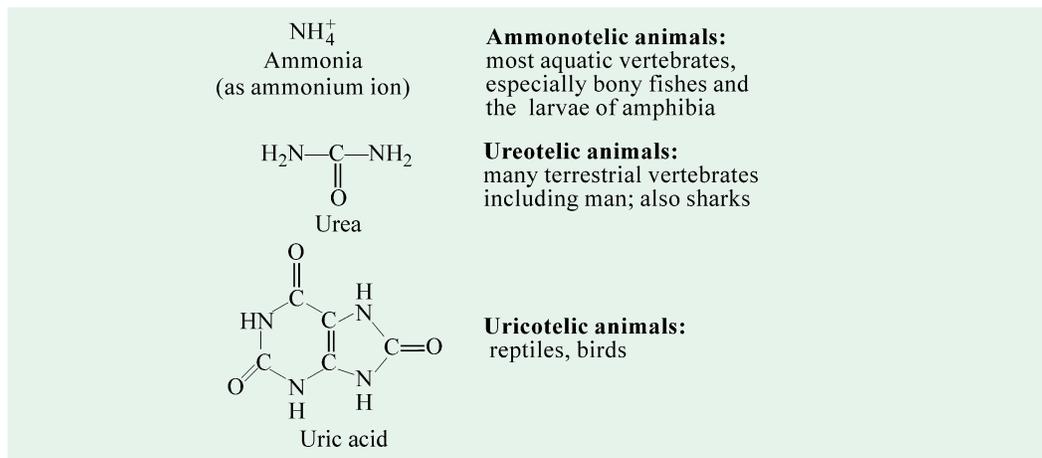


Fig. 26-3. Excretory forms of amino group nitrogen in different forms of life

Note that the C atoms of urea and uric acid are at a high oxidation state. And the organism discards carbon only when it has obtained most of its available energy of oxidation.

The first step in the catabolism of most of the L-amino acids is the removal of the α -amino group (*i.e.*, transamination) by a group of enzymes called **aminotransferases** (= **transaminases**). In these reactions, the α -amino group is transferred to the α -carbon atom of α -ketoglutarate, leaving behind the corresponding α -keto acid analogue of the amino acid (Fig. 26-4). There is no net deamination in such reactions because the α -ketoglutarate becomes aminated as the α -amino acid is deaminated. *The effect of transamination reactions is to collect the amino groups from many different amino acids in the form of only one, namely L-glutamate.* Cells contain several different aminotransferases, many of which are specific for α -ketoglutarate as the amino group acceptor. The amino-transferases differ in their specificity for the other substrate (*i.e.*, the L-amino acid that donates the amino group) and are named for the amino group donor. The reactions catalyzed by the aminotransferases are freely reversible, having an equilibrium constant of about 1.0 ($\Delta G^{\circ} \approx 0$ kJ/mol).

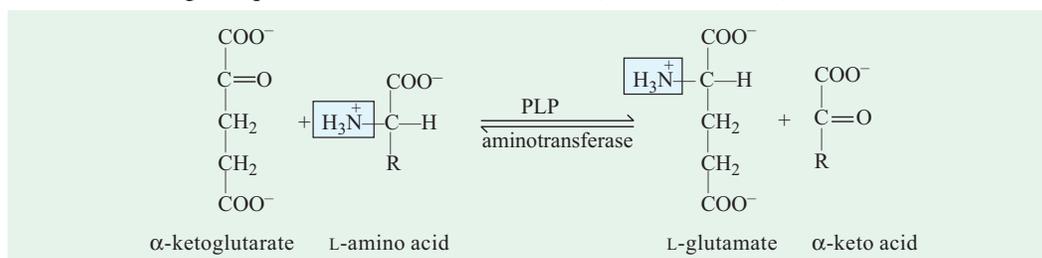


Fig. 26-4. The transamination (or the aminotransferase) reaction

Note that in many aminotransferase reactions, α -ketoglutarate is the amino group acceptor. All aminotransferases have pyridoxal phosphate (PLP or PALP) as cofactor.

All aminotransferases possess a common prosthetic group and have a common reaction mechanism. The prosthetic group is pyridoxal phosphate, which is the coenzyme form of pyridoxine or vitamin B₆. Besides acting as a cofactor in the glycogen phosphorylase reaction, PALP also participates in the metabolism of molecules containing amino groups. PALP functions as an intermediate carrier of amino groups at the active site of aminotransferases. It undergoes reversible transformations between its aldehyde form (pyridoxal phosphate, PALP) which can accept an amino group, and its aminated form (pyridoxamine phosphate, PAMP) which can donate its amino group to an α -keto acid (Fig. 26-5a). PALP is generally bound covalently to the enzyme's active site through an imine (Schiff-base) linkage to the ϵ -amino group of a lysine (Lys) residue (Fig. 26-5b).

Pyridoxal phosphate is involved in a number of reactions at the α and β carbons of amino acids. Reactions at α carbon (Fig. 26–6) include racemizations, decarboxylations and transaminations as well. PALP plays the same chemical role in each of these reactions. One of the bonds to the α carbon is broken, removing either a proton or a carboxyl group, and leaving behind a free electron pair on the carbon, *i.e.*, a carbanion. This intermediate is very unstable. PALP provides a highly conjugated structure (an *electron sink*) that allows delocalization of the negative charge, stabilizing the carbanion.

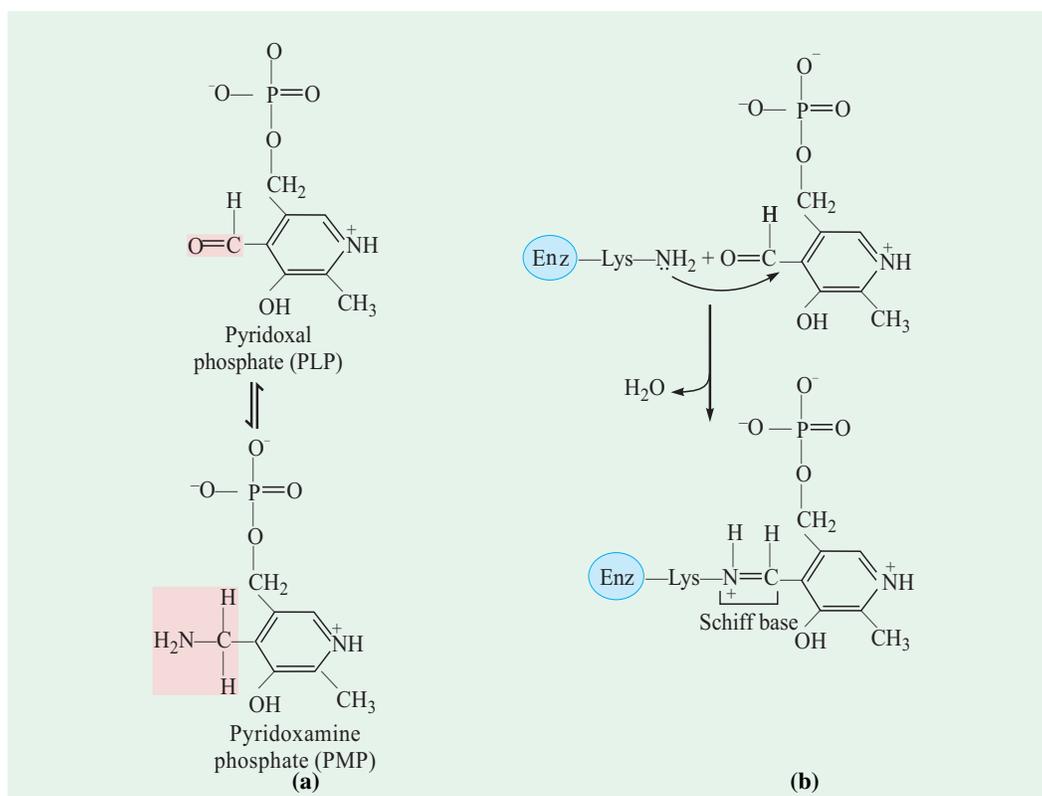


Fig. 26–5. The prosthetic group of aminotransferases

- (a) Pyridoxal phosphate and its aminated form pyridoxamine phosphate. [The functional groups involved in their action are enclosed in rectangle].
- (b) Covalent bonding between pyridoxal phosphate and enzyme's active site both through strong noncovalent interactions and through formation of an imine (Schiff-base) linkage to the ϵ -amino group of a Lys residue.

The catalytic versatility of PLP enzymes is remarkable. The PLP enzymes catalyze a wide range of amino acid transformations and transamination is only one of them. The other reactions at the α -carbon atom of amino acids are decarboxylations, deaminations, racemizations and aldol cleavages (Fig. 26–7). Besides, PLP enzymes catalyze elimination and replacement reactions at the β -carbon atom (*e.g.*, tryptophan synthetase) and at the γ -carbon atom (*e.g.*, cystathionase) of amino acid substrates. These reactions have the following **common features**:

1. A Schiff base is formed by the amino acid substrate (the amine component) and PLP (the carbonyl component).
2. Pyridoxal phosphate acts as an electron sink to stabilize catalytic intermediates that are negatively charged. The ring nitrogen of PLP attracts electrons from the amino acid substrate. In other words, *PLP is an electrophilic catalyst.*

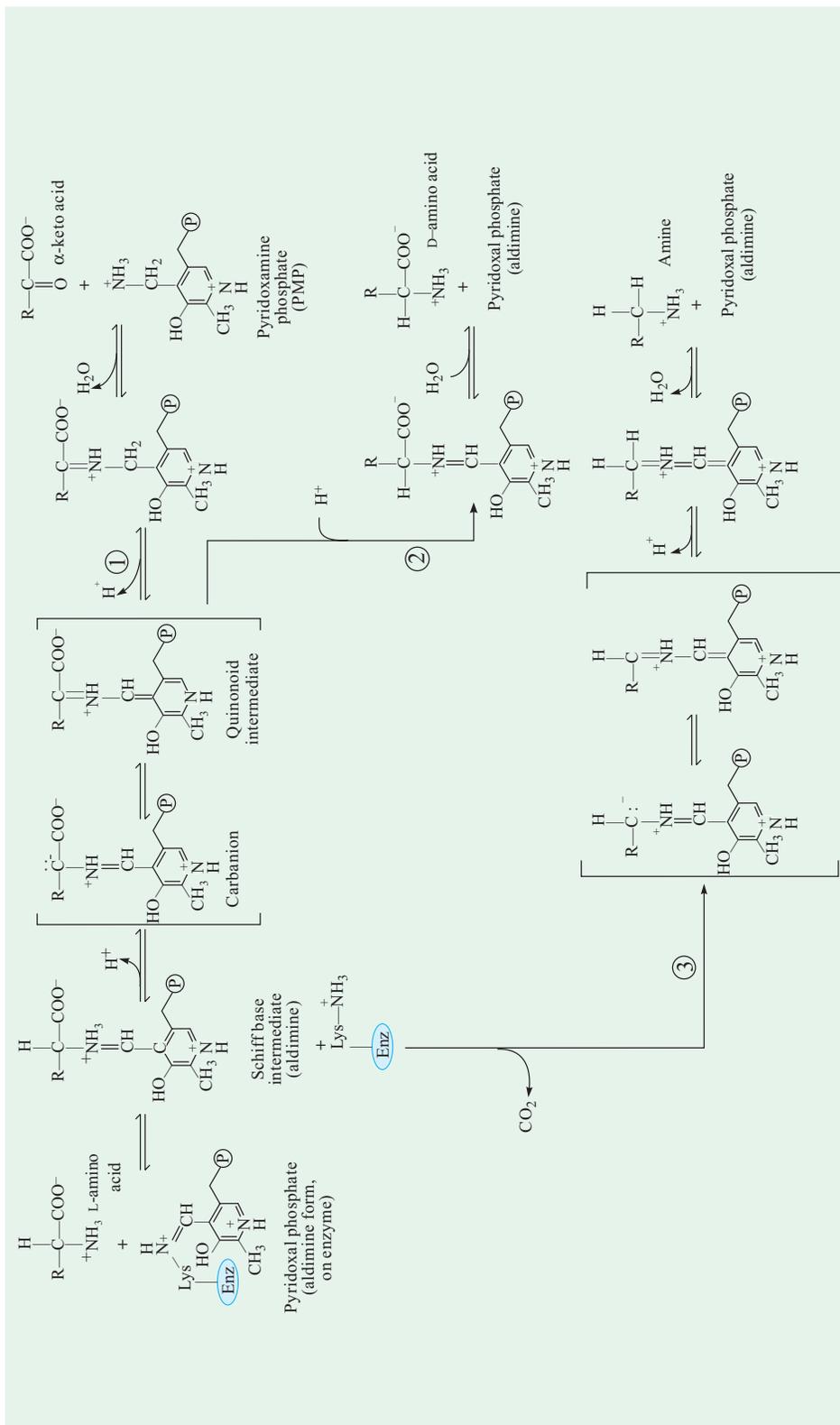


Fig. 26-6. Some amino acid transformations facilitated by pyridoxal phosphate (PLP or PALP)

PALP is usually bound to the enzyme by means of a Schiff base. Reactions begin with the formation of a new Schiff base (aldimine) between the α-amino group of the amino acid and PALP, which substitutes for the enzyme-PALP-linkage. The amino acid then can undergo 3 alternative fates: (1) transamination, (2) racemization, or (3) decarboxylation. The Schiff-base formed between PALP and the amino acid is in conjugation with the pyridine ring, which acts as an electron sink, allowing delocalization of the negative charge of the carbanion (as shown within the brackets). A quinonoid intermediate is involved in all of the reactions.

(Adapted from Lehninger, Nelson and Cox, 1993)

3. The product Schiff base is then hydrolyzed.

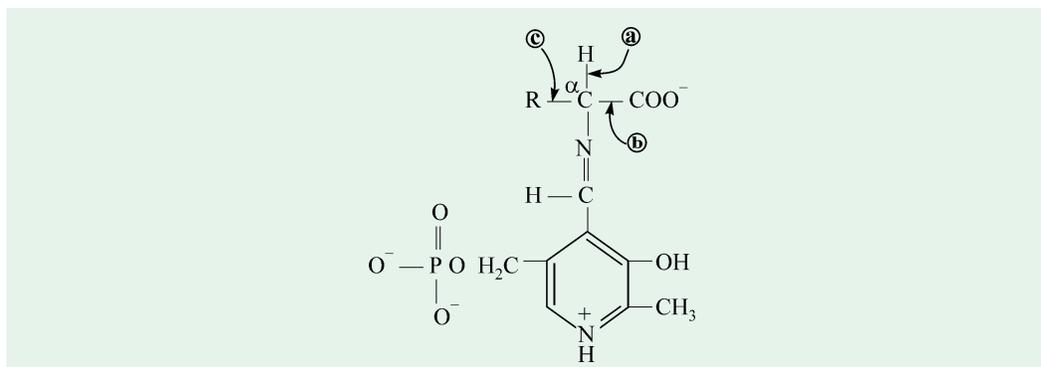
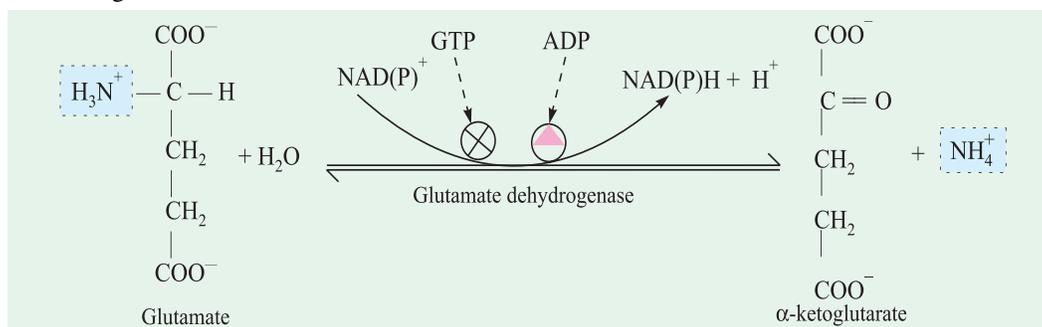


Fig. 26–7. Versatility of the pyridoxal phosphate enzymes

PLP enzymes labilize one of the 3 bonds at the α -carbon atom of an amino acid substrate. For example, bond (a) is labilized by transaminases, bond (b) by decarboxylases, and bond (c) by aldolases. PLP enzymes also catalyze reactions at the β and γ carbon atoms of amino acids.

B. Removal of Amino Groups from Glutamate

Glutamate is transported from the cytosol to the mitochondria, where it undergoes **oxidative deamination** catalyzed by L-glutamate dehydrogenase (GD). GD can employ NAD^+ or NADP^+ as cofactor and is allosterically regulated by GTP and ADP. The combined action of the aminotransferases and GD is referred to as **transdeamination**. A few amino acids bypass the transdeamination pathway and undergo direct oxidative deamination.



Glutamate dehydrogenase (Mr 330,000) is a complex allosteric enzyme and is present only in the mitochondrial matrix. The enzyme molecule consists of 6 identical subunits. It is influenced by the positive modulator ADP and by the negative modulator GTP. Whenever a hepatocyte needs fuel for the citric acid cycle, GD activity increases, making α -ketoglutarate available for the citric acid cycle and releasing NH_4^+ for excretion. On the contrary, whenever GTP accumulates in the mitochondria due to high activity of the citric acid cycle, oxidative deamination of glutamate is inhibited.

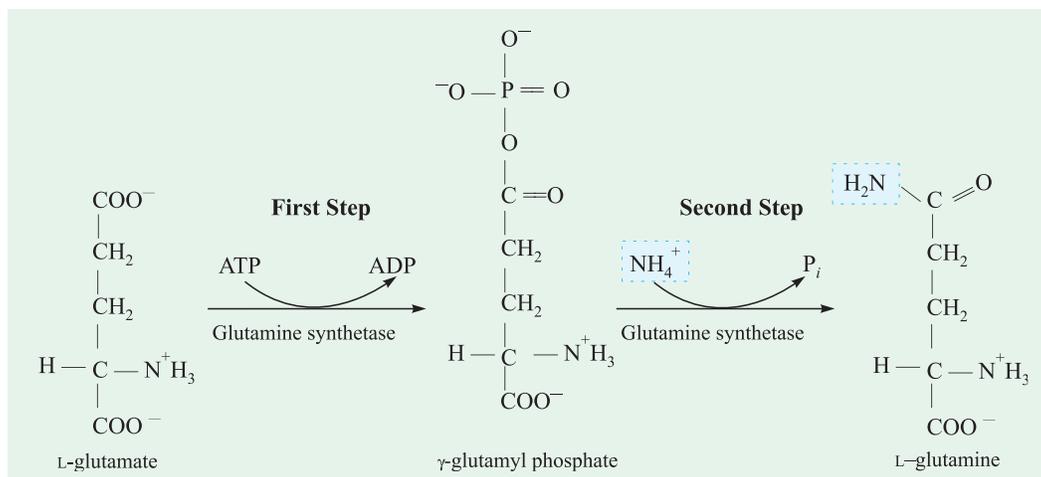
C. Transport of Ammonia Through Glutamine to Liver

In most animals excess ammonia, which is toxic to the animal tissues, is converted into a nontoxic compound before export from extrahepatic tissues into the blood and thence to the liver or kidneys. This transport function is accomplished by **L-glutamine** and not by glutamate which is so critical to amino group metabolism. In many tissues, ammonia enzymatically combines with glutamate to yield glutamine by the action of glutamine synthetase. The reaction, which requires ATP, takes place in 2 steps:

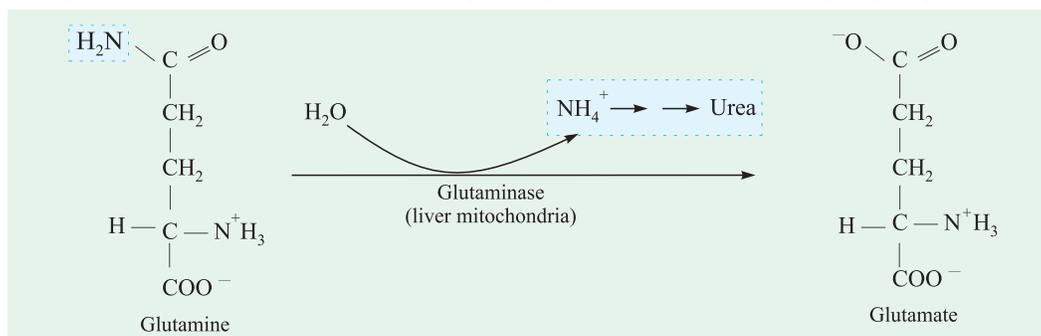
I. *First step:* glutamate and ATP react to form ADP and γ -glutamyl phosphate

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II. *Second step:* γ -glutamyl phosphate reacts with ammonia to produce glutamine and inorganic phosphate.



Glutamine is a nontoxic, neutral compound that can readily pass through cell membranes, whereas glutamate which bears a negative net charge, cannot. In most terrestrial animals, glutamine is carried through blood to the liver. The amide nitrogen of glutamine, like the amino group of glutamate, is



released as ammonia only within liver mitochondria, where the enzyme glutaminase converts glutamine to glutamate and NH_4^+ . *Glutamine is, thus, a major transport form of ammonia.* It is normally present in blood in much higher concentrations than other amino acids.

D. Transport of Ammonia from Muscle to Liver Through Alanine (= Glucose—Alanine Cycle)

Alanine also plays a special role in transporting amino groups to the liver in a nontoxic form by **glucose—alanine cycle** (Fig. 26–8). In muscle and certain other tissues that degrade amino acids for fuel, amino groups are collected in glutamate by transamination (refer Fig. 26–2). Glutamate may then either be converted to glutamine for transport to the liver, or it may transfer its α -amino group to pyruvate, a readily-available product of muscle glycolysis, by the action of **alanine aminotransferase**. Alanine passes into the blood and is carried to the liver. As in the case of glutamine, excess nitrogen carried to the liver as alanine is ultimately delivered as ammonia in the mitochondria. During a reversal of this alanine aminotransferase reaction, alanine transfers its amino group to α -ketoglutarate, forming glutamate in the cytosol. Some of this glutamate is transported into the mitochondria and acted upon by glutamate dehydrogenase, releasing NH_4^+ . Alternatively, transamination with oxaloacetate moves amino groups from glutamate to aspartate, another nitrogen donor in urea synthesis.

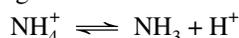
Vigorously contracting skeletal muscles operate anaerobically and produce not only ammonia from protein degradation but also large amounts of pyruvate from glycolysis. Both these products

must find their way to the liver—*ammonia* for its conversion into urea for excretion and *pyruvate* for its incorporation into glucose and subsequent return to the muscles. The animals thus solve two problems with one cycle (*i.e.*, glucose—alanine cycle):

- They move the carbon atoms of pyruvate, as well as excess ammonia from muscle to liver as alanine.
- In the liver, alanine yields pyruvate, the starting material for gluconeogenesis and releases NH_4^+ for urea synthesis.

The energetic burden of gluconeogenesis is, thus, imposed on the liver rather than on the muscle, so that the available ATP in the muscle can be devoted to muscle contraction.

Ammonia is toxic to animals and causes mental disorders, retarded development and, in high amounts, coma and death. The protonated form of ammonia (ammonium ion) is a weak acid, and the unprotonated form is a strong base:



Most of the ammonia generated in catabolic process is present as NH_4^+ at neutral pH. Although most of the reactions that produce ammonia yield NH_4^+ a few reactions produce NH_3 . Excessive amounts of ammonia cause alkalization of cellular fluids, which has multiple effects on cellular metabolism.

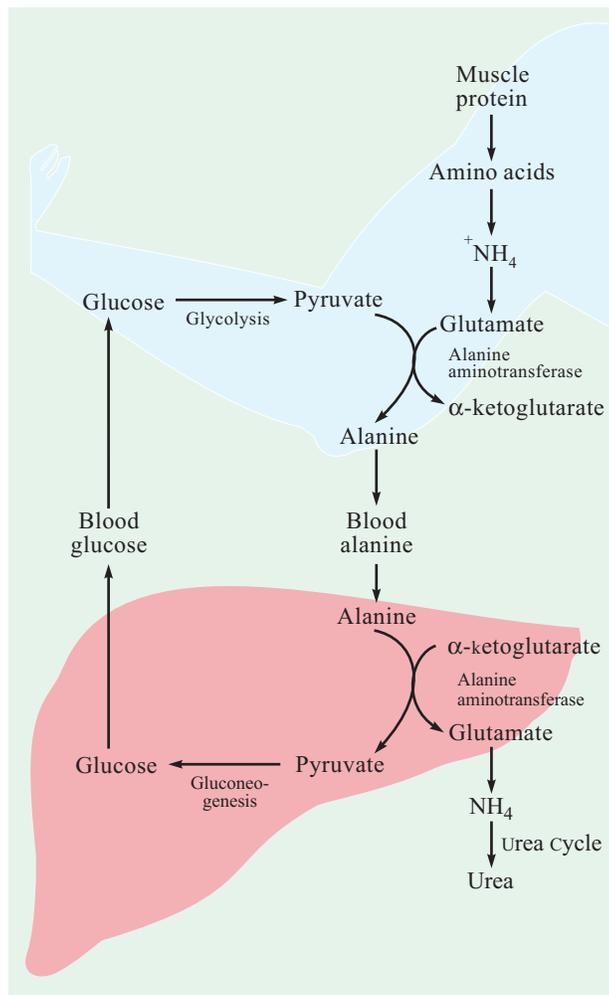


Fig. 26-8. The glucose—alanine cycle

Alanine serves as a carrier of ammonia equivalents and of the carbon skeleton of pyruvate from muscle to liver. The ammonia is excreted, and the pyruvate is used to produce glucose, which is returned to the muscle.

(Adapted from Lehninger, Nelson and Cox, 1993)

NITROGEN EXCRETION

The amino nitrogen is excreted in 3 different forms in various types of life-forms (Fig. 26-3).

- as *ammonia* in most aquatic vertebrates, bony fishes and amphibian larvae (**ammonotelic animals**)
- as *urea* in many terrestrial vertebrates including man, also sharks and adult amphibian (**ureotelic animals**)
- as *uric acid* in reptiles and birds (**uricotelic animals**)

Plants, however, recycle virtually all amino groups, and nitrogen excretion occurs only under highly unusual circumstances. *There is no general pathway for nitrogen excretion in plants.*

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In ureotelic organisms, the ammonia in the mitochondria of liver cells (= hepatocytes) is converted to urea *via* the **urea cycle**. This pathway was discovered by Hans Adolf Krebs and a medical student associate, Kurt Henseleit in 1932, five years before the elucidation of the citric acid cycle. In fact, *the urea cycle was the first cyclic metabolic pathway to be discovered*. They found that the rate of urea formation from ammonia was greatly accelerated by adding any one of the 3 α -amino acids: ornithine, citrulline or arginine. Their structure suggests that they might be related in a sequence. This finding led them to deduce that a cyclic process occurs (Fig. 26-9), in which ornithine plays a role resembling that of oxaloacetate in the citric acid cycle. A mole of ornithine combines with one mole of ammonia and one of CO_2 to form citrulline. A second amino group is added to citrulline to form arginine. Arginine is then hydrolyzed to yield urea with concomitant regeneration of ornithine. Ureotelic animals have large amounts of the enzyme arginase in the liver. This enzyme catalyzes the irreversible hydrolysis of arginine to urea and ornithine. The ornithine is then ready for the next turn of the urea cycle. The urea is passed *via* the bloodstream to the kidneys and is excreted into the urine.

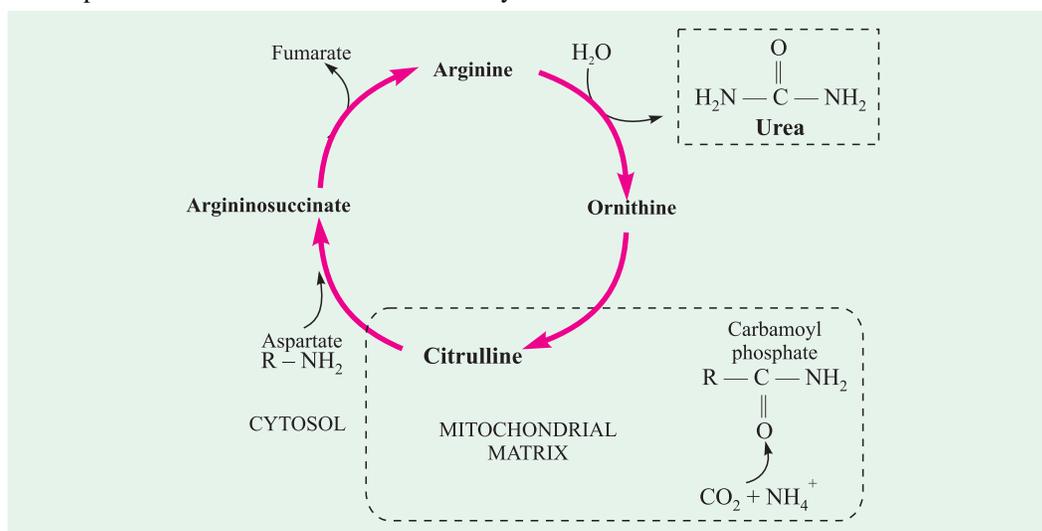


Fig. 26-9. The urea cycle

Note that ornithine and citrulline can serve as successive precursors of arginine. Ornithine and citrulline are nonstandard amino acids that are not found in proteins.

A. Production of Urea from Ammonia: The Urea Cycle

A moderately-active man consuming about 300 g of carbohydrate, 100 g of fat and 100 g of protein daily must excrete about 16.5 g of nitrogen daily. Ninety-five per cent is eliminated by the kidneys and the remaining 5% in the faeces. The major pathway of N_2 excretion in humans is as urea which is synthesized in the liver, released into the blood, and cleared by the kidney. In humans eating an occidental diet, urea constitutes 80-90% of the nitrogen excreted. The urea cycle spans two cellular compartments (Figs 26-9 and 26-10). It begins inside the mitochondria of liver cells (= hepatocytes), but 3 of the steps occur in the cytosol. The first amino group to enter the urea cycle is derived from ammonia inside the mitochondria, arising by multiple pathways described above. Whatever its source, the NH_4^+ generated in liver mitochondria is immediately used, together with HCO_3^- produced by mitochondrial respiration, to form **carbamoyl phosphate** in the matrix. This ATP-dependent reaction is catalysed by **carbamoyl phosphate synthetase I**, a regulatory enzyme present in liver mitochondria of all ureotelic organisms including man. In bacteria, glutamine rather than ammonia serves as a substrate for carbamoyl phosphate synthesis.

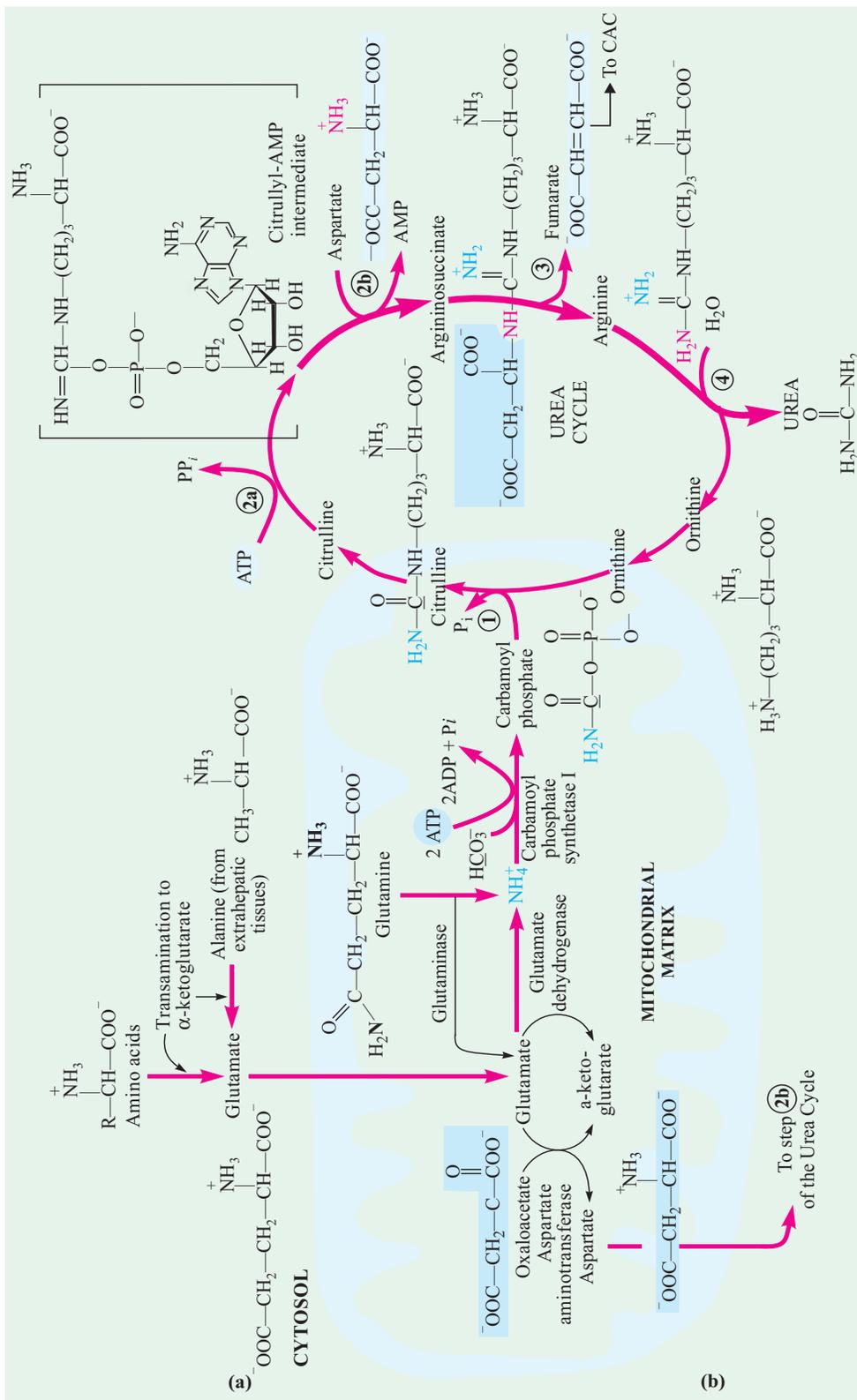


Fig. 26-10. The urea cycle and the reactions that feed amino groups into it

Note that one of the nitrogen atoms of the urea synthesized by this pathway is derived from an amino acid, aspartate. The other nitrogen atom is derived from NH_4^+ and the carbon and the nitrogen atom comes from CO_2 . Ornithine, a nonprotein amino acid, is the carrier of these carbon and nitrogen atoms. Also note that the enzymes catalyzing these reactions are distributed between the mitochondrial matrix and the cytosol.

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The carbamoyl phosphate now enters the urea cycle, which itself consists of 4 enzymatic steps. These are:

Step 1: Synthesis of citrulline

Carbamoyl phosphate has a high transfer potential because of its anhydride bond. It, therefore, donates its carbamoyl group to ornithine to form citrulline and releases inorganic phosphate. The reaction is catalyzed by **L-ornithine transcarbamoylase** of liver mitochondria. The citrulline is released from the mitochondrion into the cytosol.

Step 2: Synthesis of argininosuccinate

The second amino group is introduced from aspartate (produced in the mitochondria by transamination and transported to the cytosol) by a condensation reaction between the amino group of aspartate and the ureido (= carbonyl) group of citrulline to form argininosuccinate. The reaction is catalyzed by **argininosuccinate synthetase** of the cytosol. It requires ATP which cleaves into AMP and pyrophosphate and proceeds through a citrullyl-AMP intermediate.

Step 3: Cleavage of argininosuccinate to arginine and fumarate

Argininosuccinate is then reversibly cleaved by **argininosuccinate lyase** (= **argininosuccinase**), a cold-labile enzyme of mammalian liver and kidney tissues, to form free arginine and fumarate, which enters the pool of citric acid cycle intermediates. These two reactions, which transfer the amino group of aspartate to form arginine, preserve the carbon skeleton of aspartate in the form of fumarate.

Step 4: Cleavage of arginine to ornithine and urea

The arginine so produced is cleaved by the cytosolic enzyme **arginase**, present in the livers of all ureotelic organisms, to yield urea and ornithine. Smaller quantities of arginase also occur in renal tissue, brain, mammary gland, testes and skin. Ornithine is, thus, regenerated and can be transported into the mitochondrion to initiate another round of the urea cycle.

In the urea cycle, mitochondrial and cytosolic enzymes appear to be clustered and not randomly distributed within cellular compartments. The citrulline transported out of the mitochondria is not diluted into the general pool of metabolites in the cytosol. Instead, each mole of citrulline is passed directly into the active site of a molecule of argininosuccinate synthetase. This channeling continues for argininosuccinate, arginine and ornithine. Only the urea is released into the general pool within the cytosol. Thus, the compartmentation of the urea cycle and its associated reactions is noteworthy. The formation of NH_4^+ by glutamate dehydrogenase, its incorporation into carbamoyl phosphate, and the subsequent synthesis of citrulline occur in the *mitochondrial matrix*. In contrast, the next 3 reactions of the urea cycle, which lead to the formation of urea, takes place in the cytosol.

A perusal of the urea cycle reveals that of the 6 amino acids involved in urea synthesis, one, N-acetylglutamate functions as an enzyme activator rather than as an intermediate. The remaining 5 amino acids — aspartate, arginine, ornithine, citrulline and argininosuccinate — all function as carriers of atoms which ultimately become urea. Two (aspartate and arginine) occur in proteins, while the remaining three (ornithine, citrulline and argininosuccinate) do not. The major metabolic roles of these latter 3 amino acids in mammals is urea synthesis. Note that urea formation is, in part, a cyclical process. The ornithine used in Step 1 is regenerated in Step 4. There is thus no net loss or gain of ornithine, citrulline, argininosuccinate or arginine during urea synthesis; however, ammonia, CO_2 , ATP and aspartate are consumed.

B. The “Krebs Bicycle”

The citric acid cycle and urea cycle both are linked together (Fig. 26-11). The fumarate produced

in the cytosol by argininosuccinate lyase reaction is also an intermediate of the citric acid cycle. Fumarate enters the citric acid cycle in the the mitochondrion where it is first hydrated to malate by fumarase which, in turn, is oxidized to oxaloacetate by malate dehydrogenase. The oxaloacetate accepts an amino group from glutamate by transamination, and the aspartate thus formed leaves the mitochondrion and donates its amino group to the urea cycle in the argininosuccinate synthetase reaction; the other product of this transamination is α -ketoglutarate, another intermediate of the citric acid cycle. Because the reactions of the urea and citric acid cycles are inextricably intertwined, cumulatively they have been called “Krebs bicycle”.

C. Energetics of the Urea Cycle

The urea cycle is energetically expensive. It brings together two amino groups and HCO_3^- to form a mole of urea which diffuses from the liver into the bloodstream. The overall reaction of the urea cycle is:

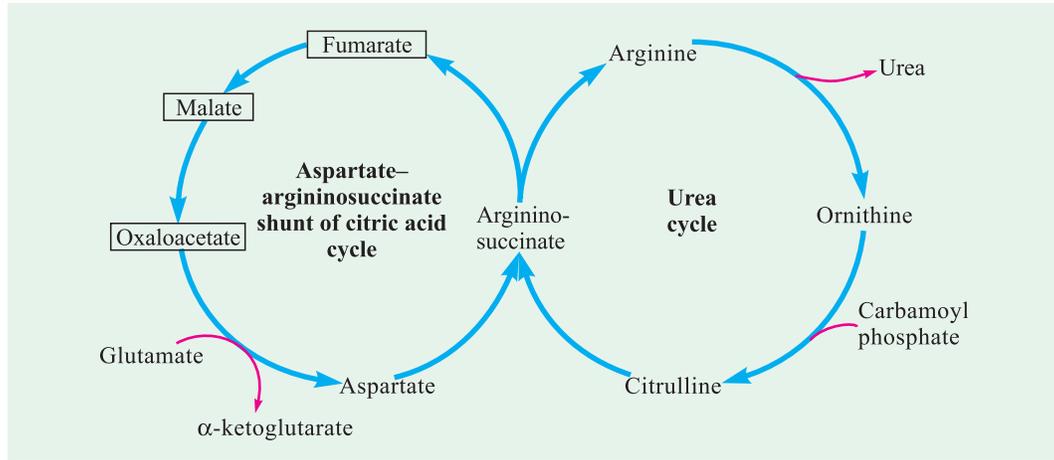
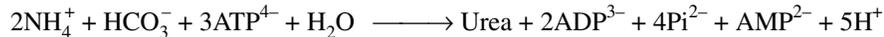


Fig. 26–11. The Krebs bicycle

It is composed of the urea cycle on the right, which meshes with the aspartate—argininosuccinate shunt of the citric acid cycle on the left. Note that the urea cycle, the citric acid cycle and the transamination of oxaloacetate are linked by fumarate and aspartate. Intermediates in the citric acid cycle are boxed



The synthesis of one mole of urea requires four high-energy phosphate groups. Two ATPs are required to make carbamoyl phosphate, and one ATP is required to make argininosuccinate. In the latter reaction, however, the ATP undergoes a pyrophosphate cleavage to AMP and pyrophosphate which may be hydrolyzed to yield two P_i .

Carbamoyl phosphate is the official nomenclature for the $-\text{CO}-\text{NH}_2$ group, but carbamyl is sometimes used.

D. Genetic Defects in the Urea Cycle

The synthesis of urea in the liver is the major pathway of the removal of NH_4^+ . A blockage of carbamoyl phosphate synthesis or any of the 4 steps of the urea cycle has serious consequences because there is no alternative pathway for the synthesis of urea. *They all lead to an elevated level of NH_4^+ in the blood (hyperammonemia)*. Some of these genetic defects become evident a day or two after birth, when the afflicted infant becomes lethargic and vomits periodically. Coma and irreversible brain damage may ensue. The high levels of NH_4^+ are toxic *probably* because elevated levels of glutamine, formed from NH_4^+ and glutamate, lead directly to brain damage:

All or part of the carbon skeletons of *ten* of the amino acids are finally broken down to yield acetyl-CoA. *Five* amino acids are converted into α -ketoglutarate, *four* into succinyl-CoA, *two* into fumarate and *two* into oxaloacetate. The individual pathways for the 20 amino acids will be summarized by means of **flow diagrams**, each leading to a specific point of entry into the citric acid cycle. Note that some amino acids appear more than once which means that their carbon skeleton is broken down into different fragments and each of which enters the citric acid cycle at a different point. *The strategy of amino acid degradation is to form major metabolic intermediates that can be converted into glucose or be oxidized by the citric acid cycle.* In fact, the carbon skeletons of the diverse set of 20 amino acids are funneled into only 7 molecules (refer Fig. 26–13), viz., pyruvate, acetyl-CoA, acetoacetyl-CoA, α -ketoglutarate, succinyl-CoA, fumarate and oxaloacetate. Thus, *here we have an example of the remarkable economy of metabolic conversions.*

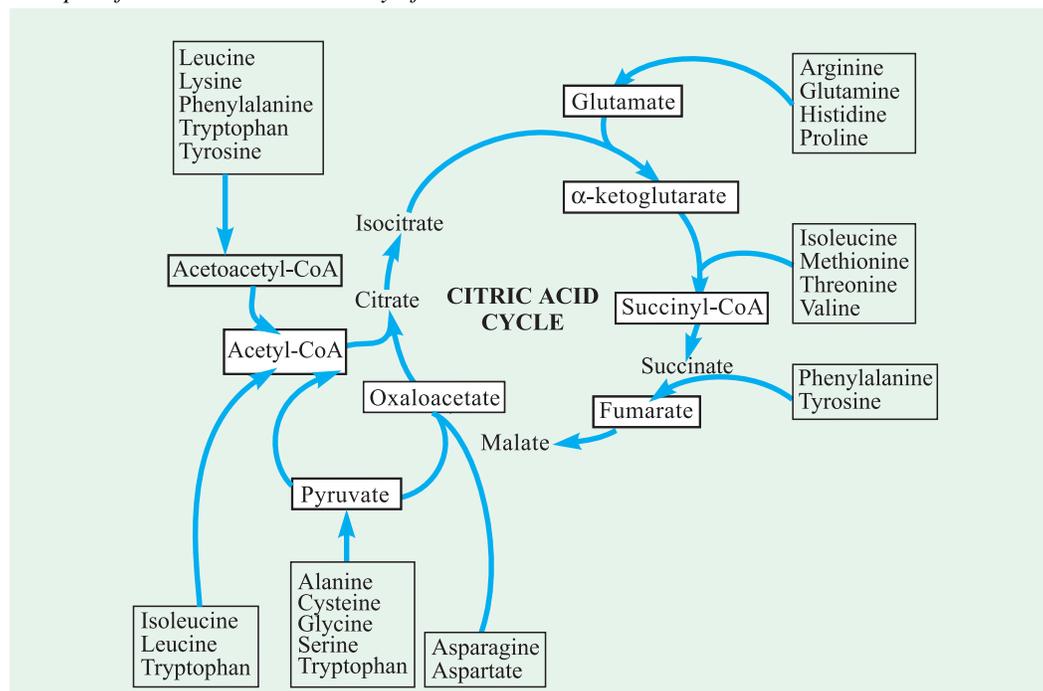


Fig. 26–13. Entry points of standard amino acids into the citric acid cycle

This scheme represents the major catabolic pathways in vertebrates, but there are minor variations from one organism to another. Some amino acids are listed more than once, reflecting the fact that different parts of their carbon skeletons have different fates. The 7 molecules into which the carbon skeletons of the diverse sets of 20 amino acids are funneled are shown within thick-lined boxes.

Based on their catabolic products, amino acids are classified as glucogenic or ketogenic (Table 26–2):

- those amino acids that generate precursors of glucose, *e.g.*, pyruvate or citric acid cycle intermediate (*i.e.*, α -ketoglutarate, succinyl-CoA, fumarate or oxaloacetate), are referred to as **glucogenic**. Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser, Thr and Val belong to this category. Net synthesis of glucose from these amino acids is feasible because these glucose precursors can be converted into phosphoenolpyruvate (PEP) and then into glucose.
- those amino acids that are degraded to acetyl-CoA or acetoacetyl-CoA are termed as **ketogenic** because they give rise to ketone bodies. Their ability to form ketones is particularly evident

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in untreated diabetes mellitus, in which large amounts of ketones are produced by the liver, not only from fatty acids but from the ketogenic amino acids. Leucine (Leu) exclusively belongs to this category.

The remaining 5 amino acids (Ile, Lys, Phe, Trp and Tyr) are both glucogenic and ketogenic. Some of their carbon atoms emerge in acetyl-CoA or acetoacetyl-CoA, whereas others appear in potential precursors of glucose. Thus, the division between glucogenic and ketogenic amino acids is not sharp. Whether an amino acid is regarded as being glucogenic, ketogenic or both depends partly on the eye of the beholder.

Table 26–2. Classification of amino acids as glucogenic or ketogenic

<i>Glucogenic</i>		<i>Ketogenic</i>	<i>Glucogenic and ketogenic</i>
Alanine	Glycine	Leucine	Isoleucine
Arginine	Histidine		Lysine
Asparagine	Methionine		Phenylalanine
Aspartate	Proline		Tryptophan
Cysteine	Serine		Tyrosine
Glutamate	Threonine		
Glutamine	Valine		

Table 26–3 lists the catabolic end products of the 20 protein (or standard) amino acids. It may be seen that nearly all of the amino acids yield on breakdown either an intermediate of the citric acid cycle, pyruvate or acetyl-CoA. Five amino acids (Leu, Lys, Phe, Trp and Tyr) are exception to this since they give rise to acetoacetic acid. Since, however, this compound also forms acetyl-CoA, carbon skeletons of all of the amino acids are ultimately oxidized *via* the citric acid cycle.

Table 26–3. End products of amino acid metabolism

<i>Amino acid(s)</i>	<i>End product</i>
Alanine, Cysteine (Cystine), Glycine, Serine and Threonine (2)	Pyruvic acid
Leucine (2)	Acetyl-CoA
Leucine (4), Lysine (4), Phenylalanine (4), Tryptophan (4) and Tyrosine (4)	Acetoacetic acid (or its CoA-ester)
Arginine (5), Glutamic acid, Glutamine, Histidine (5) and Proline	α -ketoglutaric acid
Isoleucine (4), Methionine and Valine (4)	Succinyl-CoA
Phenylalanine (4) and Tyrosine (4)	Fumarate
Asparagine and Aspartic acid	Oxaloacetic acid

* The figures in parentheses specify the number of carbon atoms in the amino acid that are actually converted to the end product listed.

A. Ten Amino Acids are Degraded to Acetyl-CoA.

The carbon skeletons of 10 amino acids yield acetyl-CoA, which enters the citric acid cycle directly. Five of the ten are degraded to acetyl-CoA *via* pyruvate. Alanine, cysteine, glycine, serine and tryptophan belong to this category. In some organisms, threonine is also degraded to form acetyl-CoA as shown in Fig. 26–14; in men, it is degraded to succinyl-CoA, as described later. The other five amino acids are converted into acetyl-CoA and/or acetoacetyl-CoA which is then cleaved to form acetyl-CoA. Leucine, lysine, phenylalanine, tryptophan and tyrosine come under this category.

Degradation to acetyl-CoA via pyruvate

Alanine yields pyruvate directly on transamination with α -ketoglutarate. The side chain of **tryptophan** is cleaved to yield alanine and thus pyruvate. **Cysteine** is converted to pyruvate in 2 steps: one to remove the S atom and the other a transamination. Serine is converted (or deaminated) to pyruvate by serine dehydratase. Both the α -amino and the β -hydroxyl groups of serine are removed in this single PLP-dependent reaction (an analogous reaction with threonine is shown in Fig. 26–15).

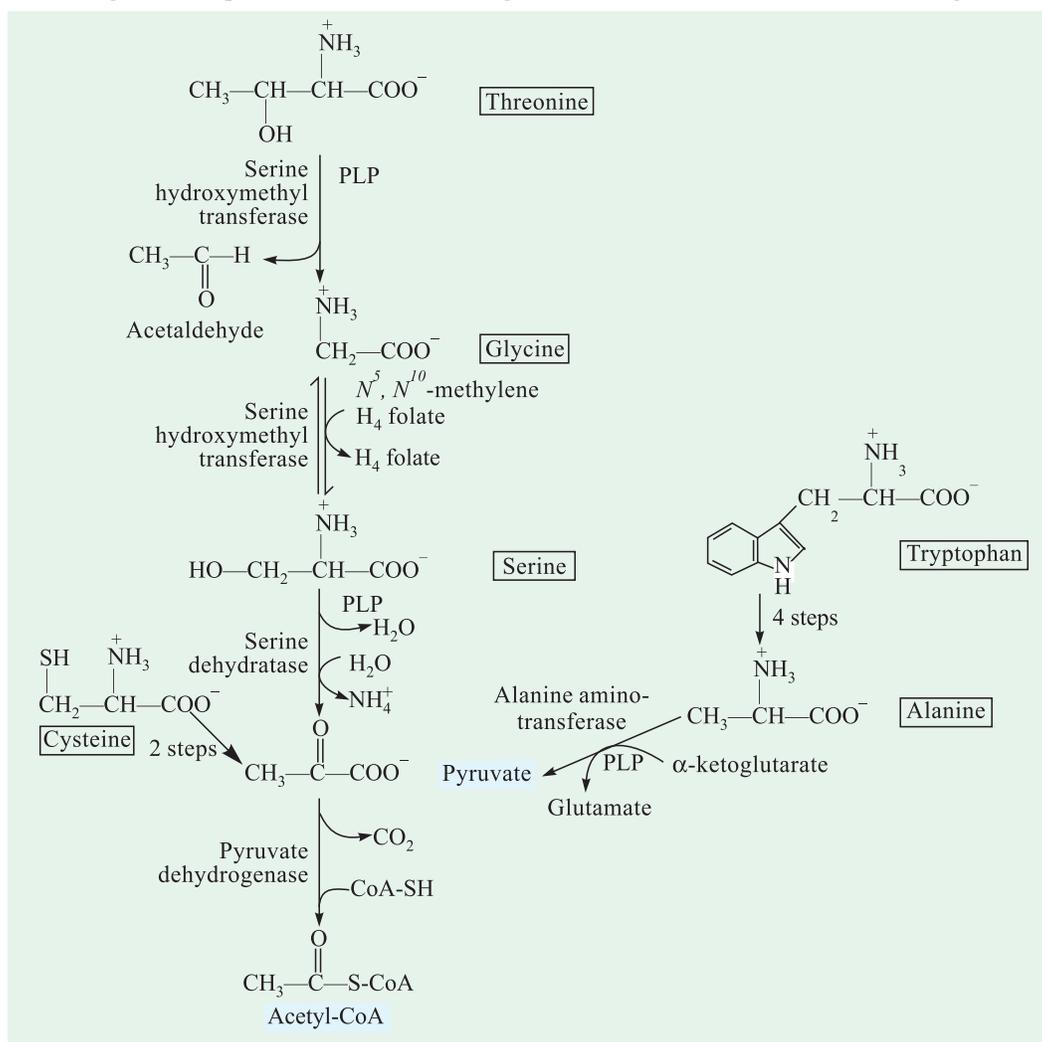


Fig. 26–14. Catabolic fates of glycine, serine, cysteine, tryptophan, alanine and also threonine

The fate of the indole group of tryptophan is shown in Fig. 26–16. Details of the two pathways for glycine are shown in Fig. 26–15.

Glycine has 2 routes. It can be converted to serine by enzymatic addition of a hydroxymethyl group (Fig. 26–15a). This reaction, catalyzed by serine hydroxymethyl transferase, requires two coenzymes, tetrahydrofolate and pyridoxal phosphate. The other route for glycine, which predominates in animals, involves its oxidative change into CO_2 , NH_4^+ and a $\text{-CH}_2\text{-}$ group (Fig. 26–15b). This readily reversible reaction, catalyzed by glycine synthase, also requires tetrafolate which accepts the methylene ($\text{-CH}_2\text{-}$) group. In this oxidative cleavage pathway, the 2 carbon atoms of glycine do not enter the citric acid cycle. One is lost as CO_2 , and the other becomes the methylene group of N^5, N^{10} -methylene-tetrahydrofolate.

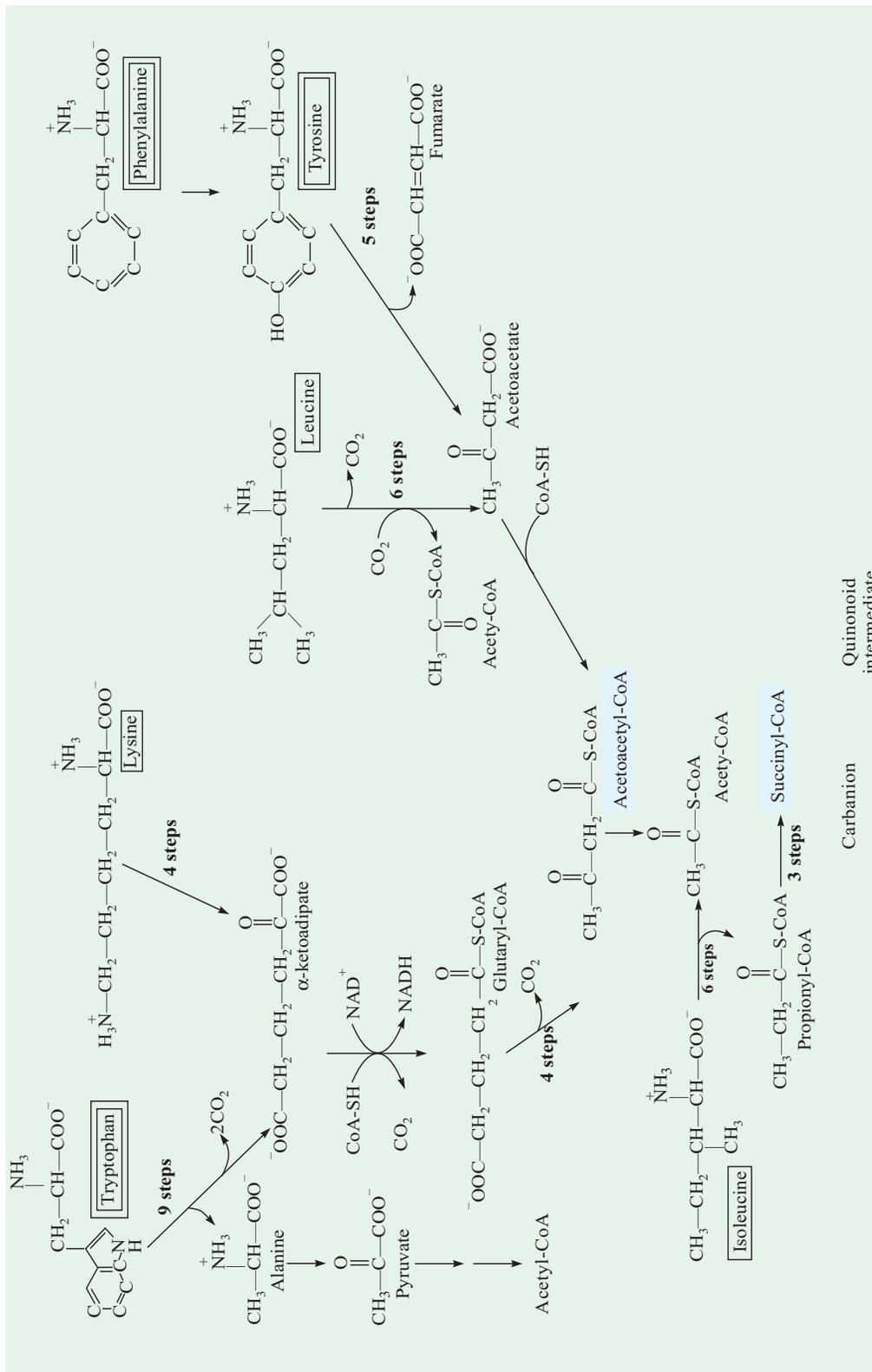


Fig. 26-16. Catabolic pathways for tryptophan, lysine, phenylalanine, tyrosine, leucine and isoleucine

All these amino acids donate some of their carbons to acetyl-CoA. Tryptophan, phenylalanine, tyrosine and isoleucine also contribute carbons as pyruvate or citric acid cycle intermediates.

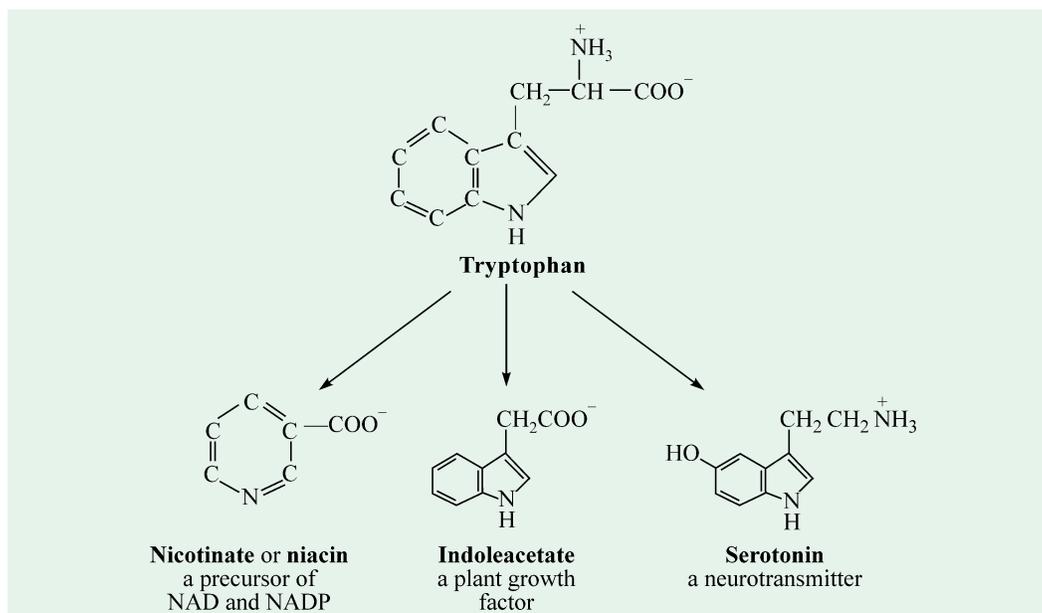


Fig. 26–17. Some of the intermediates of tryptophan catabolism

Atoms in boldface are used to trace the source of the ring atoms in nicotinate.

The next step is the transamination of tyrosine to produce an α -keto acid, *p*-hydroxyphenylpyruvate in a reaction catalyzed by **tyrosine aminotransferase**. *p*-hydroxyphenylpyruvate then reacts with O_2 to form homogentisate. In this reaction, a carbon atom is lost as CO_2 and a hydroxyl group is added to the phenyl ring to yield 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. Next follows the oxidative cleavage of the phenyl ring to produce 4-maleylacetoacetate. This reaction is catalyzed by another dioxygenase called **homogentisate oxidase**. In fact, *nearly all cleavages of the aromatic rings in biological systems are catalyzed by dioxygenases*, a class of enzymes discovered by Osamu Hayaishi. The 4-maleylacetoacetate is then isomerized by **4-maleylacetoacetate isomerase** to produce its *trans* isomer, 4-fumarylacetoacetate. In the last step, 4-fumarylacetoacetate is hydrolyzed by **fumarylacetoacetate** to generate fumarate and acetoacetate. The latter product, acetoacetate can subsequently be activated by **β -ketoacyl-CoA transferase** (with succinyl-CoA serving as the second substrate) to produce acetoacetyl-CoA, which can be oxidized aerobically.

Thus, we see that phenylalanine and tyrosine are both degraded into 2 fragments, each of which can enter the citric acid cycle, but at different points. Four of the 9 carbon atoms of phenylalanine and tyrosine yield acetoacetate, which is converted into acetoacetyl-CoA. A second four-carbon fragment of phenylalanine and tyrosine is recovered as fumarate. Eight of the 9 carbon atoms of these two amino acids thus enter the citric acid cycle; the remaining carbon is lost as CO_2 .

B. Five Amino Acids are Converted into α -ketoglutarate.

The carbon skeletons of 5 amino acids — arginine, histidine, proline, glutamate, glutamine — enter the citric acid cycle at α -ketoglutarate (Fig. 26–20). Proline, glutamate and glutamine have 5-carbon skeletons. The cyclic structure of **proline** is opened by oxidation of the carbon furthest from the carboxyl group to create a Schiff base and hydrolysis of the Schiff base to a linear semialdehyde, glutamate γ -semialdehyde. This is further oxidized at the same carbon to produce **glutamate**. By the action of glutaminase, **glutamine** donates its amide nitrogen to some acceptor and gets converted to glutamate. Transamination or deamination of glutamate produces the citric acid cycle intermediate.

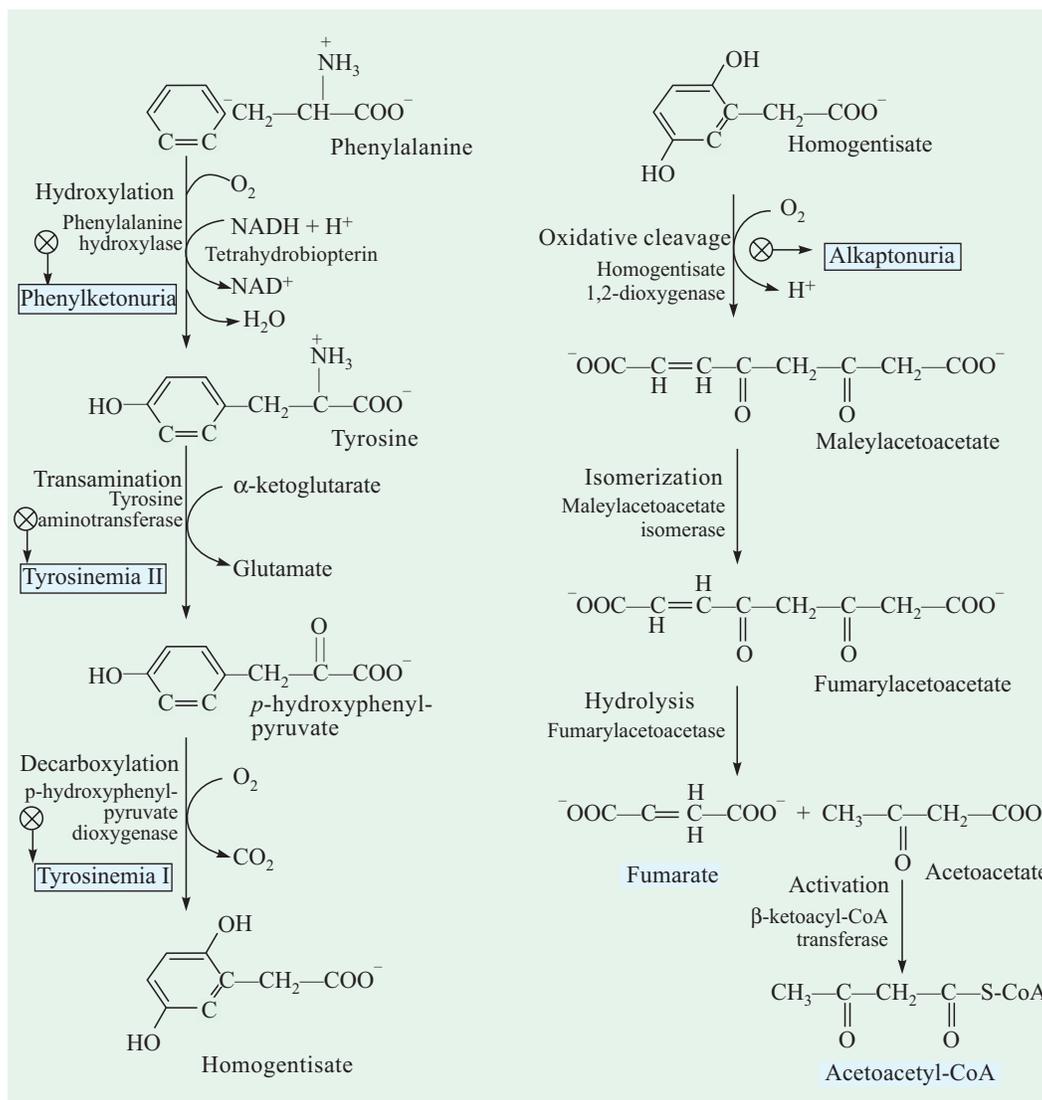


Fig. 26–18. The normal catabolic pathway of phenylalanine and tyrosine in human beings

Note that the pathway ultimately results in the production of fumarate and acetoacetyl-CoA. The genetic defects in each of the first four enzymes in this pathway are known to cause inheritable human diseases, shown here in rectangular boxes. The path of the 4 carbon atoms of acetoacetyl-CoA from phenylalanine downwards is traced by depicting them in boldface.

Arginine and histidine contains 5 adjacent carbons and a 6th carbon attached through a nitrogen atom. The catabolic conversion of these two amino acids to glutamate is, hence, slightly more complex than the path starting from proline or glutamine to glutamate. Arginine is converted to the 5-carbon skeleton of ornithine by arginase in the urea cycle (see Fig. 26–9), and the ornithine is transaminated to glutamate semialdehyde.

The conversion of **histidine** to the 5-carbon glutamate occurs in a 4-step pathway (Fig. 26–21). Histidine is first converted to urocanate by **histidine ammonia lyase**. Urocanate is then converted into 4-imidazolone 5-propionate enzymatically by the action of **urocanate hydratase**. The amide bond in the ring of this intermediate is hydrolyzed to the *N*-formimino derivative of glutamate

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enzymatically by **imidazolonepropionase**. The *N*-formiminoglutamate is then converted into glutamate by transfer of its formimino group to tetrahydrofolate, a carrier of activated one-carbon units. The enzyme **glutamate formimino transferase** catalyzes the reaction. The extra carbon is, thus, removed in this last step.

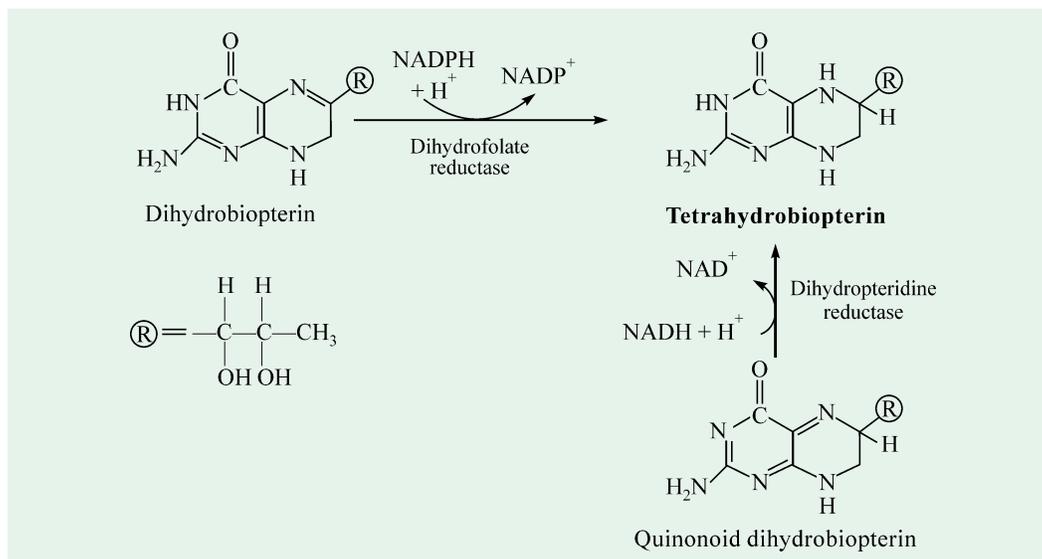


Fig. 26-19. Formation of tetrahydrobiopterin by reduction of either of the two forms of dihydrobiopterin

C. Four Amino Acids are Converted into Succinyl-CoA.

The carbon skeletons of methionine, isoleucine, threonine and valine are degraded by pathways that produce succinyl-CoA (Fig. 26-22). Methylmalonyl-CoA is an intermediate in the breakdown of these 4 amino acids.

Methionine donates its methyl group to one of the many possible acceptors through *S*-adenosylmethionine and 3 of the four remaining atoms of its carbon skeleton are converted into those of propionate as propionyl-CoA. **Isoleucine** first undergoes transamination and then oxidative decarboxylation of the resulting α -keto acid. The remaining 5-carbon skeleton derived from isoleucine undergoes further oxidation, producing acetyl-CoA and propionyl-CoA. In human beings, **threonine** is also converted into propionyl-CoA. The degradation of **valine** follows a path similar to those for methionine, isoleucine and threonine. After transamination, decarboxylation and a series of oxidation reactions, valine is converted to propionyl-CoA.

The pathway from propionyl-CoA to succinyl-CoA is especially interesting. Propionyl-CoA is carboxylated at the expense of an ATP to yield the *D*-isomer of methylmalonyl-CoA. This carboxylation reaction is catalyzed by **propionyl-CoA carboxylase**, a biotin enzyme that has a catalytic action like that of acetyl-CoA carboxylase and pyruvate carboxylase. The *D*-isomer of methylmalonyl-CoA is unusually racemized to the *L*-isomer. Lastly, *L*-methylmalonyl-CoA is converted to succinyl-CoA by an *intramolecular rearrangement*, using the enzyme **methylmalonyl-CoA mutase**, which is one of the two mammalian enzymes known to possess a derivative of vitamin B₁₂ as its coenzyme. The –CO–S–CoA group migrates from C–2 to C–3 in exchange for a hydrogen atom.

D. Three Branched-chain Amino Acids are Degraded in Extrahepatic Tissues

Although most of the amino acids are mainly catabolized in liver, the 3 amino acids with branched side chains (*viz.*, leucine, isoleucine and valine) are oxidized as fuels primarily in the extrahepatic tissues such as muscle, adipose, kidney and brain tissue. These 3 amino acids share the first two

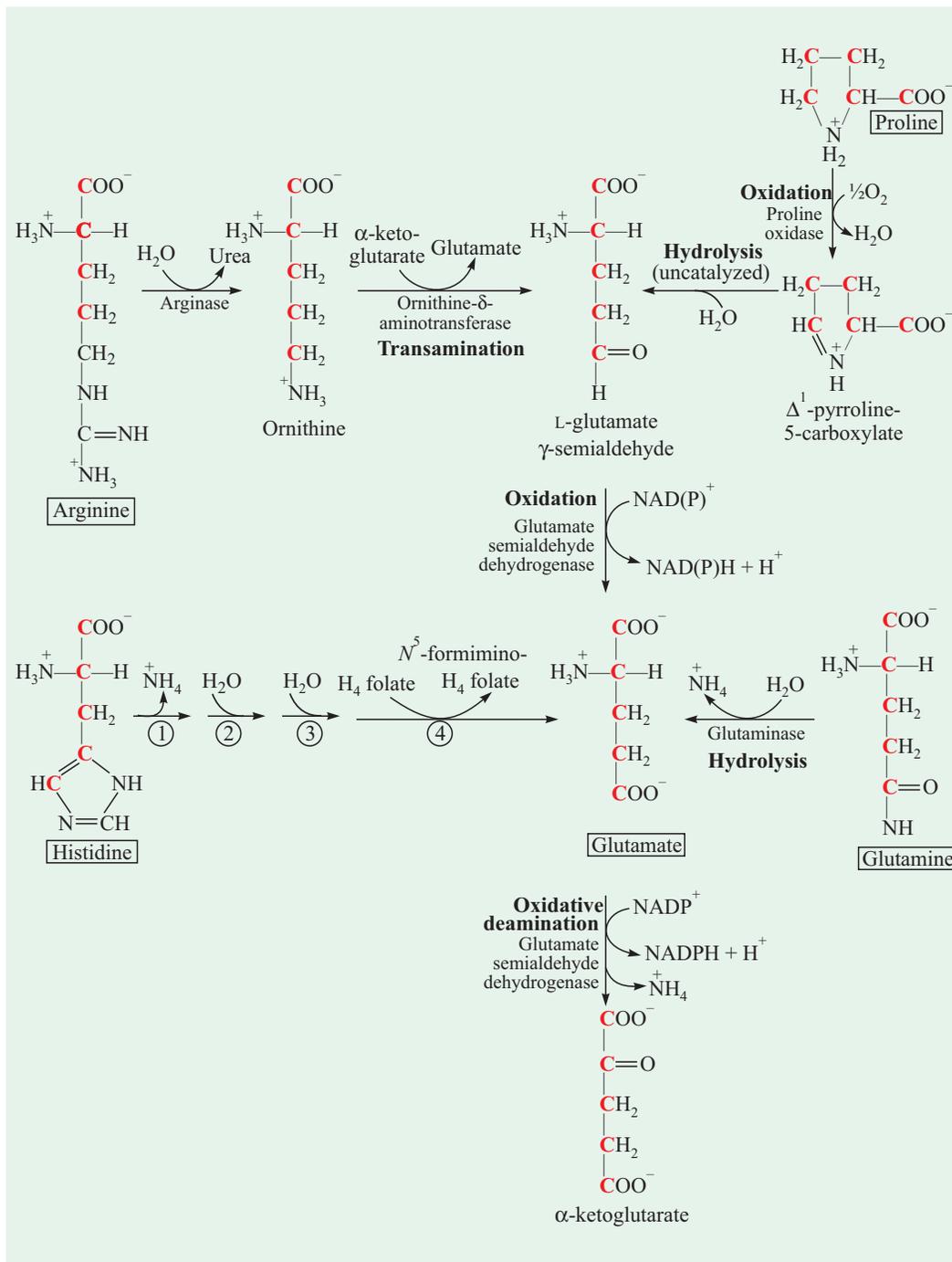


Fig. 26-20. Catabolic pathways for arginine, histidine, proline, glutamate and glutamine

Note that all these amino acids are converted to α -ketoglutarate. For the details of the breakdown of histidine to glutamate, which is a 4-step mechanism, refer Fig. 26-21. The numbered steps in the histidine pathway are catalyzed by (1) histidine ammonia lyase, (2) urocanate hydratase, (3) imidazolonepropionase, and (4) glutamate formimino-transferase.

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enzymes in their catabolic pathways, which occur in extrahepatic tissues. The first of these 2 enzymes, **aminotransferase**, not present in liver, acts on all 3 branched-chain amino acids to produce the corresponding α -keto acid (Fig. 26–23). The second enzyme, the **branched-chain α -keto acid dehydrogenase** catalyzes oxidative decarboxylation of the corresponding α -keto acid, releasing the carboxyl group as CO_2 and producing the acyl-CoA derivative. This dehydrogenase complex is formally analogous to the pyruvate and α -ketoglutarate dehydrogenases. In fact, all 3 enzymes are closely homologous in structure and the reaction mechanism is essentially the same for all. Five cofactors (TPP, FAD, NAD, lipoate and coenzyme A) participate and the 3 proteins in each of these complexes catalyze homologous reactions. The subsequent reactions are like those of fatty acid oxidation. Isoleucine yields acetyl-CoA, whereas valine yields methylmalonyl-CoA.

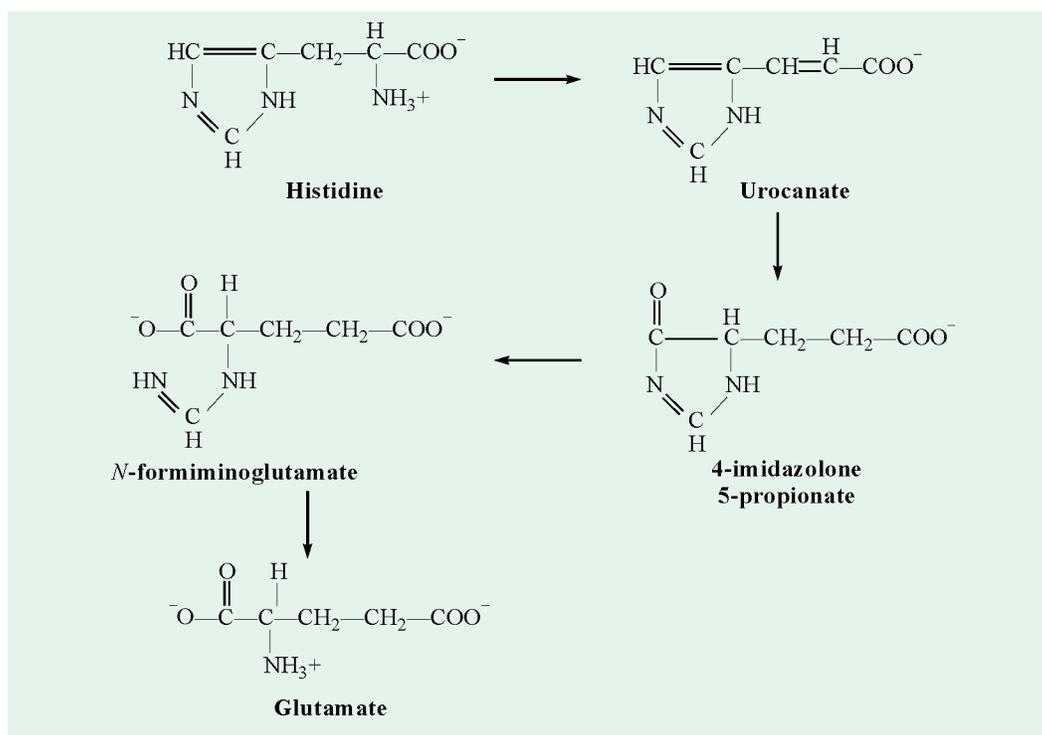


Fig. 26–21. Conversion of histidine into glutamate

E. Two Amino Acids are Degraded to Oxaloacetate.

The carbon skeletons of asparagine and aspartate ultimately enter the citric acid cycle via oxaloacetate. **Asparagine** is hydrolyzed by the enzyme **asparaginase** to NH_4^+ and aspartate (Fig. 26–24). **Aspartate** then undergoes a transamination reaction with α -ketoglutarate to yield glutamate and oxaloacetate. The latter enters the citric acid cycle. Recall that aspartate can also be converted into fumarate by the urea cycle (refer page 539). Fumarate is also a point of entry for half the carbon atoms of phenylalanine and tyrosine.

Thus, we have now seen that how the 20 different protein amino acids, after losing their nitrogen atoms, are degraded through dehydrogenation, decarboxylation and other reactions to yield portions of their carbon skeleton in the form of 5 central metabolites that can enter the citric acid cycle. Here they are completely oxidized to CO_2 and H_2O . During electron transfer, ATP is generated by oxidative phosphorylation, and in this way, the amino acids contribute to the total energy supply of the organism.

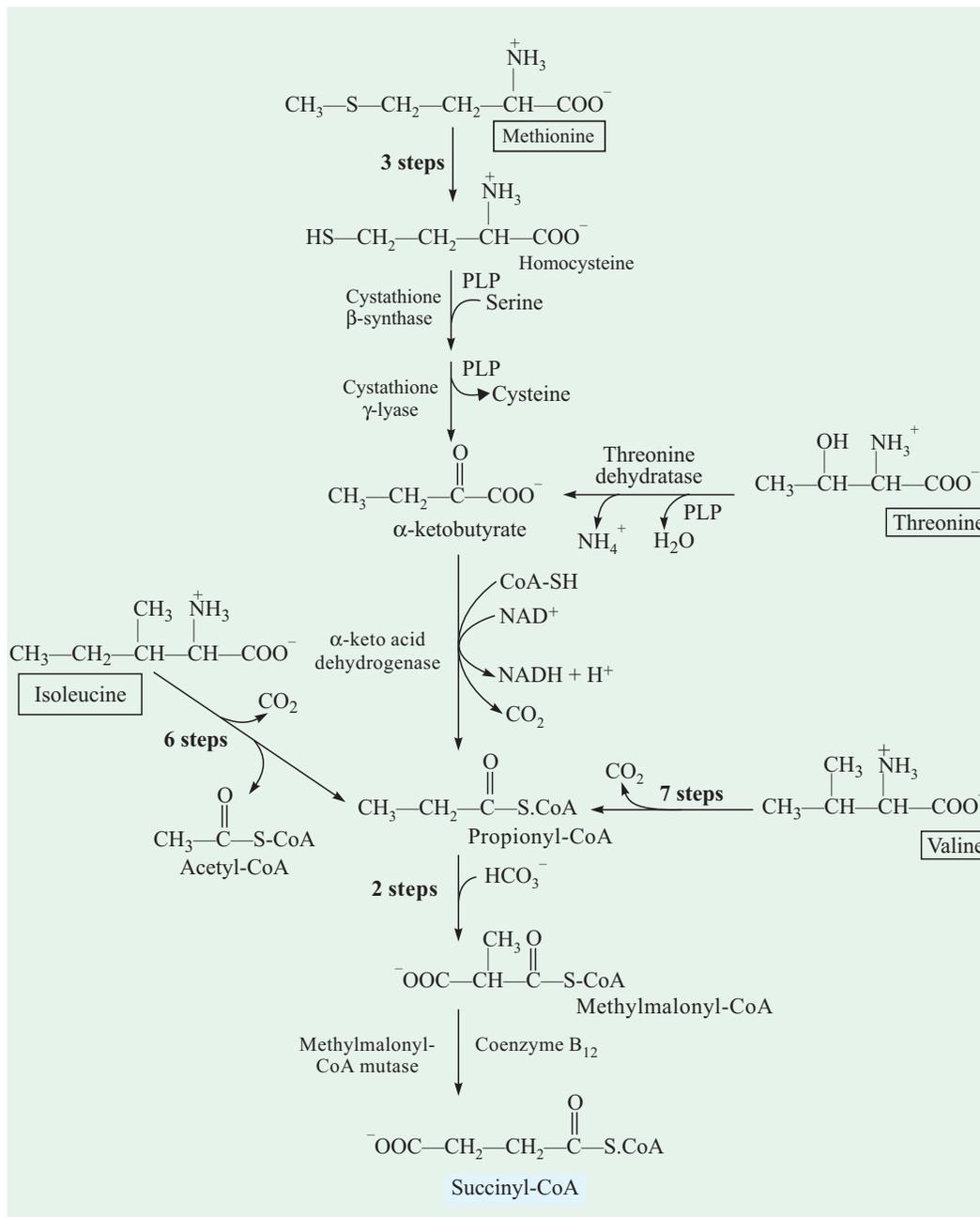


Fig. 26-22. The catabolic pathways for methionine, isoleucine, threonine and valine

Note that isoleucine also contributes two of its carbon atoms to acetyl-CoA. The threonine pathway shown here occurs in human beings. Another pathway for threonine degradation is shown in Fig. 26-14.

INBORN ERRORS OF AMINO ACID CATABOLISM

Many different genetic defects in amino acid metabolism have been identified in humans (Table 26-4). Most such defects cause specific intermediates to accumulate a condition that can cause defective neural development and mental retardation.

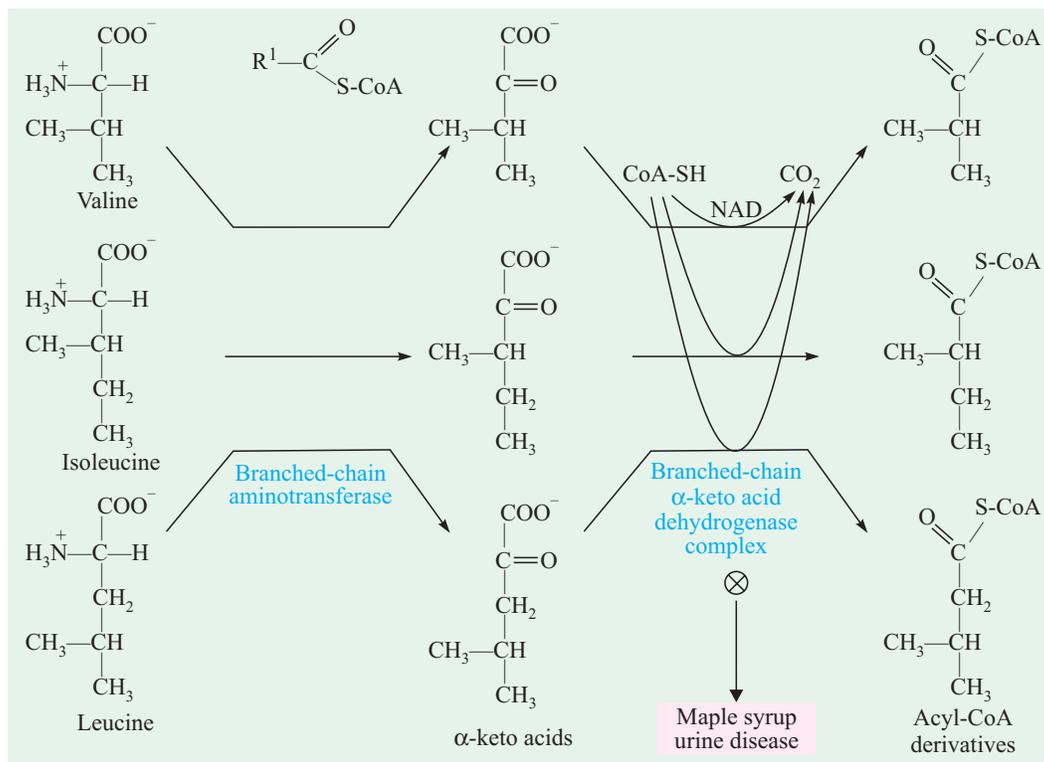


Fig. 26-23. The catabolic pathway for leucine, isoleucine and valine

Note that these 3 branched-chain amino acids share the first two enzymes in their catabolic pathways, which occur in extrahepatic tissues. The second enzyme, the branched-chain α -keto acid dehydrogenase complex is defective in people suffering from maple syrup urine disease. This dehydrogenase complex is analogous to the pyruvate and α -ketoglutarate dehydrogenases and requires the same 5 cofactors, some of which are not shown here.

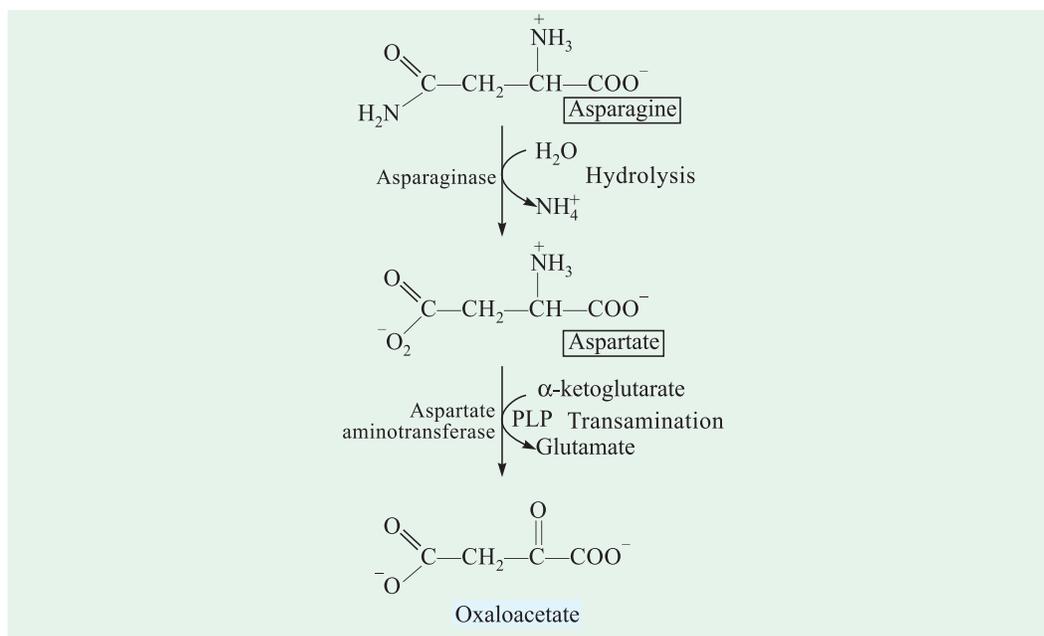


Fig. 26-24. The catabolic pathway for asparagine and aspartate

Table 26-4. Some human genetic disorders affecting amino acid catabolism

Medical condition	Approximate incidence (per 1 lac births)	Defective process	Defective enzyme	Symptoms and effects
Albinism*	3	Melanin synthesis from tyrosine	Tyrosine 3-monooxygenase (= tyrosinase)	Lack of pigmentation, White hair, Pink skin
Alkaptonuria*	0.4	Tyrosine degradation	Homogentisate 1,2-dioxygenase	Dark pigment in urine, Late developing arthritis
Argininemia	< 0.5	Urea synthesis	Arginase	Mental retardation
Argininosuccinic acidemia	1.5	Urea synthesis	Argininosuccinate lyase	Vomiting, Convulsions
Carbamoyl phosphate synthetase I deficiency	> 0.5	Urea synthesis	Carbamoyl phosphate synthetase I	Lethargy, Convulsions, Early death
Homocystinuria	0.5	Methionine degradation	Cystathione β -synthase	Faulty bone development, Mental retardation
Maple syrup urine disease* (= Branched-chain ketoaciduria)	0.4	Isoleucine, leucine and valine degradation	Branched-chain α -keto acid dehydrogenase complex	Vomiting, Convulsions, Mental retardation, Early death
Methylmalonic acidemia	< 0.5	Conversion of propionyl-CoA into succinyl-CoA	Methylmalonyl-CoA mutase	Vomiting, Convulsions, Mental retardation, Early death
Phenylketonuria* (= Hyperphenylalaninemia)	8	Conversion of phenylalanine to tyrosine	Phenylalanine hydroxylase	Neonatal vomiting, Mental retardation

* Disorders marked with an asterisk are described in the text.

(Adapted from Lehninger AL, Nelson DL and Cox MM, 1993)

Several metabolic disorders were termed by Garrod **inborn errors of metabolism** because each is present throughout life and is hereditary. In 1902, he showed that alkaptonuria, one of the metabolic syndromes, is transmitted as a single recessive Mendelian trait and is due to the absence of a metabolic enzyme which, 45 years later, was identified as homogentisate 1,2-

Sir Archibald E. Garrod (LT, 1858-1936), an English pediatrician and often called the '*father of biochemical genetics*', is an example of a scientist ahead of his time. Although Garrod had developed his hypothesis by 1902, it is of interest to note that the word gene was not coined until 1911 and the first enzyme was not crystallized until 1926. His contributions were summarized in the Croonian lectures in 1908. His classic and prescient book, "*Inborn Errors of Metabolism*" (1909) was a most imaginative and important contribution to Biology and Medicine. As in the case of Gregor Johann Mendel, Garrod's perception was well ahead of his time and consequently had little impact. But some 4 decades after, Garrod's findings were well appreciated and acknowledged.

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dioxygenase with the help of liver biopsy analysis. In essence, he proposed *one gene-one enzyme hypothesis*, which Beadle and Tatum later elucidated in 1940. Garrod, thus, perceived the direct relationship between genes and enzymes. The inborn errors, listed by Garrod in 1908, were cystinuria, alkaptonuria, pentosuria and albinism. Hundreds of metabolic alterations have since been described that have a genetic basis.

1. Alkaptonuria

Alkaptonuria is an inherited metabolic disorder found in infants (approximately one in every 2,00,000 live births) and caused by the absence of **homogentisate 1,2-dioxygenase**. Homogentisate is a normal intermediate in the degradation of phenylalanine and tyrosine (refer Fig. 26–18) and it accumulates in alkaptonurics because its breakdown is blocked. Homogentisate is then excreted in the urine which turns dark on standing open to the atmosphere as homogentisate is oxidized and polymerized to a melanin-like substance. Alkaptonuria is a relatively benign condition and results in no serious ill effects. But it has historical importance for being the first such disease to be associated with an inborn error. The alkaptonurics live until well into reproductive age with no difficulty other than whatever esthetic offense the darkening urine may represent. Many in their fourth or fifth decade develop arthritis. The degeneration of the connective tissue in the joints is apparently associated with a deposition of pigment (**ochronosis**), presumably resulting from further oxidation of homogentisate in cartilage.

Alkaptonuria is also spelt as **alcaptonuria**.

2. Albinism

Another inborn error associated with phenylalanine and tyrosine metabolism is albinism, an autosomal recessive trait. The biochemical defect involves melanin production, for which tyrosine is the precursor (Fig. 26–25). One type of albinism is believed to be due to a deficiency of **tyrosinase** (= **tyrosine 3-monooxygenase**), a copper-containing enzyme needed for melanin synthesis.

Melanin (*melan*^G = black) is a black pigment present in the skin and hair. This polymeric pigment is formed in granules called *melanosomes* that are rich in *tyrosinase*, a monooxygenase enzyme.

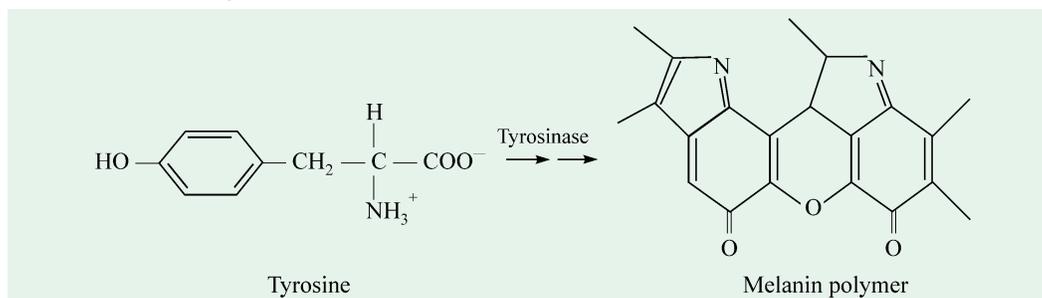


Fig. 26–25. Production of melanin

3. Phenylketonuria or Hyperphenylalaninemia

Phenylketonuria (PKU), the commonest inborn error of metabolism, is so named because of high levels of phenylalanine, a type of phenylketone, in the urine of individuals afflicted with this disease. It is also known as **phenylpyruvic oligophrenia** because the condition results in early neurological damage preventing normal intellectual development. It was among the first human genetic defects of metabolism discovered. Phenylketonuria can have devastating effects in contrast with alkaptonuria. Untreated individuals with PKU are nearly always *severely mentally retarded* (a mean I.Q. of 20). In fact, about 1% of the institutionalized patients (those admitted in mental institutions) have phenylketonuria. The weight of the brain of these individuals is below normal, myelination of their nerves is defective and their reflexes are hyperactive. The life expectancy of untreated phenylketonurics

is drastically shortened. Nearly half are dead by age 20, and three-quarters by age 30. Very fair skin and light blonde hair are two characteristics of phenylketonuria.

Phenylketonuria is caused by an absence or deficiency of **phenylalanine hydroxylase (= phenylalanine 4-monooxygenase)** or, more rarely, of its tetrahydrobiopterin cofactor. Phenylalanine cannot be converted to tyrosine and so there is an accumulation of phenylalanine in all body fluids. Under such conditions, a secondary pathway of phenylalanine metabolism, normally little used, comes into play. In this minor pathway (Fig. 26–26), phenylalanine undergoes transamination with pyruvate to form **phenylpyruvate**. Much of the **phenylpyruvate** is either decarboxylated to produce **phenylacetate** or reduced to form **phenyllactate**. Phenylacetate imparts a characteristic odour to the urine that has been used by nurses to detect PKU in infants.

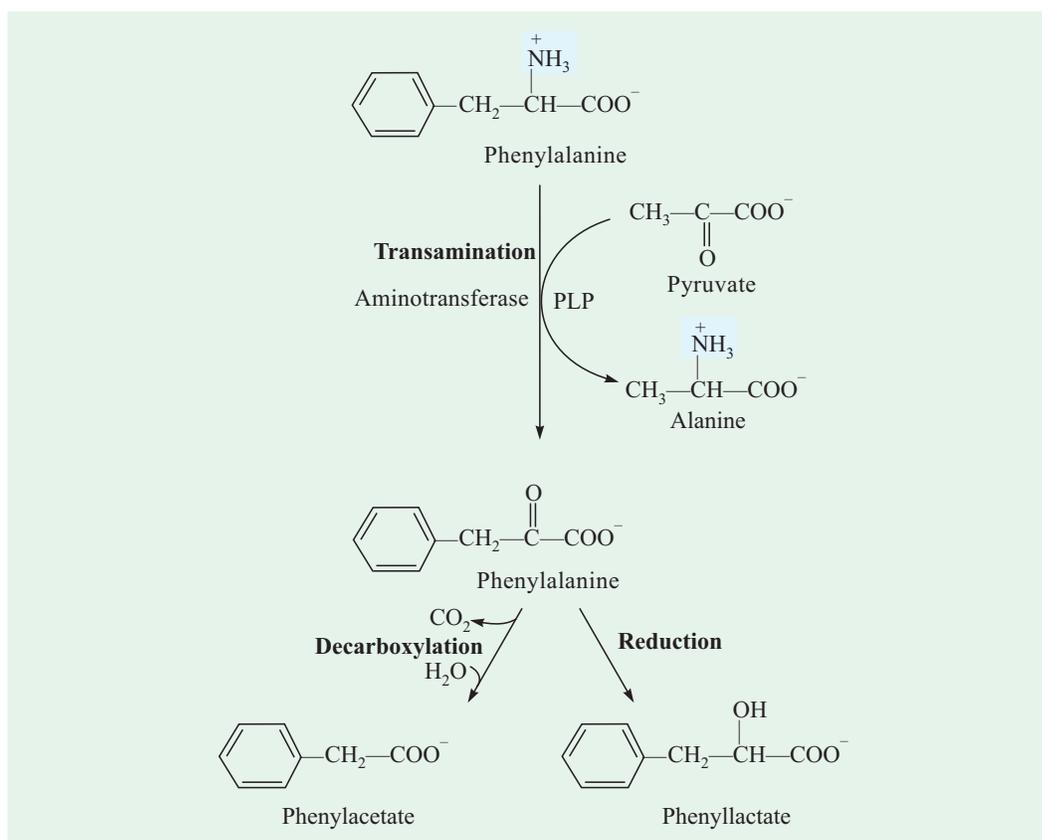


Fig. 26–26. Alternative pathways for catabolism of phenylalanine in phenylketonurics

Phenylpyruvate accumulates in the tissues, blood and urine. Both phenylacetate and phenyllactate can also be found in the urine.

At a first glance, it might be assumed that the inability to synthesize tyrosine, the precursor of melanin, is responsible for the observed lack of pigmentation. However, tyrosine is not lacking since food proteins (*e.g.*, casein of milk) furnish adequate amounts of the amino acid. Melanin production is impaired because high levels of phenylalanine effectively compete with tyrosine as substrate, *i.e.*, competitive inhibition.

Phenylketonurics appear normal at birth but are severely defective by age one, if untreated. PKU can be cured by giving low phenylalanine diet, just enough to meet the needs for growth and development. Foods that are rich in proteins must be avoided rather curtailed. Proteins with low phenylalanine content, such as casein of milk, must first be hydrolyzed and much of the phenylalanine

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removed by adsorption to provide an appropriate diet for phenylketonurics, at least through childhood to prevent irreversible brain damage. A phenylketonuric child must be maintained on this restricted low-protein diet (with no more than 200-500 mg/day of phenylalanine), and continued as long as possible or until at least by age 3, by which time brain development is complete. Although severe mental retardation can be prevented by this treatment, the phenylketonurics exhibit other physical and/or emotional problems, many of which remain biochemically unexplained. It should be noted that a high blood level of phenylalanine in a pregnant mother can result in abnormal development of the fetus. *This is a striking example of maternal-fetal relationship at the molecular level.*

Phenylketonuria is inherited as an autosomal recessive and the approximate incidence of phenylketonuria in individuals is 80 out of 10,00,000 live births. Data on the low frequency of this and other genetic disorders can mislead the unwary on the prevalence of the altered genes that cause them. In the present instance of PKU, for example, both chromosomes carrying specifications for the protein must be defective because the disease is recessive and as such both parents must have contributed defective versions of the genes. In such cases, the probability that a child will have two defective genes is the product of the probabilities for the presence of one defective gene in each chromosome. Since the gene is **autosomal**, *i.e.*, it does not occur on a sex chromosome, the probabilities are the same for males as they are for females in large populations. Therefore, the estimated gene frequency for PKU would be $(80 \times 10^{-6})^{1/2}$, which is 8.9×10^{-3} . Approximately, one out of every 57 humans has a gene for phenylketonuria in one of his paired chromosomes, an impressive incidence.

4. Maple syrup urine disease (MSUD) or Branched-chain ketoaciduria

Maple syrup urine disease is a rare autosomal, recessive, hereditary disorder in which the 3 branched-chain amino acids (leucine, isoleucine, valine) accumulate in the blood and “spill over” into the urine. MSUD presents acutely in the neonatal period. The disease is so named because of the characteristic odour of the urine which resembles that of maple syrup or burnt sugar and is imparted to the urine by the α -keto acids. In afflicted individuals, both plasma and urinary levels of 3 branched-chain amino acids and their corresponding α -keto acids are greatly elevated. For this reason, the disease has also been termed **branched-chain ketonuria**. Smaller quantities of branched-chain - hydroxy acids, formed by reduction of α -keto acids, also are present in the urine.

Although the afflicted newborn infant initially appears to be normal, the characteristic symptoms of the disease are evident by the end of the first week of extrauterine life. Besides the biochemical abnormalities mentioned above, the infant is difficult to feed, may vomit and may show alternate periods of hypertonicity and flaccidity. The patient may be highly lethargic. Extensive brain damage and demyelination occur in surviving children. If left untreated, death ensues by the end of first year of life.

The biochemical defect is the absence or highly reduced activity of the **branched-chain α -keto acid dehydrogenase complex**, which catalyzes conversion of all 3 branched-chain keto acids to CO_2 plus acetyl-CoA derivatives. All 3 branched-chain α -keto acids are competitive inhibitors of L-glutamate dehydrogenase activity.

Maple syrup urine disease is usually fatal until the patient is placed on a diet in which protein is supplied by a mixture of all the purified amino acids except leucine, isoleucine and valine. When the plasma levels of these amino acids fall within the normal range, they are restored to the diet in the form of milk and other foods in amounts adequate to supply, but not to exceed, the requirements for these branched-chain amino acids.

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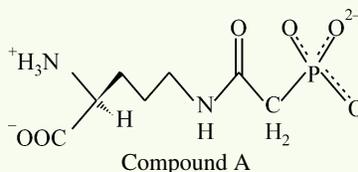
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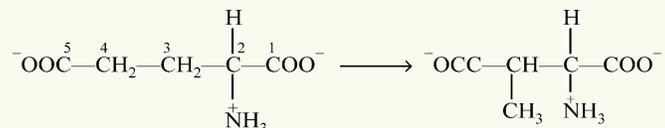
PROBLEMS

1. Name the α -ketoacid that is formed by transamination of each of the following amino acids
1, 6-bisphosphat :
- | | |
|---------------|-------------------|
| (a) Alanine | (d) Leucine |
| (b) Aspartate | (e) Phenylalanine |
| (c) Glutamate | (f) Tyrosine |
2. Compound A has been synthesized as a potential inhibitor of a urea-cycle enzyme. Which enzyme do you think compound A might inhibit ?



3. How would you treat an infant who is deficient in argininosuccinate synthetase ? Which molecules would carry nitrogen out of the body ?
4. Why should phenylketonurics avoid using aspartame, an artificial sweetener ? (**Hint:** Aspartame is L-aspartyl-L-phenylalanine methyl ester.)
5. Within a few days after a fast begins, nitrogen excretion accelerates to a higher-than-normal level. After a few weeks, the rate of nitrogen excretion falls to a lower level and continues at this low rate. However, after the fat stores have been depleted, nitrogen excretion rises to a high level.
- | |
|--|
| (a) What events trigger the initial surge of nitrogen excretion ? |
| (b) Why does nitrogen excretion fall after several weeks of fasting ? |
| (c) Explain the increase in nitrogen excretion when the lipid stores have been depleted. |
6. Isoleucine is degraded to acetyl-CoA and succinyl CoA. Suggest a plausible reaction sequence, based on reactions discussed in the text, for this degradation pathway.

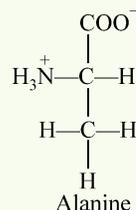
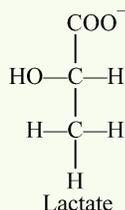
7. Use numbers 1 to 5 to identify each carbon atom in the product of this reaction. What is the coenzyme ?



8. Explain the basis for the following statement : As a coenzyme, pyridoxal phosphate is covalently bound to enzymes with which it functions, yet during catalysis the coenzyme is not covalently bound.
9. Briefly discuss how a yeast cell might contain two glutamate dehydrogenases, one specialized for nitrogen assimilation and one for amino acid catabolism, and not dissipate energy in the futile cycle,
- $$\text{glutamate} \rightleftharpoons \alpha\text{-ketoglutarate.}$$
10. Draw the structure and give the name of the α -keto acid resulting when the following amino acids undergo transamination with α -ketoglutarate :
- (a) Aspartate (c) Alanine
(b) Glutamate (d) Phenylalanine
11. If your diet is rich in alanine but deficient in aspartate, will you show signs of aspartate deficiency ? Explain.
12. A two-year-old child was brought to the hospital. His mother indicated that he vomited frequently, especially after feedings. The child's weight and physical development were below normal. His hair, although dark, contained patches of white. A urine sample treated with ferric chloride (FeCl_3) gave a green color characteristic of the presence of phenylpyruvate. Quantitative analysis of urine samples gave the results shown in the table below.

Substance	Concentration in patient's urine (mM)	Normal concentration in urine (mM)
Phenylalanine	7.0	0.01
Phenylpyruvate	4.8	0
Phenyllactate	10.3	0

- (a) Suggest which enzyme might be deficient. Propose a treatment for this condition ?
- (b) Why does phenylalanine appear in the urine in large amounts ?
- (c) What is the source of phenylpyruvate and phenyllactate ? Why does this pathway (normally not functional) come into play when the concentration of phenylalanine rises ?
- (d) Why does the patient's hair contain patches of white ?
13. The three carbons in lactate and alanine have identical states of oxidation, and animals can use either carbon source as a metabolic fuel. Compare the net ATP yield (moles of ATP per mole of substrate) for the complete oxidation (to CO_2 and H_2O) of lactate versus alanine when the cost of nitrogen excretion as urea is included.



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- 14.** Aspartate aminotransferase has the highest activity of all the mammalian liver aminotransferases. Why ?
- 15.** A weight-reducing diet, heavily promoted some years ago, required the daily intake of “liquid protein” (soup of hydrolyzed gelatin), water, and an assortment of vitamins. All other food and drink were to be avoided. People on this diet typically lost 10 to 14 lb in the first week.
 - (a)* Opponents argued that the weight loss was almost entirely water and would be regained almost immediately when a normal diet was resumed. What is the biochemical basis for the opponent’s argument ?
 - (b)* A number of people on this diet died. What are some of the dangers inherent in the diet and how can they lead to death ?
- 16.** Blood plasma contains all the amino acids required for the synthesis of body proteins, but they are not present in equal concentrations. Two amino acids, alanine and glutamine, are present in much higher concentrations in normal human blood plasma than any of the other amino acids. Suggest possible reasons for their abundance.
- 17.** An inability to generate tetrahydrobiopterin would have what specific effects on the metabolism of phenylalanine, tyrosine, and tryptophan ?
- 18.** Untreated phenylketonuria patients, in addition to mental retardation, have diminished production of catecholamines and light skin and hair. If the defect is in phenylalanine hydroxylase itself, a diet lacking phenylalanine but including tyrosine alleviates these conditions. If the defect is in the ability to produce tetrahydrobiopterin, the same dietary treatment may alleviate the mental retardation and light hair but not the diminished catecholamine production. What is the rationale explaining these findings ?